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Design and evaluation of the microfluidic magnetic isolating method for aquaculture pathogens detection

J.J. Guo [1,2], R.B. Zhang[1*], N. Yang[1]

[1]*S chool of Electrical and Information Engineering, Jiangsu University, Zhenjiang, PR China*

[2]*School of Electrical and Photoelectronic Engineering，Changzhou Institute of Technology，Changzhou 213002，China*

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The spread of infectious diseases has a serious impact on the aquaculture industry. But aquaculture pathogens detection in a water environment often relies on traditional laboratory techniques which are difficult to use、time-consuming and lower degree of automation. The paper proposes a magnetic isolating method based on microfluidic chip for aquaculture pathogens detection. We designed a dedicated microfluidic chip with automatic sample injecting、magnetic isolation, and set up the experiment platform based on a microfluidic magnetic isolating system. The optimum magnetic pole current and switching frequency in the microfluidic isolating system were determined and as an example of *Salmonella typhimurium* the performance of the system was experimentally evaluated. The experimental results showed that compared with the passive isolation by barriers, the capture efficiency of the magnetic isolating system increased by 32%, more than 93%, which benefits pathogens efficient separation and high precision detection in the prevention of aquaculture disease.

Keywords: Microfluidic chip; aquaculture pathogens;magnetic isolation; capture efficiency

INTRODUCTİON

Nowadays aquaculture diseases have been the biggest barrier to the sustained, rapid and sound development of the aquaculture industry in the world. Among all the causes, many aquaculture pathogens(.i.e.*Aeromonas hydrophila*、*Escherichia coli*、*Salmonella typhimurium*) are major factors of aquaculture diseases, which have caused enormous economic loss. Therefore, rapid aquaculture pathogen detection is the key to prevent the occurrence of aquatic product breeding diseases and the quality improvement of aquatic products[1-4].Compared with pathogens detection technique in the traditional laboratory,The lab-on-a-chip (LOC) devices based on a microfluidic chip is characterized by its high sensitivity, high determination speed, less dosages and high automation [5-9].

The aquiculture water environment are usually diverse and complex, so how to rapidly isolate pathogens from the samples is especially critical to improve subsequent microfluidic detection precision.Conventional pathogens separation methods include filtration, centrifugation, dielectric electrophoresis. Filtration and centrifugation can need multiple procedures, consume a long time and have a difficulty to screen target pathogens. Dielectric electrophoresis is likely to damage the target pathogens and has a low separation efficiency.The immunomagnetic isolating method applys immunomagnetic nanoparticles (MNPs) coupling pathogen antibodies to capture and separate the target pathogens [10-13]. Nevertheless, The microchip with micro-nano scale structure can cause fluid laminar flow, which cannot bring out the magnetic nanoparticles and target pathogens to sufficiently mix and immunological reaction. In the end the lower isolating efficiency of magnetic nanoparticles can lead to poor detection accuracy.

This paper proposes a microfluidic magnetic isolating method for the aquaculture pathogens detection system and takes *Salmonella typhimurium* for example to experimentally evaluate the proposed method.

A MAGNETIC ISOLATING SYSTEM FOR PATHOGENS MICROFLUIDIC DETECTION

The basic principle

By Maxwell's law the total current is showed as follows[14-15]

$$\oint_L Hdl = \sum I \qquad (1)$$

In the equation, H is the magnetic field intensity, L is coil winding length, I is the current of electromagnetic coil, Based on equation (1), the intensity of the magnetic field is proportional to the driving current of the electromagnetic coil.

During the process of the magnetic separation of the pathogens, the magnetic field exerts the

* To whom all correspondence should be sent:
E-mail:474820848@qq.com

magnetic force on MNPs.Meanwhile, the viscous resistance is exerted on MNPs by the microfluid. Therefore,the magnetic force of the single magnetic nanoparticle exerted by the outer magnetic field is:

$$\overrightarrow{F_m} = \mu_0 \chi_{eff} V_P \left(\overrightarrow{H} \cdot \nabla\right) \overrightarrow{H} \qquad (2)$$

In the equation: μ_0 is the permeability of vacuum, V_p is the volume of the magnetic nanoparticle,χ_{eff} is the effective magnetic susceptibility of the magnetic nanoparticle.

Based on equations (1) and (2), when other parameters are constant, the magnetic force exerted on the magnetic nanoparticle by the outer magnetic field is proportional to the magnetic field intensity, which is proportional to the drive current of the role coil.

The viscous resistance exerted on single magnetic nanoparticle by the microfluid is:

$$\overrightarrow{F_d} = -6\pi\eta r_P \left(\overrightarrow{v_f} - \overrightarrow{v_P}\right) \qquad (3)$$

In the equation: η is the fluid viscosity, r_p is the semidiameter of the magnetic nanoparticle, v_f is the fluid velocity, v_p is the speed of the magnetic nanoparticle.

Based on Newton's second law :

$$m_p \frac{d\overrightarrow{v_p}}{dt} = \overrightarrow{F_m} + \overrightarrow{F_d} + F_g \qquad (4)$$

In the equation: m_p is the mass of the magnetic nanoparticle, F_g is the sum of the magnetic nanoparticle's gravity and buoyancy.

Due to the minuteness of the magnetic nanoparticle, F_g is far less than the magnetic force and the viscous vesistance and can be ignored.

$$\overrightarrow{F_m} + \overrightarrow{F_d} = 0 \qquad (5)$$

According to the equation (5),the magnetic force balances with the viscous resistance exerted by the microfluid.The capture and separation of pathogens with MNPs are affected by the magnetic force F_m.

Based on the Navier-Stokes equation of the incompressible fluid while in laminar flow

$$\rho_f \frac{\partial v_f}{\partial t} + \rho_f \left(\overrightarrow{v_f} \cdot \nabla\right) \overrightarrow{v_f} = -\nabla P + \eta \nabla^2 \overrightarrow{v_f} + c\overrightarrow{F_m}\delta(f) \quad (6)$$

In the equation: ρ_f is the fluid density, P is the pressure intensity of the microfluid, c is the concentration of the magnetic nanoparticles, $\delta(f)$ is the periodic function varying with the magnetic field frequency f.

Based on equation (6), the capture and separation of pathogens with MNPs are affected by the magnetic field frequency and the concentration of MNPs.

The magnetic capture efficiency of pathogens can be defined (Capture efficiency,CE),

$$CE = \frac{C_m}{C_0} \times 100\% \qquad (7)$$

In the equation: C_m is the concentration of pathogens after magnetic isolation, C_0 is the initial concentration of pathogens.

the capture efficiency can quantitively evaluate the performance of the magnetic isolating method of microfluidic detection system.

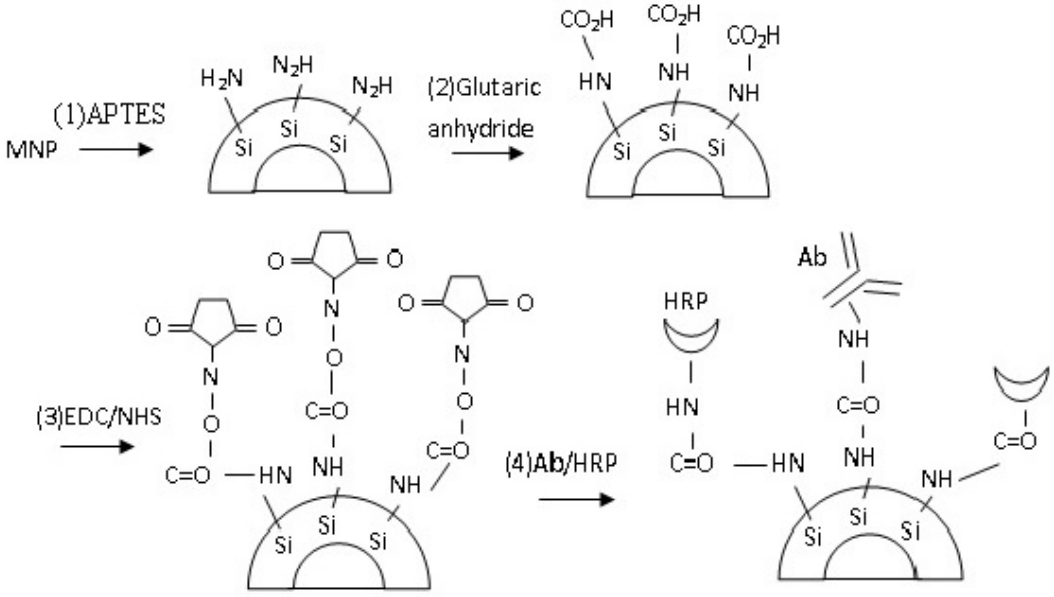

Fig. 1. Functionalization scheme of magnetic nanoparticles.

Functionalization of the magnetic nanoparticles

This experiment used the magnetic nanoparticle with 10nm silica shell and 160nm iron oxide core. As a proof of the immunoreaction principle, 180nm magnetic nanoparticles were functionalized with anti-*Salmonella typhimurium* Ab and horse radish peroxidase (HRP) to generate Ab-HRP-MNPs. The scheme for the MNPs immune-functionalization is shown in Fig.1. Treatment of the magnetic nanoparticles with (3-aminopropyl) triethoxysilane (APTES) produced a self-assembled monolayer containing surface amino groups that were converted to carboxyl groups by reaction with glutaric anhydride. The carboxyl groups were activated by sequential reactions with 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) and N-hydroxysuccinimi-de (NHS). The resulting NHS groups were used for chemical conjugation of anti-S. typhimurium Ab and HRP enzyme to generate Ab-HRP-MNP [16].

Design of a microfluidic chip

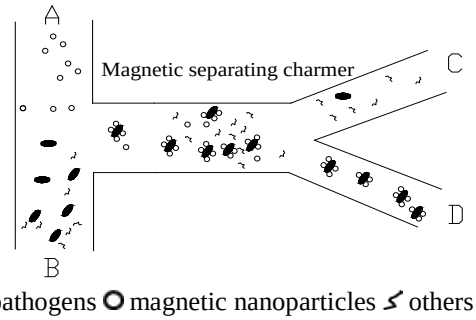

Fig.2. Structure model of the microfluidic chip

As shown in figure 2, A is the inport of immunomagnetic nanoparticles and B is the inport of pathogens. Immunomagnetic nanoparticles and pathogens are simultaneously injected into the magnetic separating chamber by an injector pump. C is the impurity outlet and D is the detection outlet after magnetic separation. The microfluidic chip adopts port on-off method to realize the flow control of the sample fluid. Port A and B adopts the injector pump to run and stop the flow. Outlet C and D adopt manual clips with teflon catheter to control the on-off flow. The material of the chip is polydimethylsiloxane (PDMS), which is bonded to a glass substrate.The micro channel volume on the chip is 8.2×10^{-3}mL.

The magnetic separating chamber is mainly used for the complete mixture of magnetic nanoparticles and pathogens, which cause specific immune-response under the action of an external magnetic field to realize the capture and separation of the pathogens. The inadequate separation will yield great errors of subsequent detection.

Experimental platform

Fig.3 is the photo of the overall experimental platform. The microfluidic chip is set on the +Z iron core terminal of the magnetic field generator, which is in the center of the generator.The Microinjector is connected to an injection pump (0.098 μm/pace, flow accuracy of CVs ＜ 1%). The pathogen samples and MNPs solution (particle size 180 nm, concentration 10 mg/mL) are injected into the microfluidic chip through the port A and B. The outlet C is connected to the impurity collecting tube and the outlet D is connected to the sample collecting tube after the magnetic separation.

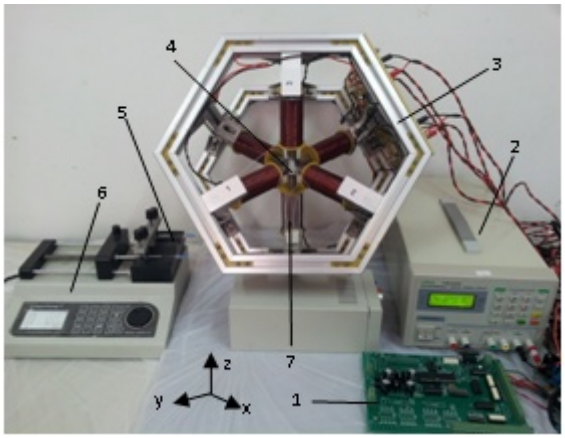

Fig.3. Photo of the microfluidic magnetic isolating system:1-Power controlled panel,2-DC stabilized supply power, 3-Magnetic field generator, 4-Microfluidic chip, 5 - Microinjector, 6 - Injection pump, 7 - test tube.

The magnetic field generator is driven by role currents from six iron core coils and generates parallel magnetic fields in six positive and negative directions. The magnetic induction intensity in each direction at the center of the magnetic separation chamber can be adjust between 0~65mT. The power control board can manage the DC stabilized power supply (YB3205, 0-5A) by a program supplying power in all directions. the X-Y-Z magnetic field were varied to realize magnetic separation of the pathogens.

The size of applied magnetic nanoparticles is 180 nm in the experiments and their surface is conjugated with *Salmonella typhimurium* specific antibodies which can have specific binding with *Salmonella typhimurium* and produce the magnetic nanoparticles/ pathogens compounds.

Separated pathogens are collected into the test tube and adopted the plate counting method to count the pathogens colony. The magnetic capture

efficiency of pathogens is calculated to verify the effect of the separation of the chip.

Experimental procedure

Water environment of aquiculture usually has a lower pathogens content in unit volume, so the pathogens detection needs to adopt the vacuum pump with special membrane (<0.45μm)to enrich pathogens，and need to rinse and dilute to earn pathogens samples . The standard detection method applys plate counting method. The sample is applied to the color culture medium, and put into the oscillation incubator with constant temperature (37^0C, 200r/min) to conduct a 48h enrichment culture.In the end the concentration of the pathogen samples is obtained by the pathogens colony counting.

Salmonella typhimurium is used as the target pathogens, while Escherichia coli and Staphylococcus aureus are used as interference pathogens in the experiment. The pathogens strain is provided by the Food Engineering Experiment Center of Jiangsu University in China. Three kinds of strains are blended into pathogens solution through phosphate buffer solution (PBS) and series of concentration samples are obtained after gradient dilution. The plate counting method is adopted to obtain the concentration of pathogens in standard samples. Three kinds of pathogen samples are blended into the synthetic samples of the pathogens in aquaculture according to a certain concentration ratio.We mix 0.1mL of *Salmonella typhimurium* samples with 0.9mL of Escherichia coli and Staphylococcus aureus.Synthetic samples of *Salmonella typhimurium* with series of concentrations are obtained after a 10^1~10^8 gradient dilution.

Four magnetic poles of the X-Y plane of the magnetic field generator are driven to produce parallel magnetic field to magnetic separating chamber of chips. Then the samples are disturbed with MNPs movement to speed up immunological reaction to produce MNPs/pathogen compounds, which is shown in Fig.2. After a certain period of time, the magnetic pole in the X-Y direction stops and the magnetic pole in the -Z direction starts to attract the beads wrapped pathogens cells to the underside of the separation chamber. The B port is replaced by PBS buffer injection washing separation chamber and the magnetic pole in the -Z direction stops releasing MNPs/pathogen compounds to realize the separation of pathogens from samples. Samples from the output D are taken to test tube to carry out plate colony counting and The magnetic capture efficiency of the pathogens is calculated using equation（7).

WORKING PARAMETERS OPTIMIZATION OF THE MAGNETIC SEPARATİON SYSTEM

The main parameters of the microfluidic magnetic separation system include the magnetic pole current and magnetic frequency.

The synthetic sample of 1mL *Salmonella typhimurium* (2.8×10^3 CFU/mL) and 1mL of MNPs solution are injected into the microfluidic chip. Different parameters are adopted to detect the magnetic capture efficiency and determine the system's optimal working parameters .

Based on equation (1) and (2), the magnetic capture efficiency is connected with the magnetic force of the magnetic nanoparticles. When the magnetic pole current increases, the magnetic field intensity increases and so does the magnetic force of the magnetic nanoparticles. When the magnetic field intensity meets or exceeds its saturation level, the magnetic force no longer increases. Therefore, the experiments adopt 7 kinds of magnetic current (0.5~3.5A) to detect magnetic capture efficiency. As observed in Fig.4, the results show that when the magnetic pole current is more than 3A, the change of the magnetic capture efficiency is not obvious, which the magnetic nanoparticles reach magnetic saturation.The magnetic force of the magnetic nanoparticles exerted by the magnetic field is a maximum.Thus, the optimum magnetic role current of the system is 3A.

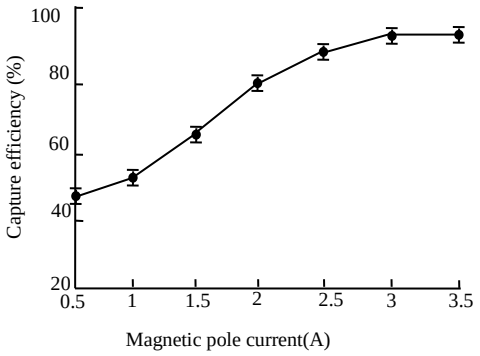

Fig. 4. Capture efficiency VS. Magnetic pole current.

Based on equation (6), the switching frequency of the magnetic field varied to disturb the flow of the sample fluid,which improve the immunological reaction of magnetic nanoparticles and pathogens and thus increase the magnetic capture efficiency.When the switching frequency is too low, the disturbance of the magnetic force is too slow and the magnetic capture efficiency is not high. The capture efficiency increases with the increase of the magnetic switching frequency.When the frequency

is too high, the magnetic nanoparticles lag the disturbance of the magnetic force owing to the action of the viscous liquid resistance.At the same time, the magnetic nanoparticles can't be fully released owing to the action of the rapidly changing magnetic force. So the magnetic field with the further increase of the magnetic switching frequency will lead to the decline of the magnetic capture efficiency.The experiments adopt 7 kinds of magnetic switching frequency (1~4 Hz).As observed in Fig.5, the results show that when the magnetic frequency is over 3Hz ,the magnetic capture efficiency starts to decline significantly.So the optimal magnetic frequency is 3Hz.

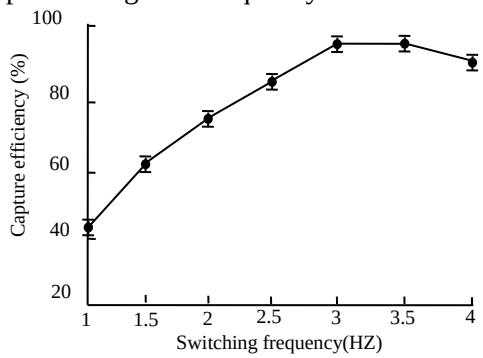

Fig.5. Capture efficiency *vs.* switching frequency.

RESULTS AND DISCUSSION

The performance of the microfluidic magnetic separation system mainly include the capture efficiency, the separation speed and the degree of automation.

We uses the aboved optimum parameters of the system to analyze the results of the magnetic separation of the samples with different concentrations. The pathogen sample(1mL *Salmonella typhimurium* 3×10^8 CFU/mL)is taken as detection target. Meanwhile,two kinds of interfering pathogens with different concentrations is added and mixed.After gradient dilution, 7 kinds of target pathogens detection samples are obtained. The target detection sample and 1 mL magnetic nanoparticles solution are injected into the microfluidic chip at the same time and the magnetic separation detection is conducted under the condition of the optimum parameters. A quantitative relationship between the magnetic capture efficiency and the target pathogens concentrations in samples is obtained, as shown in Fig.6.

The results illustrates that when the target pathogens concentration is within the range from 3×10^2CFU/mL to 3×10^6CFU/mL, the capture efficiency of target pathogens is over 93% on average. When the concentration of target pathogens is over 3×10^6CFU/mL, the magnetic capture efficiency of the system is below 93%. Thus, the magnetic separation method has a high capture efficiency to pathogens with both a high concentration(10^6CFU/ml) and a low concentration （10^2 CFU/mL).

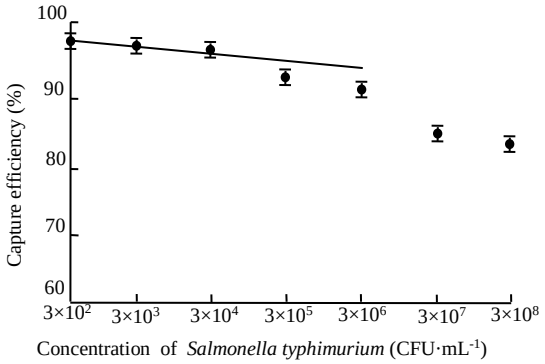

Fig. 6. Capture efficiency *vs.* the concentration of *Salmonella typhimurium.*

In order to analyze and verify the performance of the designed microfluidic magnetic separation system, after adding interfering pathogens to conduct detection 6 groups of target pathogens samples with random gradient concentration within the range from 4×10^2CFU/mL to 4×10^7CFU/mL are injected into the system. Passive microfluidic chips mentioned in the document [15] are adopted to conduct comparison and analysis of pathogens separation.

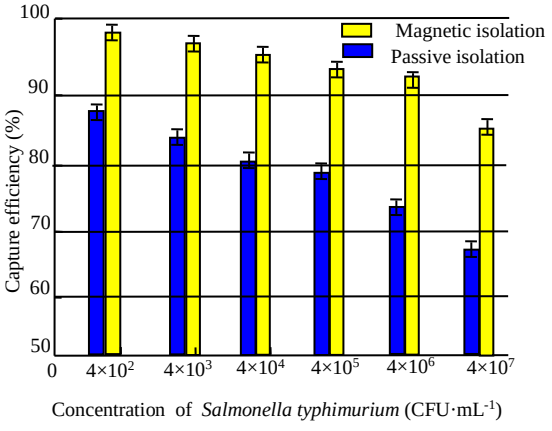

Fig. 7. compared analysis for the proposed method and passive isolation method

Fig.7 is the comparison histogram of *Salmonella typhimurium* capture efficiency with two kinds of methods. As shown in Fig 7, compared with the passive separation method, the target pathogens capture efficiency of the magnetic separation method increases about 32% and realizes the high efficiency separation of the aquatic microfluidic pathogens concentration. It is also convenient to

improve subsequent detection accuracy and efficiency of pathogens.

CONCLUISIONS

This paper integrates magnetic separation on a microfluidic chip，and establishes a microfluidic magnetic isolating system for aquaculture pathogens detection. Compared with the passive capture separation method, the capture efficiency of the proposed method increases by 32% and realizes the high efficiency isolation of the aquatic pathogens. It is also convenient to improve the subsequent detection accuracy of the pathogens. The proposed method will provide prospective references for the research of aquaculture disease by early warning and prevention .

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ПРОЕКТИРАНЕ И ОЦЕНКА НА МЕТОД С МИКРО-ФЛУИДНА ИЗОЛАЦИЯ ЗА ОТКРИВАНЕ НА ПАТОГЕНИ В АКВАКУЛТУРИ

Дж.Дж. Гуо [1,2], Р.Б. Джан[1*], Н. Ян[1]

[1]*Училище по електро- и информационно инженерство, Университет "Джиандзу", Дженджиян 212013, Китай*
[2]*Училище по електро- и фотоелектронно инженерство，Технологичен институт в Чанджоу, Чанджоу 213002，Китай*

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(Резюме)

Разпространението на инфекциозни болести има сериозно въздействие върху аквакултурната промишленост. Откриването на патогените във водна средачесто зависи от традиционни лабораторни методи, при които включват изолация и продължителни операции, при ниска степен на автоматизация. Тук се предлага метод с магнитна изолация за откриването на патогени в аквакултури, основан на микрофлуиден чип. Ние проектирахме микрофлуиден чип с автоматично впръскване на пробата, магнитна изолация и устройство за експериментална платформа. Определени са оптималния полярен магнитен поток и превключващата честота в изолационната система и като пример експериментално е изследвана културата *Salmonella typhimurium*. Опитните резултати показват, че в сравнение с пасивната изолацияна бактериите ефективността на улавяне им нараства с 93%, което благоприятства ефективното отделяне на патогените и високата точност при откриването им при предпазването на аквакултурите от заболявания