

Control of Signal Transmission Across the Liquid Circuits of Biological Network Through Freeze Switch

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Abstract : The electrically turning off properties of the liquid circuit in a biological network or a generalized conductive microfluidic system based on a new conception electrical switch was proposed. By selectively freezing part of the electric aqueous solution inside the circuit , the ions to conduct the electric current along a specific direction can be completely shut off due to the formation of the frozen insulation solution. After thawing , the current along the microfluidic circuit will resume its turning-on status again. For a preliminary demonstration , several conceptual experiments were performed to test the transient electrical impedances of the solution or biological system subject at freezing and heating , the convincing results were obtained. The working performances of the freezing electric switch on controlling the conductive liquid circuit were summarized , and its potential applications in electrophoretic analysis , liquid chromatogram analysis or especially controlling the signal transmission across the neural network , are suggested.

Key words : microfluidic system ; biological network ; wet circuit ; liquid circuit ; TECD ; nano electrical switch ; freezing and thawing

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冻结开关控制生物网络 液体电路中的信号传输

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摘要 : 通过引入一种新概念型电开关 , 首次提出研究生物网络液体电路或具有普遍意义的导电性微流体系统中的电学关断问题 , 通过有选择性地对通路中的水性电溶液实施冻结 , 则其内在特定方向上传输电流的离子可因溶液冻结后绝缘性的形成而被完全阻断 , 而一旦复温融化后 , 则此微流体电路中的电流又恢复其开启状态。为初步证实上述设想 , 设计了相应的概念性试验 , 通过检测溶液或生物系统在冻结及加热时的电阻瞬态响应规律 , 证实了方法的可行性。总结了冻结性电开关在控制导电性液体电路方面的工作性能及其在电泳分析、液相色谱分析以及在控制神经网络中信号传输方面的潜在应用。

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关键词：微/纳米流体系统；生物网络；湿性电路；液体电路；半导体制冷片；纳米电开关；冻结及融化

1 Introduction

Liquid circuit, the construction of fluidic devices similar to electronic circuits, had ever been an active research field over the 1960s and early 1970s^[1]. Recently, with the advent of miniaturized micro/nano fluidic technologies, there is an increasing demand for logical control of such devices. Some newly emerging fluidic devices such as oscillators, flip-flops, amplifiers, and logic gates were built and integrated into sophisticated control systems^[1-3]. In fact, nature has produced a great many different liquid circuits in biological system. For example, the neural system serves as just one of such typical circuits, which were wired together to make up a complex network through which the information spreads and processes. Through diverse and complicated biochemical reactions, neuron is able to receive process and transmit the signals over the synapses. The electrical impulses can pass through the body of the neurons by means of changes in the membrane potential^[2]. Up to now, some new techniques have been developed to operate the growth of myocytes and neurons to assemble a functional network^[4,5]. The neurons also have been inoculated onto the silicon chip to form a part of the neuron-semiconductor hybrid circuit^[6], which will help to study the function of neurons and provide an opportunity to reveal the physical mechanisms of thinking. Clearly, if a micro/nano-scale switch can be fabricated to flexibly turn on/off the neuron, it should be an important aid for such endeavors.

However, operating living system is not as easy as controlling a solid electrical circuit. With the complexity and nonlinearity that can be introduced into the system through the use of multi-

phase flows or chemically responsive materials, few controlling or actuating elements have been demonstrated for flexibly operating the liquid circuit especially the biological network. Usually the commonly adopted switch is through a physical cutting to stop the current of electrical charges. But within a micro or even nano fluidic system, it is difficult to completely cut the current in the flow just by a mechanical valve^[3]. The troubles mainly come from the following reasons. Firstly, traditional micromachined valves are complex and have many components, which will make the control systems fragile and thus unreliable, saying nothing of their high cost. Secondly, most of them consist of a bulk micromachined orifice and a deflectable sealing element. The sealing element generally can be a membrane, a ring mesa, a cantilever or a float^[7-10]. These valves execute their functions through actions between the orifice and the sealing element. Because of the existence of such moving elements, the electrical current could not be completely turned off due to the flow leakage around the valve. Unfortunately it is difficult to solve this problem through the traditional way. Lastly, if the valves are made of electrically conductive materials, the electrical current is not controllable. In this side, the recently established ice valve^[3] can basically resolve most of the foregoing problems, which turns out to be a good candidate for the electrical control of liquid circuit. As revealed before, electrical impedance of biological materials could become almost infinitely large during the processes of freezing and thawing^[11].

Therefore freezing the target circuit will significantly increase the electrical resistance and thus prevent the electrical signal from transmission across there. Here, aiming at establishing a flexi-

ble tool of electric switch for studying the biological circuit network or the like, this paper will focus on testing the new approach of using freezing to stop the electrical signal transmission along a liquid circuit or some biological systems.

2 Freezing electric switch for liquid circuit

Here, the proposed electrical switch is different in conception from that of the previously established ice valve, which is for mechanically turning off the flow. A typical form of such electrical switch is consisted of a thermoelectric cooling device (TECD) and the auxiliary parts. As illustrated in Fig.1, there run both the solutions and the electrical currents in the working channel. The electrical signal switch, which is the main component of the circuit, is controlled by a TECD (or other freezing methods such as employing a pre-cooled heat-sink) to freeze the solution in the channel. Besides, a fin-like heat dissipater was connected with TECD to keep its working temperature under a safe range. Meanwhile, effective encapsulation was adopted to cover the whole system, which is to avoid disturbance from the surrounding environment.

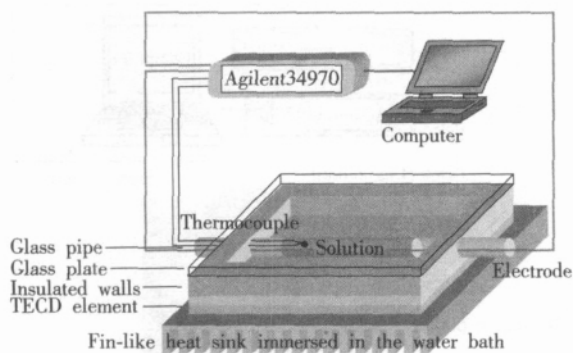


Fig.1 The visual view on the experimental setup

图1 实验台示意图

When a positive electric voltage is applied on TECD, the working fluid running or resting inside

the channel begins to be frozen in a short time. With the phase change of the solution, the flow itself will be blocked. Meanwhile, the liquid circuit turns to be switched off due to formation of the ice, which has extremely large electrical resistance. If switching the electric voltage to its opposite direction, the TECD begins to heat the frozen water, the "ice" in the blocked channel will resume to its flowing state again and ions in the aqueous solution are then free to move and electrical circuit is connected. Clearly, such freeze switch puts an end to potential short circuit, which basically comes from the possible leakage in a mechanical switch. Therefore without leakage of fluidic solution, the freezing based electrical switch could produce a strong enough resistance to cut the electrical current. Particularly, since no external elements were introduced into the fluidic circuit, the freeze switch appears very clean. The other charming property of this switch still lies in that no moving elements are required in the flow channel. Therefore complexity and maneuverability thus involved can be avoided. When the freezing process occurs, the electriferous ions are frozen within the crystal water molecules. The current was cut off as soon as the movement of the ions was stopped. In this sense, flow and electric current of the working fluid is broken just by itself. As gradually known that, a high-density microfluidic circuit chip would consist of hundreds of functional logical calculators, memories and individually addressable reaction chambers^[1]. With self-supporting capability, both the electrically controlled freeze switch and fluidic circuit will find interesting applications in such systems.

3 Experimental setup

To demonstrate the feasibility of using the freeze switch to control the fluidic circuit, several experiments were carried out to characterize their

working performance based on the principle described above. As schematically shown in Fig.1, the micro channel is chosen as a circular pipe and it can generally be made as various geometries for different purposes or fabrication techniques. A transparent thin vitreous tube with dimension $\Phi 2.0 \text{ mm} \times 0.5 \text{ mm} \times 100 \text{ mm}$ (outer diameter \times thickness \times length) was mounted by an aluminum film on the upper surface of a TECD with square size of $30 \text{ mm} \times 30 \text{ mm}$. A fin structure made of aluminum was fixed on the lower bottom surface of the TECD and immersed in the water bath at temperature of about $17.5 \text{ }^\circ\text{C}$ to enhance heat dissipation. Silicon oil has been filled between the pipe and the cold portion of TECD to improve the heat transfer performance of the two parts. To reduce the heat release whatever comes in or out, a heat insulation layer made of sponge was covered on the switch of the microfluidic channel circuit including the pipe, aqueous solution and cold portion of TECD. At last a transparent vitreous slice was covered on the whole set though which one can observe the specific phase change and operate the experimental devices. To measure the electric impedance on the solution column, a pair of electrodes was positioned respectively at the two terminal parts of the liquid column and the electric signal was recorded by the computer to give out the data for synchronous impedance of the solution during the freezing process. At the same time, a thermocouple was inserted into the center of the aqueous solution to measure its temperature transient, but the terminal of the probe of thermocouple must not pass beyond the tip of the electrodes. Both leads for the electrode and thermocouple should be covered with isolation lacquer to avoid short circuit among them. A direct current (DC) power supply ranging from 0 to 2.5 A was adopted to drive the TECD.

To approximately simulate the practical situa-

tion in operating a neuron, the nerve of *Carassius auratus* (commonly called crucian) has also been tested under the experimental circumstance as above. About 30 mm long crucian sidetrack nerve material was carefully taken out from the tail of a fresh crucian which is about 180 mm long and immediately tested. In the experiment, crucian nerve with length of about 10 mm was selectively frozen. Further, tests were also performed on a typical biological system, the earthworm, which is dug out from the garden and unidentified genus was settled on a two-stage TECD. The earthworm was anaesthetized by alcohol solution with a concentration of 10%. In Fig.2 the earthworm was fixed on an insulated plate and the two-stage thermoelectric cooler is utilized to freeze the body of the earthworm in order to detect the freezing and thawing effects on the signal re-sponse of the living system. As schematically shown in Fig.2, two electrodes respectively disposed at both the head and tail of the earthworm, and electrical voltage waves with sinusoidal, quadrate and triangular forms with a frequency of about 0.03 Hz and the amplitude of about $V_{\text{signal}} = 1.0 \text{ V}$ produced by the signal generator, were loaded on the earthworm. The measurement circuit consisted of an earthworm and a $600 \text{ k}\Omega$

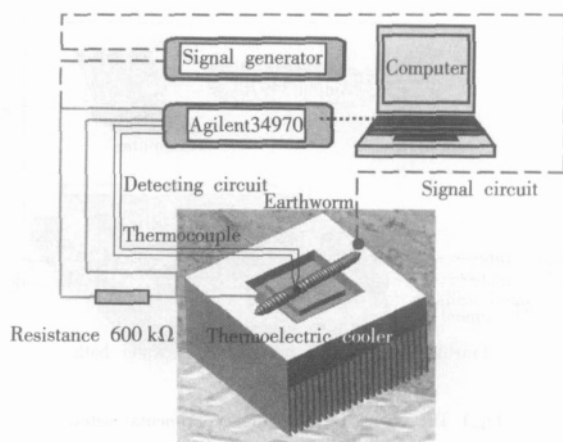


Fig.2 A schematic diagram describing details on measuring the earthworm

图2 蚯蚓试验示意图

resistance. The signals were detected by the Agilent 34970 Acquisition System (USA).

4 Results and discussion

4.1 Switching off the general liquid circuit

Depicted in Fig.3 is the transient impedance of an aqueous solution column inside a pipe, which was subjected to freezing by TECD, i.e. the freeze switch. Here, tap water was directly used as the aqueous solution. One can observe that the water in the freeze switch kept staying at a static state for a short time after the TECD began to refrigerate. Gradually the solution temperature will decrease until reaches a sufficiently low supercooling point, then the column of solution will be suddenly frozen and its temperature goes up again due to heat release during the phase changing process. Later soon, it resumes decreasing and reaches a steady state due to continuous cooling by TECD. With the start of the congealing process, the impedance of the solution began to increase smoothly in the first period, but this trend did not take long. Then an acute and huge increase in the impedance occurred and the solution would become into insulation state as soon as the solution finishes the phase change transit.

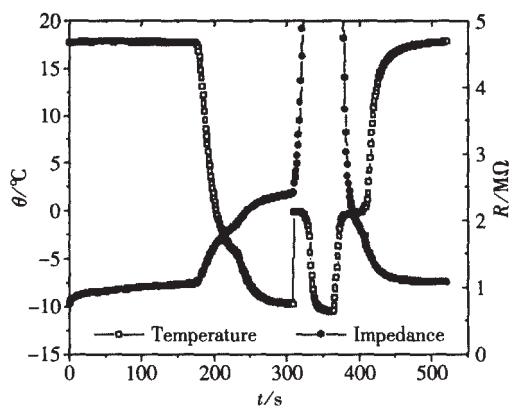


Fig.3 Transient temperature and impedance of the aqueous solution without nano-size powder

图3 无纳米粉水溶液的瞬态温度及电阻响应

In practical situation, the aqueous solution generally contains a lot of particles such as cells, proteins or the other tiny particles for detection or reaction purposes. The freeze switch especially has potential applications in such fields. To better understand the operation performances of the switch, experiments on aqueous solution suspended with tiny particles of nano aluminum particles were particularly performed. Some of the results were presented in Fig.4, which indicates the transient temperature and impedance of the aqueous solution with nano-size aluminum powder mixture. The curves appear almost the same as that in the former experiments except for a few differences. One is that the impedance of the mixed solution appears a little higher than that of the pure solution since the oxygenized nano-scale particles mixed in the solution might have disturbed the motion of ions and weakened electrical conduction of the ions. Another is that the impedance of the solution has a drop following its temperature rise at the phase change point where certain heat was released due to the latent heat of phase change. This special phenomenon perhaps has certain applications in the near future. Further, the crucial nerve was also tested as a simulation of neurons being turned on-off by the freeze switch. The results were given

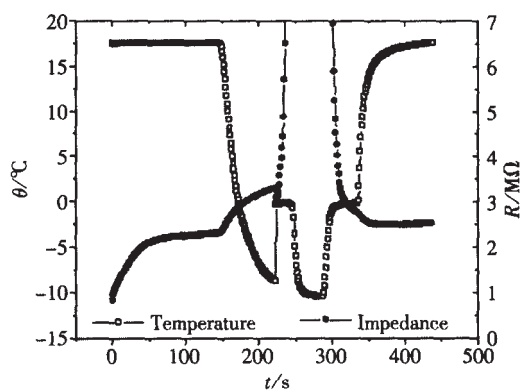


Fig.4 Transient temperature and impedance of the aqueous solution mixed with nano-size aluminum powder

图4 掺有纳米铝粉的水溶液的瞬态温度及电阻响应

in Fig.5. It depicts the transient temperature and impedance of the nerve material during the freezing and thawing. And again, the figure reveals the homologous character with the aqueous solution and a distinctive electrical signal intermitting and resuming.

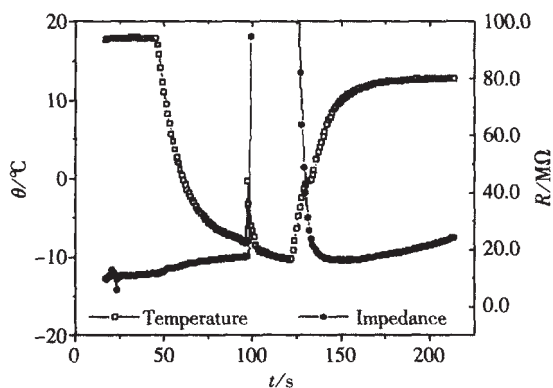


Fig.5 Transient temperature and impedance of the neurons of a crucian nerve during a freezing and thawing process

图5 鲫鱼神经在冷冻及融化过程中的瞬态温度及电阻响应

4.2 Switching off the liquid circuit in small living system

To further demonstrate the controllability of freeze switch in turning off part of the liquid electric circuit in a small living system, experiments on earthworm had also been performed in the similar way as described above. With the application of the electrical voltage with a sinusoidal wave signal came from the signal generator, Fig.6 presents the transient temperature and voltage signal detected from two positions (with a distance of 2 cm) of the earthworm. Clearly, with the working of the TECD, the amplitude of the detected signal wave gradually decreases until finally drops to close to zero. The reasons for this phenomenon can be attributed to the same as occurred in the above fluidic channel that the impedance of the partial body of earthworm increased sharply during freezing and electrical signal intensity therefore fell down. To test the reliability and repeatability of the experi-

ments, some other types of the signal were also measured. In Fig.7 a quadrate wave was applied and the similar results were obtained, so did in Fig.8 with a triangular wave. Observing the results in these figures, even the organic living system had been frozen, there still existed a tiny wave signal. The reason can be attributed to the formation of a thin layer of liquid water film at the top surface of the earthworm, which was exposed in the surrounding room environment. Therefore signal leakage of the electrical voltage on the above surface can not be avoided. But the voltage leakage

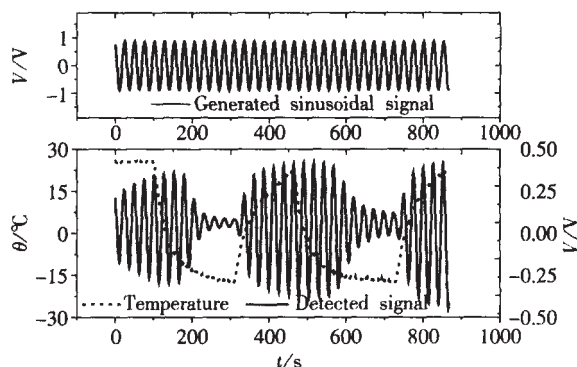


Fig.6 Transient temperature and voltage signal detected from the earthworm during a freezing process (The upper sinusoidal wave signal came from a signal generator)

图6 蚯蚓在冻结过程中的瞬态温度及电压信号(上图的正弦波产生于信号发生器)

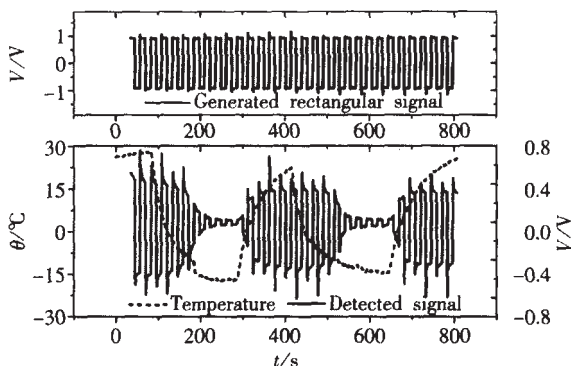


Fig.7 Transient temperature and voltage signal detected from the earthworm during a freezing process (The upper rectangular wave signal came from the signal generator)

图7 蚯蚓在冻结过程中的瞬态温度及电压信号(上图的方波产生于信号发生器)

effect can be prevented by applying much stronger freezing. Fig.9 shows the transient temperature and voltage signal detected from the earthworm during the freezing process with a copper block pre-cooled by liquid nitrogen and the sinusoidal wave signal came from the signal generator. Clearly, the signal transmission through the earthworm during the freezing was ultimately cut off.

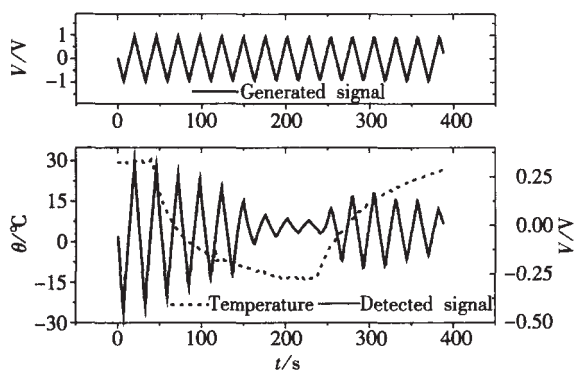


Fig.8 Transient temperature and voltage signal detected from the earthworm during a freezing process (The upper triangular wave signal came from the signal generator)

图 8 蚯蚓在冻结过程中的瞬态温度及电压信号 (上图的三角波产生于信号发生器)

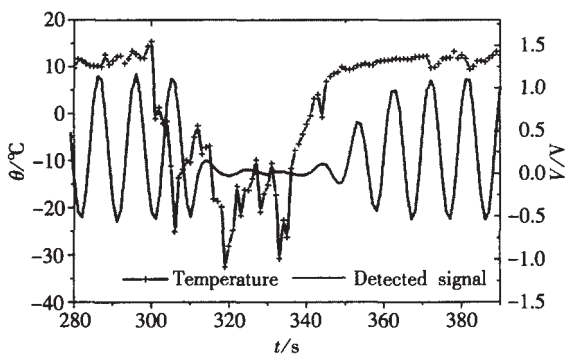


Fig.9 Transient temperature and voltage signal detected from the earthworm during a freezing process with a copper block pre-cooled by liquid nitrogen (The sinusoidal wave signal came from the signal generator)

图 9 蚯蚓受液氮预冷铜块冻结时的瞬态温度及电压信号 (正弦波产生于信号发生器)

4.3 Interpretation of the freeze switch of electrical signal

As a common sense, the existing state for a

substance depends on the balance between the kinetic energies and the bonding forces of its particles. Kinetic energy tends to move the particles, while bonding forces serve to pull them together. With the decrease of temperature, the bonding forces of the particles may exceed their kinetic energies and liquid would become solid, and vice versa. Due to having hydrogen-bonds between the water molecules, water like aqueous solution generally has special features different from other liquids. Whether water can conduct electrical current depends on that there are a lot of water molecules and/or the electrolyte dissolved in water. They have electrolyzed to hydronium ions, hydroxide ions and/or electrolytic ions, which are all the carriers of electricity. With the movement of the ions the solution becomes conductor. Conversely, if the concentration of ions is reduced to a very low level and free ions are absent, the solution will become an insulator. Therefore fettering the free locomotors ions in water means to cut the conduction of the electrical current. Having a higher melting temperature, the aqueous solution can be easily frozen which then blocks the currents for both the fluid flow and the electricity. In this side, the micro TECD appears very simple and can be integrated into the micro fluidic and circuitual system.

As a solution to turn on/off the microfluidic circuit, the concept of freeze electric switch is expected to be useful in a wide range of engineering fields. For example, it is an excellent controller for electrophoretic or liquid chromatogram analysis systems, as shown in Fig.10. Another significant application of the micro/nano freeze switch could be found in the study of electric signal transmission across the nerve network. Nerve signals often travel over long distances in the body. Dozens of neurons are involved in such a process, necessitating a sophisticated communication system to rapidly convey signals between cells. Also, because length

of individual neurons can be up to nearly 1 m long, a rapid-relay mechanism within the neurons themselves is required to transmit each signal from the site where it is received to the site where it is passed on to a neighboring cell. Inside the cells, electrical signals are conveyed along the cell membrane. For communication between different cells, the electrical signals generally are converted into chemical signals conveyed by small messenger molecules called neurotransmitters. The freeze switch can therefore be employed to turn off or on such signal transmittal. The mechanism underlying signal transmission within neurons is based on voltage potentials that exist between the interior and the exterior of the cell. The membrane potential is created by the uneven distribution of electrically charged particles, or ions, the most important of which are sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^{2+}). Ions enter and exit the cell through specific protein channels over the cell membrane, which would "open" or "close" in response to neurotransmitters or to changes in membrane potential of the cell. The resulting redistribution of electric charge may alter the voltage difference across the membrane. A decrease in the voltage difference is called depolarization. If depolarization exceeds a certain threshold, an impulse will travel along the neuron. Various mechanisms ensure that the action potential propagates in only one direction, toward the axon

tip. The generation of an action potential is sometimes referred to as "firing" [11].

For a comprehensive investigation on such detailed behavior, a hybrid circuit for a semiconductor chip with synaptically connected neurons can be constructed. Individual nerve cells were immobilized on a silicon chip as schematically shown in Fig.11. They formed a network with electrical synapses after outgrowth in brain conditioned medium. Pairs of neurons were electronically interfaced for noninvasive stimulation and recording. Voltage pulses were applied to a capacitive stimulator on the chip to excite the attached neuron. Signals were transmitted in the neuronal network and elicited an action potential in a second neuron. Later the postsynaptic excitation modulated the current of a transistor on the chip [12]. As schematically shown in Fig.11, one can assume that there is a circuit made of a series of neurons and the freeze switches are set on the circuit plate at some specific positions of axons or dendrites of neurons. Or the freeze switch can be fabricated in micro or even nano scale and placed on axons or dendrites. Thus when it acts, the cytoplasm will be congealed. On one hand the freezing would block the movement of ions and stop the electric pulse signal. Meanwhile conduction of all types of neurotransmitters is ended as the result of the fetter due to formation of the ice crystals. Besides, the frozen area can be confined in a small range and kept away from the nucleolus to avoid possible harm to the nerve cell. In this way, one can control the neurons network by using the micro/nano scale freeze system. In addition, a modeling of the nerve cell communication can also be mechanical performed by micro fluidic and electric system. This simulation assumes that an artificial neural system can be constructed by using micro channel to conduct electrical signal through the aqueous solution. Multiple nerves can then be connected to-

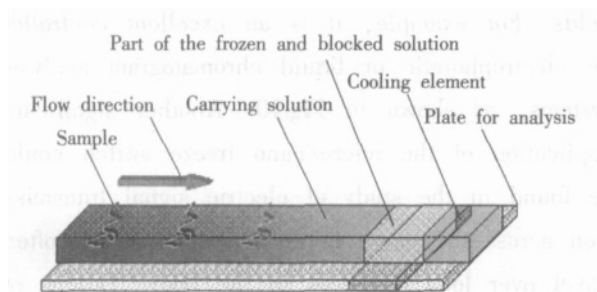


Fig.10 An application of the freeze switch in the liquid chromatogram or electrophoresis as a controller

图 10 冻结开关用于液相色谱或电泳的控制

gether to form an artificial neural system^[13].

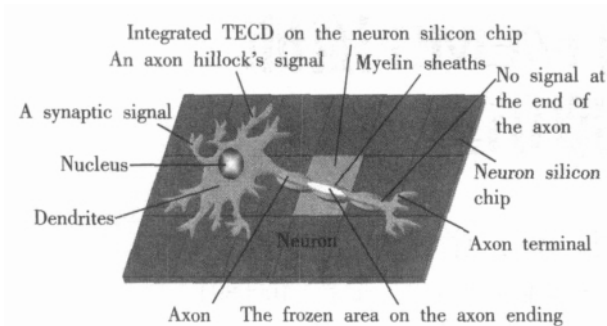


Fig.11 A schematic diagram of a neuron silicon chip using freeze switch to control the electrical signal transmitting through the neuron

图 11 采用冻结开关控制神经元硅芯片中神经电信号传输的情形

5 Conclusion

The presently demonstrated electrical switch based on freezing the fluid has many new characters, such as flexible, durable and can easily be fabricated. This new conceptual switch is suitable for an in depth study on synaptic modulation in neuronal networks that are connected by chemical synapses and communication between neurons in vitro. One can test and intentionally turn off some synapse made of elaborate treelike structures called dendrite to probe into the specialized functions for the neurons to carry out within their respective networks^[14]. This may lead to new understanding on how dendrites contribute to neuronal computation in the mammalian brain. For all of these applications, the present freeze switch should be a very interesting and powerful tool. Further study along this direction is worth of carrying out in the near future.

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