

Coding by Different Diameter Impedance, Detection Method of Microspheres Which are Used to Produce Liquid Biochip

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Abstract

This research was aiming at developing liquid biochip which was coded by impedance microsphere of different diameter and providing research foundation for its detecting device. Adopting the method of loading constant current source between both ends of micropore in ruby, it were tested that three sorts of microspheres' impedance with particle sizes of 2 μ m, 5 μ m, 10 μ m. It was resultantly found that microsphere diameter is in direct proportion to impedance and voltage between both ends of micropore. In other words, microsphere of different size could be distinguished from each other by their impedance. Our research drew the indication that impedance of microspheres in different sizes could be theoretically calculated as well as measured and automatically distinguished, offering experimental and theoretical foundation for liquid biochip development.

Keywords: Impedance of microspheres coded by different sizes; Liquid biochip

Introduction

Liquid biochip refers to multiple microspheres diagnostic technique which is based on principle of coding analytes by microspheres. Fluorescence color-code microspheres biochip was produced by bioengineer company and has been put into clinic application and practice. At the same time, it has played significant role in fields of disease diagnosis, medical scientific research, etc. [1-3]. Nevertheless, color of microspheres is not the only feature to code analytes, there still are other features of microspheres when coding analytes [4].

Impedance is one of material basic physical properties. The author proposed a new technique which is characterized by liquid biochip impedance-coded microsphere. Moreover, it obtained intellectual property right in 2007 [5]. The amount of information from microspheres impedance supposes to be infinite in the side of theory, as well as the categories of substance which are used to determine its impedance. The technique of impedance-code microsphere may well be more significant than color-code one. It gains potential developing prospect while it lacks relevant research in China and the world as a whole.

Detecting-tech for microspheres of different impedance is the skill crux and critical step of the biochip technology. So vital are the corresponding theories and experiments in research and development of impedance-code microspheres.

The impedance differs from different diameters of microspheres in one material. If their electronic signal could be detected automatically, these microspheres have the capacity to code analytes in the prospect of theory. Therefore, we set microspheres which are in three different diameters and in the same materials as research stuff. After programming the device of loading constant current source between the both ends of micropore in ruby, it automatically detected impedance of microsphere by inspecting voltage between the both ends of micropores when microspheres flowed in. It provides research foundation for manufacturing and developing liquid biochip of impedance-code microspheres in different sizes.

Materials and Methods

Preparation and observation of microspheres in different sizes

Preparation of microspheres in different sizes: According to literature [6], we manufactured polystyrene microspheres in varied micrometer sizes, by adopting the method of dispersion polymerization. We infused the compound of phenylrthylene, ethanol and AIBN (azobisisobutyronitrile) into 100ml flask with four necks which was connected with ventilation device and electric agitator. Then, we added ethanol and PVP (polyvinylpyrrolidone) mixture solution to it, stirred with the speed of 200r/min. In order to get rid of oxygen, argon has been past into it for 30min. After that, we heated it up to 70 °C and kept in reaction for 12 hours in thermostatic water bath. Once the reaction was finished, we rinsed the emulsion with water-ethanol mixture and centrifugated it in the rate of 6000r/min. After repeating the process six times, we have got seed beads. Blended with 2.0 g cyclohexane as swelling agent, we added a handful of seed beads with 30 g SDS (sodium dodecyl sulfate) solution though ultrasonic device. Disposed seed beads were swelling for 12 hours in 250ml flask with four necks, under the temperature of 30. Next, compound of phenylrthylene, BPO (benzoyl peroxide) and SDS solution were infused proportionally into the

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Received August 13, 2011; **Accepted** December 08, 2011; **Published** January 07, 2012

Citation: Wang Z, Hu T, Hu X, Song T, Lei W, et al. (2012) Coding by Different Diameter Impedance, Detection Method of Microspheres Which are Used to Produce Liquid Biochip. J Biosens Bioelectron 3:111. doi:[10.4172/2155-6210.1000111](https://doi.org/10.4172/2155-6210.1000111)

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flask for swelling 12 hours. Styrene, BPO and SDS were proportionally added to the flasks so as to swell for 12 hours. Then, we added 80 mL PVA solution to the mixture, kept in 80 water bath for reaction, lasted as long as 12 hours. After the reaction, microspheres were washed with alcohol/water mixture, and centrifuged at the speed of 6000 r/min. After repeating six times, the products were dehydrated in vacuum drying oven. The control of monomer content of microspheres and swelling ratio which were finished in our experiment have provided a wide range of polystyrene impedance-code microspheres with the diameter of 2.0 μm , 5.0 μm and 10.0 μm . The photographs of polystyrene impedance microspheres with different particle sizes are shown in Figure 1a,b,c.

Width detection of volume distribution in microspheres with different sizes: Using tri-classification blood counting instrument provided by Tekang technology Limited Company of Jiangxi, we detected saline solution mixed with polystyrene microspheres in different sizes. As the volume of these microspheres has not gone beyond the volume of leukocyte, erythrocyte and thrombocyte, which are 10-15 μm , 6-9 μm and 2-4 μm in particle size, so the blood counting instrument could display results in histogram. Finally, it is available for us to calculate the average size and coefficient of variation of three sorts of polystyrene microspheres through the histogram of red blood cells or mean platelet volume (MCV or MPV) and their coefficient of variation of the volume distribution width (RDW or PDW).

The principle, device and its methods of detecting impedance of microspheres in different sizes

The principle of detecting impedance of microspheres in different sizes: A micropore drilled in ruby was loaded with constant current source between its both ends. Infused with electrolyte, the impedance of micropore was extremely small, so were the voltages between the both ends. Because polystyrene microspheres are much more superior to electrolyte in impedance. So when microspheres flow into micropore, the larger microspheres volumes are, the less electrolytes were re-

mained in micropore. As a consequence, the amplitude of voltage is in direct proportion to diameter of microspheres. Oscilloscope could display the voltage processed by amplified electrical circuit. The principle of detecting impedance of microspheres in different sizes is shown in Figure 1d. The setup of microsphere impedance detecting experiment is shown in Figure 1e.

Detecting device, technical parameters, and oscilloscope regulation parameter of impedance of microspheres in different sizes: Opening the main case of tri-classification blood counting instrument offered by Tekang technology Limited Company of Jiangxi, we have found the output connector in the circuit board, and linked it to input connector of UT-3000 E type oscilloscope made by Uni-Trend technology (dong guan) Limited Company. The micropore is 160 μm in diameter, and 345 μm in length. The voltage of constant current source between the both ends of micropore is 16.5 V, and solution resistance rate is $5.9510 \times 10^{-2} \pm 0.63 \mu\text{m}\cdot\text{cm}$. The oscilloscope was set one grid in X axis as 2V, and one grid in Y axis as 10 ms with full bandwidth (200 MHz).

The method for detecting impedance of microspheres in different sizes: In our design of biochip, software is used to identify analytes. While single electronic signal of microsphere impedance was analysed one after another, software can complete the identification according to the relation between different analytes and their impedance. Hence, it is of great significance for the feasibility of this detecting technology that collecting the information of voltage changes when one microsphere flows into the micropore. By diluting the microspheres solution, we have gauranteed microspheres came into the micropore one after another. Then, the changes displayed on the screen of oscilloscope have been recorded.

Result

The test result of volume distribution width

The test results of three sizes of microspheres were 2.1 ± 0.1 , 5.3 ± 0.2 and $9.7 \pm 0.4 \mu\text{m}$, which are shown in Table 1. Coefficient of variation were less than 5%, and the uniformity of microspheres in one certain size is fine.

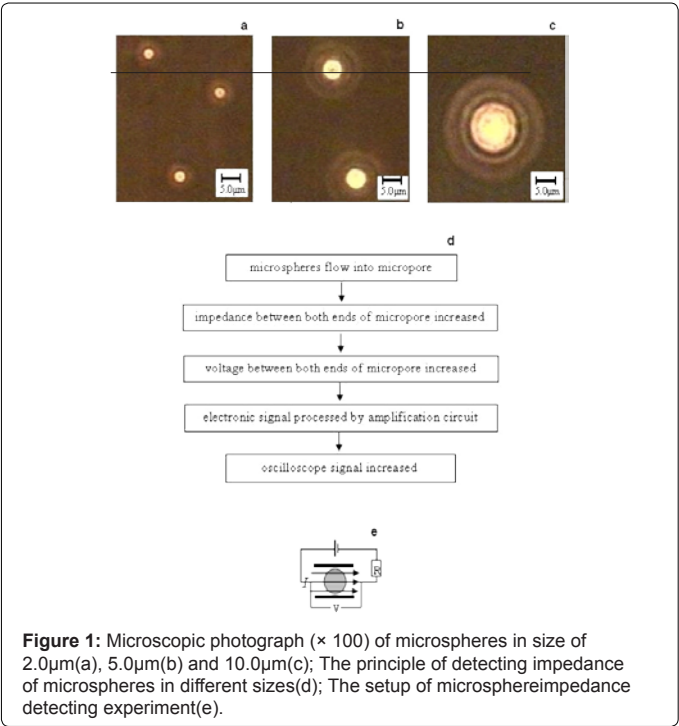
The voltage change results between both ends of micropore

When microspheres flowed into the micropore: The result showed that when a microsphere flowed into the micropore, it generated a electronic signal pulse. When polystyrene microspheres in the size of 10 μm , 5 μm and 2 μm were respectively moving through the micropore, enlarged by the amplifier, oscilloscope screen can display the increased voltage individually (Figure 2 a,b,c). Those screenshots of mixture of the three sorts of microspheres are shown in Figure 2d. We have randomly shot 10 sheets from the screen in oscilloscope with the each size of microsphere. Calculated voltage peak value and standard deviation of microspheres impedance were presented in table 1.

Discussion

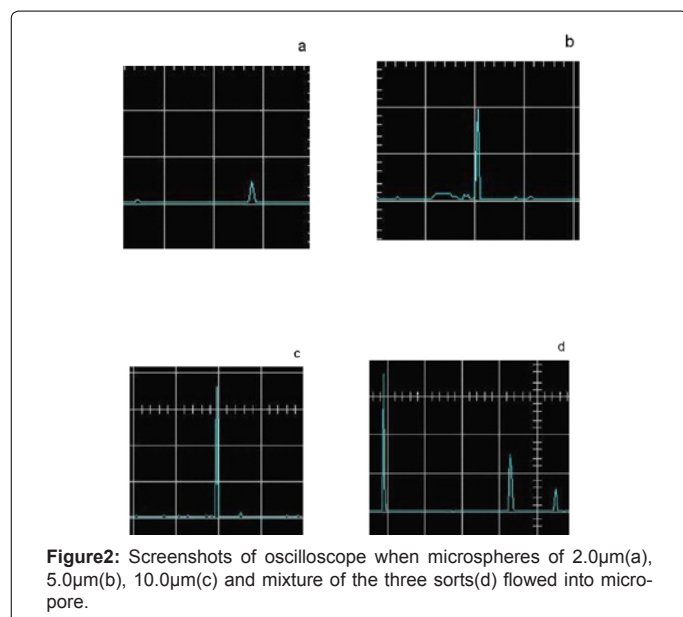
The principle of automatically detecting impedance of microspheres in different sizes

Blood counting instrument is based on Coulter Principle, and it can



particle sizes (μm)	2.1 ± 0.1	5.3 ± 0.2	9.7 ± 0.4
voltage summit (v)	1.0 ± 0.1	4.0 ± 0.3	7.6 ± 0.3

Table1: voltage summit and standard deviation for three sorts of microspheres.



distinguish different cells. To be specific, it can detect the impedance information of different cell by micropore technology in order to estimate the category of cells. Scabbard liquid streaming technology can make different particle size of particle arranged in a single line [7]. As the impedance is one of properties of microsphere, different particle sizes of them can generate different voltage signal when entering the micropore. Scabbard liquid streaming technology can make microspheres in one single line and detect the impedance information of microspheres one by one. This experiment did not adopt Scabbard liquid streaming technology because we only want to know whether single microsphere in different sizes could be identified by micropore technology. By diluting the concentration of microsphere solution, the information of voltage change microspheres was collected as the microspheres got in the micropore one after another.

The significance of different particle size of impedance microsphere detected automatically

The result of this experiment shows that different particle size of polystyrene impedance microsphere can be detected and classified by micropore technology. As the resistivity of polystyrene impedance microsphere is far larger than the resistivity of electrolyte in the micropore, microspheres in certain size flowed into micropore and electrolyte solution was spilled in the same volume. Impedance between the two sides of the micropore is in direct proportion to voltage signal between the two sides of the micropore as well as the peak value of voltage pulse shown by the oscilloscope. Absolute value of the impedance is not the ultimate meaning of coding microsphere technology; instead, it is the fact whether the device can tell the small change of impedance. Theoretically, as long as the device can distinguish different impedance, we are capable of coding two different substances in demanding.

The technology of measuring intensity of optical signal on the surface of microsphere is a quiet mature. Though there is no report about whether impedance of microspheres in different particle sizes can be detected automatically by other peer scholar, the method offers crucial precondition and foundation in developing coding liquid biochip in a new technology platform. This experiment is a tentative research of detection technology of coding liquid biochip. As the result shows,

different particle size of impedance microsphere can be detected successfully, which means developing different particle size of impedance microsphere in a new technology platform is feasible. Microsphere in different material with the same size can also be used for coding the substance to be determined, which is the researching content of our further work.

Conclusion

According to our experiment, the micropore device in blood counting instrument can detect electronic signal of impedance of microspheres in sizes. The result showed that the diameter of the microsphere with the same material is in direct proportion to the voltage signal between the two sides of micropore. This technology is feasible and it provides foundation for developing detection device of liquid biochip coded by impedance of microspheres in sizes.

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