Invited review

Potential applications of non-invasive micro-test technology in individualized tumor treatments

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Abstract: Non-invasive Micro-test Technology (NMT) is a technology used for studying the physiological functions of living materials. It can detect the three-dimensional flux information of molecules/ions entering and leaving living organisms without damaging the samples. Due to functionalities such as in vivo detection, non-invasive testing, high-resolution imaging, dynamic real-time results, etc., NMT is often used to explore physiological characteristics that are difficult to measure using other techniques. NMT is widely used in medical physiology, botany, zoology and other fields, and has become an essential technology for the study of plant adversity. As an emerging technology in the 21st century, it is becoming more and more refined in the research direction of various disciplines. However, there are still many gaps in NMT research. At present, in the context of the continued high mortality rate of malignant tumors around the world, the achievements of non-invasive micro-test technology in tumor research are increasing each day. This paper introduces the potential application of NMT in the study of tumor physiology, especially in the individualized treatment of tumors in recent years. These include applications related to tumor research such as the nervous system and the digestive system.

Key words: Non-invasive Micro-test Technology; tumor; flux; ion; molecule

1. Non-invasive micro-test technology

1.1. The concept of non-invasive micro-test technology

Non-invasive Micro-test Technology (NMT) is an ultra-high-sensitivity, contactless, flux-based technology that detects the concentration and gradient of ions/molecules outside the sample material.

1.2. Origin of non-invasive micro-test technology

NMT was created based on the theory of American scientist Lionel Jaffe's ion-selective vibrating-microelectrode system. With inspiration and help from Dr. Tingyun Kuang, Dr. Fuyu Yang, and Dr. Kechun Lin, Non-invasive Micro-test Technology (NMT) was named by Professor Yue Xu, a former senior researcher of NASA, and founder of both YoungerUSA, LLC and Xuyue (Beijing) Science &Technology Co.

For more than 20 years, unremitting efforts have been made consistently to improve all aspects of NMT from modularization, automation, specialization, and intelligence to creating standardized technological innovations, commercialized products, industrialization, and localization. Now NMT has independent intellectual property rights for international application innovations and passed the "international leading" review by the Ministry of Science and Technology in 2021.

1.3. Technical characteristics of non-invasive micro-testing

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NMT has many advantages over similar technologies. (1) The living sample is not injured, that is, it can keep the tested sample intact and detections can be made in its real physiological environment; (2) Real-time dynamics, the real-time dynamic data of the sample is obtained over a period of time; (3) High resolution, flux resolution can reach 10^{-12} mol · cm^{-2} · s^{-1}, which is 3-6 orders of magnitude higher than the measured concentration; (4) Wide range of sample sizes, from enriched organelles to single cells, cell layers, tissues, organs, and even whole organisms can be detected; (5) Samples do not need to be labeled, which is convenient, safe and environmentally friendly; (6) There are many ion/molecule detection indicators that can be measured, such as Ca^{2+}, H^+, K^+, Na^+, Cl^-, Mg^{2+}, Cd^{2+}, NH_4^+, NO_3^-, O_2, H_2O_2, indole acetic acid (IAA), glutamic acid (Glu) and other indicators [2, 6] have been commercialized. With development of the technology, the number of ions and molecules that can be measured by NMT are also increasing, which provides an ideal experimental platform for obtaining information on the movement of ions/molecules inside and outside of biological samples. NMT has been widely used in medicine, botany, zoology, agricultural science, pharmacology, environmental science, and other research fields.

2. Research status of individualized cancer therapy

Cancer chemotherapy has progressed significantly in recent years, but there are still obvious differences in chemotherapy sensitivity among different races and even individuals. The effectiveness of current commonly used cancer treatment drugs is less than 70%, and 20%-40% of patients may even receive the wrong treatment [7]. How to identify individual differences between patients and use these differences to guide clinical medication reasonably has become the focus of increasing attention in the medical field. In the study of individualized treatment of tumors, genomics, proteomics, and metabolomics are widely used to promote the optimization and improvement of new target screening, targeted drug development, and chemotherapy regimens [7]. However, the current research is still based on the viewpoint that a "tumor is a molecular disease, and the root cause is genetic alterations" [8], and the research object is tumor cells in vitro. The surrounding environment of tumor cells in the body profoundly affects their proliferation speed, mode, and other characteristics, and cells cultured in vitro proliferate in a special environment, and are very likely to have different phenotypes from orthotopic tumor cells. With the emergence of tissue microstructure theory and stem cell theory [9, 10] in the post-genomic era, researchers have found that tumor cells and tissue microenvironment play a pivotal role in individualized tumor treatment. However, how to accurately measure the material and energy exchange between living tumor cells and tissues and their microenvironment has become an obstacle in this research.

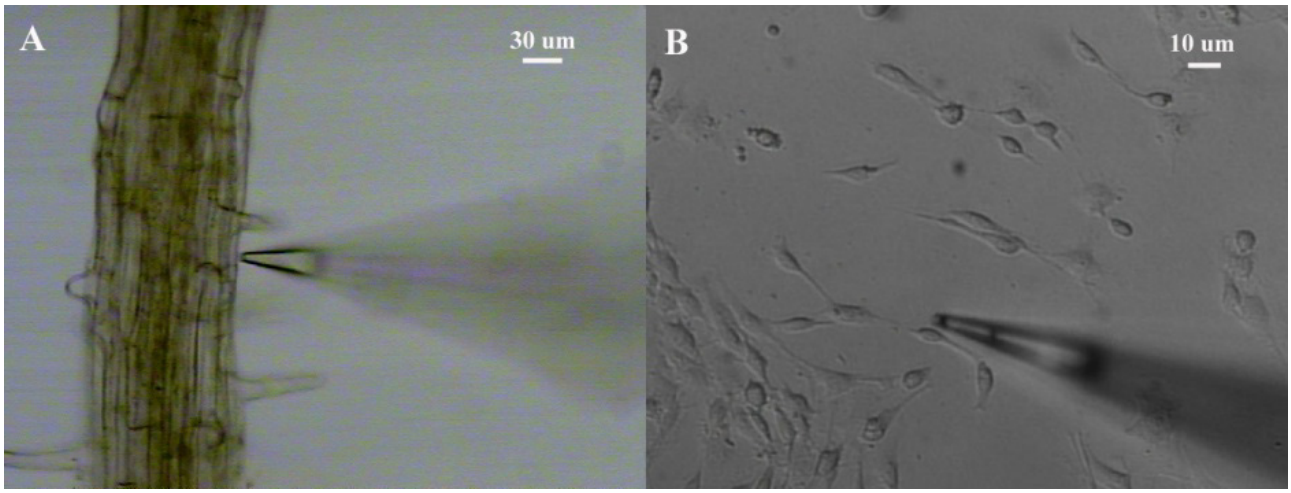

Figure 1. Real-time screenshots detected by non-invasive micro-test technology. A. Arabidopsis root maturation zone, B. Neuron cells

3. Potential application of non-invasive micro-test technology in individualized treatment of tumors

3.1. Potential applications of non-invasive micro-test technology in tumor drug susceptibility testing

3.1.1. Current status of individualization of chemotherapy drugs guided by tumor drug sensitivity testing

Among the many means of malignant tumor treatment, chemotherapy is a systemic tumor treatment method, which can kill tumor cells in the patient's body to the greatest extent. However, in clinical practice, the effect of chemotherapy is often unsatisfactory partly due to the resistance of tumor cells to chemotherapy drugs. This is a key problem that plagues cancer treatment. According to estimates from the American Cancer Society, more than 90% of cancer deaths are affected by drug resistance to varying degrees. With advances in molecular biology and cell biology, two series of in vivo and in vitro have been established, and there are now more than ten kinds of drug susceptibility testing methods.

3.1.2. NMT for cancer drug resistance research

The extracellular acidity of the tumor can effectively block the drug from entering the cell or neutralize the drug, and sequester the drug in the acidic cell vesicles to prevent the drug from reaching the intracellular target, thereby reducing its killing effect on tumor cells. Song Jin et al. [4] established a drug resistance study method (DRSM) using non-invasive micro-test technology, which can be used to study the relationship between organs, tissues, extracellular ions, molecular activities and tumor cell drug resistance. The results showed that the H^+ flux of drug-resistant breast cancer cells was close to zero before adding doxorubicin, while the H^+ flux of sensitive breast cancer cells was obvious. Both the sensitive strain and the drug-resistant strain showed H^+ efflux after adding doxorubicin, but the H^+ efflux of the drug-resistant strain was 5 times that of the sensitive strain (Figure 2). The study provides direct evidence for the correlation between extracellular H^+ activity and tumor drug resistance.

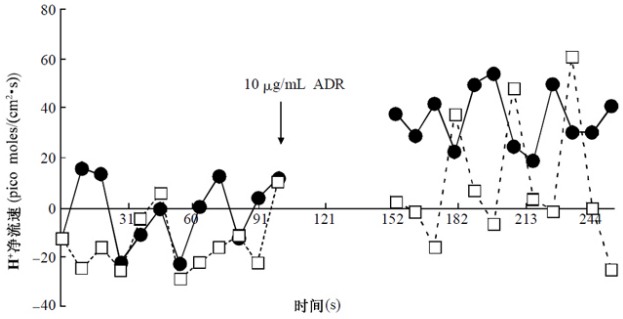

Figure 2. H^+ net flux of breast cancer cells before and after ADR (doxorubicin) treatment. A positive value indicates efflux, a negative value indicates influx.
●Drug-resistant strain MCF-7/R，□ Sensitive strain MCF-7/S7/S

3.1.3. Application of non-invasive micro-test technology in pharmacological research

Among the chemotherapeutic drug sensitivity detection methods, primary tumor cell culture is by far one of the most ideal drug sensitivity testing methods. However, recent studies have shown that changes in tissue structure provide an unfavorable microenvironment for the proliferation and differentiation of cells in the tissue, leading to the occurrence of tumors. From the study of tumor cells to the study of tumor tissues, it can explain the pathogenesis of tumors in essence. Zhu et al. [13] directly showed the detection of Cl^- flux in the colonic mucosa of rats using non-invasive micro-test technology. The results showed that entacapone induced cAMP-dependent Cl^- efflux in the colonic mucosa of rats [11, 12]. In addition, it was also found that a large amount of Cl^- efflux in the colonic mucosa of rats under the action of emodin is closely related to the degranulation of mast cells and the activation of submucosal cholinergic and non-cholinergic neurons.

3.2. Application of non-invasive micro-test technology in tumor metabolomics

3.2.1. Application of metabolomics in individualized tumor treatment

The unique metabolic characteristics of tumor cells make them produce unusual metabolites during growth and proliferation. Using metabolomics technology to analyze these specific metabolites and find new markers has achieved promising results in tumor screening and early diagnosis [14-16]. Metabolomics can detect changes in endogenous metabolites caused by drugs through the study of pharmacokinetics. Changes in endogenous metabolites caused by drugs directly reflects the biochemical process and causes in the body, and illustrates the effects of drug targets or receptors. This guides the individualized treatment of anticancer drugs to evaluate the clinical efficacy and safety.

3.2.2. Application of NMT in the study of tumor cell apoptosis markers

The relative molecular weights of molecules and ions that can be detected by NMT are less than 1000. In a broad sense, the flux detected by NMT can be classified into the category of metabolomics. Due to the in vivo detection advantages of NMT, compared with traditional metabolomics, metabolites can be detected without damaging samples, which can be called "in vivo metabolomics." At present, under the background of chemotherapy, radiotherapy and other unstable curative effects and relatively large side effects, photodynamic therapy (PDT) as a treatment method for micro-injuries has gradually come to people's attention. Song et al. [17] took oral squamous carcinoma cells (OSCC) as the research object, and used non-invasive micro-test technology to detect that after PDT treatment the O_2 influx gradually increased, and the Ca^{2+} efflux rate was also significantly higher than that of untreated cells. At the same time, the apoptosis rate, apoptosis factors and other indicators all increased. The results of this study indicated that changes in O_2 and Ca^{2+} fluxes may be early signals of PDT-induced apoptosis. Hu et al. [18] found that under normal conditions, C6 glioma cells had a small amount of Ca^{2+} efflux and K^+ influx. After PDT intervention, significant Ca^{2+} influx and K^+ efflux occurred, and the cells died. This study further revealed the molecular mechanism of PDT-induced glioma cell death, showing that the death of cancer cells may be related to the transition of Ca^{2+} influx and K^+ efflux.

3.3. Potential application of NMT in the research of targeted individualized anticancer drugs

3.3.1. Research status of targeted individualized anticancer drugs

Molecularly targeted cancer therapy refers to drugs that target specific molecular targets that play a key role in tumorigenesis and development [19]. It has recently become a new method for the treatment of malignant tumors and has received increasing clinical attention. Targeted anti-cancer drugs include targeted epidermal factor blockers, monoclonal antibodies against certain cell markers, drugs against certain oncogenes and cytogenetic markers of cancer, anti-tumor vaccines, and gene therapy etc. [20]. Huang Can et al. [21] found that the expression of p-JNK protein and cell apoptosis rate in tumor cells increased after application of SP600125 inhibitor, and also found that the JNK signal transduction pathway was involved in the multidrug resistance MDR gene and multidrug resistance protein of liver cancer. The expression of P-glycoprotein (P-gp) has a regulatory effect on tumor drug resistance. As a pro-apoptotic factor, JNK is one direction of tumor treatment research. NMT has also achieved results in the research of apoptosis and apoptosis factors.

3.3.2. Application of non-invasive micro-test technology in apoptosis research

Keefe et al. [22] detected the changes in morphology and extracellular K^+ of fertilized eggs under H_2O_2 treatment since experiments have found that apoptosis is activated by substances such as

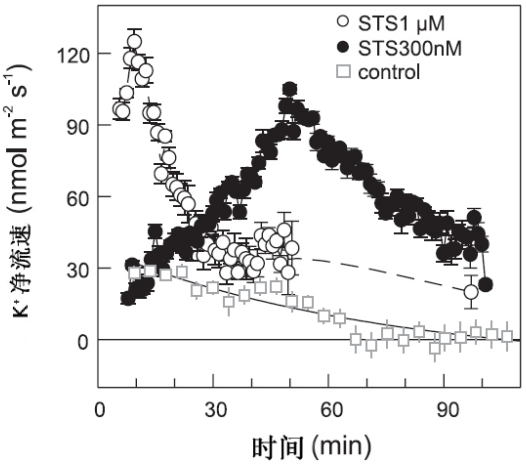

Figure 3. K^+ net flux after STS (staurosporine) treatment of T lymphocytes, positive values indicate efflux, negative values indicate influx. High concentration STS treatment and low-concentration STS treatment show faster K^+ efflux than the control as indicated with the high peaks.

H_2O_2. Figure 3 shows a significant efflux of K^+ ions using non-invasive micro-test technology. This is due to the activation of K^+ channels when cells initiate apoptosis.

Valencia et al. [23] found that after T cells were treated with 1 μM staurosporine, and while they were induced to undergo apoptosis, a rapid K^+ efflux was detected by non-invasive micro-test technique. The K^+ efflux reached a peak in about 15 minutes and then weakened as seen in Figure 3. At the same time, an increase in K^+ channel current was also recorded using the patch clamp technique, accompanied by a sharp decrease in membrane depolarization. Studies suggest that ion flux is an early event of apoptosis; K^+ efflux causes cell contraction, activates caspase, and induces apoptosis. Bcl-x_L promotes the exchange of metabolites between mitochondria and the cytosol and is the major anti-apoptotic protein in the adult brain, and Bcl-x_L overexpression increases the number and size of synapses. Alavian et al. [24] found that Bcl-x_L overexpressed neurons had higher ATP levels, and exogenous Bcl-x_L could reduce or inhibit ATP. The results of neuron O_2 flux detection showed that the O_2 influx of Bcl-x_L overexpressed neurons was relatively small (Figure 4).

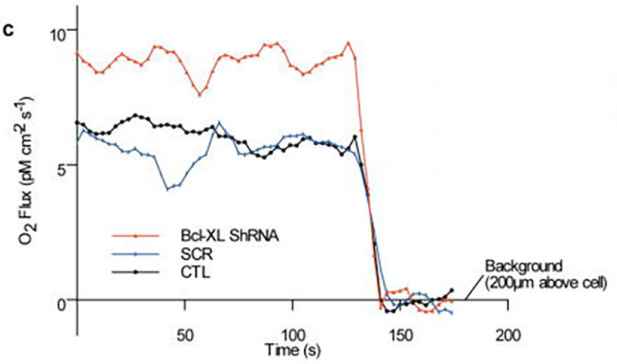

Figure 4. Neuron O_2 net flux, a positive value indicates influx, a negative value indicates efflux. The expression of neuronal Bcl-x_L is proportional to the O_2 influx rate. Ctl: normal control group, Bcl-x_L shRNA: Bcl-x_L expression suppression group, Scr.: Bcl-x_L Overexpression group; background value: measured at a distance of 200 μm from the cell in the vertical direction.

3.4. Application of non-invasive micro-test technology in diseases induced by ion imbalance

Ca^{2+} is an indispensable ion for various physiological activities of the body. It plays an important role in maintaining the biological potential on both sides of the cell membrane, nerve conduction function, normal muscle contraction and relaxation, and the action mechanism of some hormones is also expressed through Ca^{2+}.

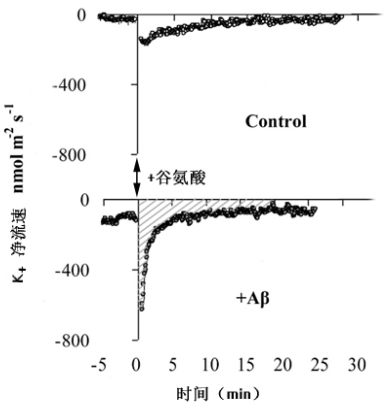

Figure 5. The net flux of K^+ under glutamate stimulation in primary cortical neurons treated with control group and Aβ (amyloid β protein). Positive values indicate influx, negative values indicate efflux. In neurons treated with Aβ, the K^+ efflux was significantly greater than that of the control group under the stimulation of glutamate.

The imbalance of Ca^{2+} can cause diseases such as diabetes, rheumatism, respiratory diseases, neurological diseases, etc., and it is also closely related to the formation of tumors.

Shabala et al. [25] studied the role of β-amyloid (Aβ) in the pathogenesis of Alzheimer's disease and found that after 1 μM Aβ treated primary cortical neurons, the rate of K^+ efflux and Ca^{2+} efflux (not shown) induced by glutamate was significantly greater than that in the control group not treated with β-amyloid protein (Figure 5). Studies have shown that neurons' loss of the ability to maintain Ca^{2+} and K^+ balance may be an indication of the early response of cells to Aβ. Chung et al. [26] found that Zn7MT-2A could prevent Cu(II)Aβ-induced oxidative stress on neurons when studying metal-chelation therapy for Alzheimer's disease. They found that Cu(II)Aβ caused K^+ efflux and Ca^{2+} influx in neurons, and the addition of Zn7MT-2A prevented the change of flux caused by Cu(II) Aβ. Zn7MT-2A maintained K^+ and Ca^{2+} ion flux, reducing the damaging effects of Cu(II)Aβ on neurons.

3.5. Application of non-invasive micro-test technology combined with patch-clamp technique in tumor research

Non-invasive micro-test technology has been combined with laser confocal technology, patch clamp technology, and other technologies to find both internal and external measurement of segregation information exchange. Yang et al. [27] used NMT and patch clamp techniques to find that nasopharyngeal carcinoma cells undergo cell regulatory volume reduction in a hypotonic environment. In the study, the K^+ efflux appears in the initial period. With the efflux of intracellular H^+ to the outer surface of the cell and the closure of K^+ channels on the membrane, the efflux of H^+ replaces the efflux of K^+. This is the first time that K^+ and Cl^- transports are uncoupled during hypotonic-induced cell regulatory volume reduction, which to some extent overturned the conclusions of previous patch clamp studies, and found that H^+ efflux in cells plays an important role in the regulation of cell volume. In the future, non-invasive micro-test technology will play an important role in the study of active transport ion or molecular pumps and co-transport carriers.

4. Outlook

Non-invasive micro-test technology has been widely used in many research fields such as plant stress, photosynthetic mechanism, neurological diseases, signal transduction, etc. Especially in the study of plant stress, a complete set of experimental system methods have been formed, and it has become an essential technology in the research field. However, the individualized treatment of tumors is in the primary stage of applied research, with many blank areas for new research and highly prospective results. As of now, relevant theoretical and practical studies around the world have confirmed the heterogeneity among tumor patients and the heterogeneity within tumors. The former has been widely recognized: the clinical course and treatment plan of any two cancer patients cannot be exactly the same, which has always been an important principle of personalized treatment [28]. The tissue microstructure theory and stem cell theory pointed out that [9, 10] the extracellular matrix component is the primary attack target of various carcinogenic factors, and the abnormal signal exchange between cells caused by it is the main reason for the uncontrolled proliferation of cells. This has opened up a new path for the current research on individualized treatment of tumors, that is, to flexibly select the corresponding treatment methods according to the characteristics of the tissue or cell microenvironment in the individual patient. Non-invasive micro-test technology provides extremely important technical support for this theory with its unique advantages of in-vivo detection, non-invasive, high-resolution, dynamic real-time, etc. Looking at the successful trajectory of non-invasive micro-test technology in the field of plant research, it will surely become an important tool for studying the physiology of living tissue and cell microenvironment.

In addition to the research on individualized treatment of tumors introduced in this article, non-invasive micro-test technology also has

very broad application prospects in other fields of tumor research. For example, in the study of the mechanism of tumorigenesis, there are still relatively few studies on the effects of physical, chemical, biological, genetic, and other carcinogenic factors on the physiological functions of normal living tissues or cells, especially the entry and exit of molecules and ions. The rate and direction (flux) of ions/molecules entering and exiting the cell membrane represent most of the physiological activities of the living tissue or cell in real time. This is of great significance for studying the mechanism of various carcinogenic factors affecting normal living tissue or cells in different parts of the human body. Non-invasive micro-test technology can not only monitor the short-term and long-term effects of carcinogenic factors by detecting one or more ion/molecule fluxes in a specific tissue or cell in real time, but it can also conduct related mechanism research. Moreover, by comparing the ion/molecule flux of normal and cancerous living tissues or cells, the flux spectrum of cancerous tissue and cell segregation can be created, which can be used as a basis to guide clinical research. To sum up, more in-depth research directly related to tumors using NMT is being noticed more and more, and will definitely become a hot spot in future research.

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