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# GATE-CONTROLLED MICROFLUIDIC CHAMBER WITH MAGNETIC BEAD FOR DNA SEQUENCING-BY-SYNTHESIS TECHNOLOGY

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## ABSTRACT

In this paper we present a novel microfluidic platform for DNA sequencing-by-synthesis methods (e.g. pyrosequencing). The proposed platform is based on the valve-controllable PDMS channel technology with DNA-coated magnetic beads. The encapsulation of the reaction of DNA polymerization in picoliter-sized wells provides for excellent isolation and control for detection. This separation prevents cross-talk amongst neighbor reactors which is one of the most limitations for higher integration of the current technologies. Through application of an external magnetic field the beads can be allocated with better accuracy. In addition this property can help mixing for the reaction. The proposed system is useful for a number of other bio-species detection and sorting templates. This paper illustrates the design and experimental results of a primary template as well as different advantages and potential applications of the Gate-Controlled Magnetic Bead (GCMB) platform in the world of DNA sequencing and genetics.

## INTRODUCTION

The revolution that the Sanger method of DNA sequencing (1) and its subsequent array automation (2) has made in biology and genetics was followed with the sequencing of the human genome and the completion of the Human Genome Project (3, 4). The Sanger dideoxy DNA sequencing method has been the most commonly used method for DNA sequencing thus far, particularly in large-scale genomic sequencing, but inherent limitations of this state-of-the-art technology such as cost, throughput and read length, and enzymatic complexity make it impractical to deliver the current needs of DNA sequencing for clinical applications, disease detection and discovery, genomics studies, diagnostics and drug delivery, and personalized medicine.

The Human Genome Project was accomplished after a reduction in the cost of DNA sequencing by three orders of magnitude. It is desired to reduce the cost by another three orders of magnitude to enable profiling of the individual genome. To achieve this goal, a highly integrated platform with high throughput and reliability will be needed.

The increase of large-scale DNA sequencing projects in recent years has driven a search for alternative methods to reduce cost and time. (6, 7, 8, 9, 12) Many efforts have been investigated toward achieving a high-throughput and low cost assay for DNA sequencing such as high-density parallel sequencing with step-wise enzymatic cleavage and ligation (5, 14), base addition with deprotection steps (21), mass spectroscopy (22, 23, 24, 25), sequencing by hybridization (26), microfluidic device sequencing-by-synthesis (14), nanopore sequencing, polymerase colonies (27, 28, 29, 30) and pyrosequencing (8, 11, 12, 15, 32, 33, 35). Amongst all the different assays, pyrosequencing more likely seems to be the

dominant one to overcome the technological challenges and has had a promising industrial progress in the last few years, starting from pyrosequencing AB (1998), toward the latest developments of 454 Life Science Corp. with a system in array based format of the technology.

In 2005, Nature published an article by Margulies et al. (10) describing beads coated with DNA in wells etched into the end of a fiber optic slide. Each well serves as a reaction chamber for the pyrosequencing method, a sequencing-by-synthesis technique (8, 11, 12, 15, 32, 33, and 35). They are making an instrument for this research built as a part of 454 Life Science Corp. which has 1.6 millions picoliter wells. However, not all of these wells can function as a reactor. (In one of the latest 454 machine as the-state-of-the-art technology, the maximum number of wells that could count as good spots with reliable results has not exceeded ~250,000 from 1.6,000,000 wells). One of the main issues that could limit chemical higher integration is the cross-talk between neighbor wells and each well. Another reason is that the platform could suffer from the effect of previous run cycles due to washing and flow issues e.g. since high pressure fluidic injection is not practical.

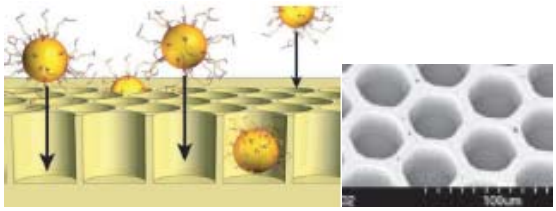

**Fig.1 Scheme of 454 Life Science Technology [Source: Margulies et al., *Nature,* 03959: 1-5 (2005)].**

Here we introduce an effective and useful microfluidic platform for DNA sequencing, especially for sequencing-by-synthesis methods (e.g. Pyrosequencing). Additionally, the proposed picoliter micofluidic system is essentially useful for a number of other bio-species detections and sorting templates (9, 18, 19, and 20). This platform is based on a PDMS gate-controlled channel with a number of wells incubated on its wall, which leads to encapsulation of the beads in these picoliter wells as sensing volumes and can significantly improve the signal to noise ratio of detection by preventing cross-talk between adjacent wells. The encapsulation of bead or reaction in the picoliter-sized vessels provides for excellent isolation and control on the detection-based systems by preventing cross-talk among neighbor reactors.

In addition, by using micron-sized magnetic beads instead of conventional sepharose or glass beads, we can gain more flexibility for allocation control, movement or holding the beads by an external magnetic force and improving the injection and washing phases, and helping to use higher pressure flow in the channel. The electromagnetic field can be applied by the use of a simple cubic magnetic bar located on the right side of the system close the wells (~5 mm)) or using a MEMS magnetic actuator which gives higher control and accuracy (in high-density production).

In conclusion, the current state-of-the-art technology could lead to a much higher sensitivity in real time.

## METHODS AND MATERIALS

The proposed micofluidic platform is based on the valve-controllable (or gate-controllable) microfluidic system made of polydimethylsiloxane (PDMS) (14, 16, 17, and 19) which contains micrometer-sized magnetic beads (e.g. 2.8 um diameter M270 DYNAL Bead (13)). Fig.2. shows the design schematic of the initial platform design for the proposed system. The red lines in Fig.2 shows the main flow line and the green lines are the control lines as a layer perpendicular in direction and on top of the flow lines (red) with about 20 µm thin PDMS membrane between these two layers which prevent mixing but the elastic property of PDMS makes it possible to close the flow line b of PDMS, which allows closure of the flow line by pressurizing the control valve (14, 17, 19). The primary channel, which has been made in Stanford Microfluidic Foundry, has a width of 100µm and height of about 10µm (the physical channel height is usually smaller than the theoretical value, due to upside pressure and some process variations). As it is shown in the design, this channel includes two inlets and one outlet plus both controlled with a top layer control valve (14, 16, and 19); In addition, this system has a rotary pump to enable rotating the liquid in the channel even during the period that all the inlet and outlet valves are closed. The right plot in the Fig.2 shows the zoom version of the right piece of channel wall which contain a number of wells (micro-vessels) in cubic shape with different geometries in length and width (e.g. for the experiment of the next section, channel wall has 10 different wells of size W*L: 10*10um2, 15*25um2, 20*20um2, 10*25um2, 30*25um2, 30*30um2, 35*35um2, 20*40um2, 30*35um2, 10*40um2 where W is the width of cross section and L is the length or depth of the cubic well). These vessels act as picoliter chambers which can incubate bio-species-immobilized magnetic beads. In the experimental set-up of the next section, biotinylated 2.8 um M270 DYNAL magnetic beads with 95-mer single-strand-DNA includes 35-mer hairpin, immobilized on the surface of these beads were used, where ROMO is a 35-mer hairpin for DNA strand.

The soft lithography technique was used for fabrication of the system (16, 17, and 34). Closing the gates is possible through pressurizing this control line (shown as green line in Fig. 2) either with gas or liquid (e.g. deionized water).

Since we were limited to eight pressure inputs from external pump for the control lines in our experimental set up, all ten micrometer-size cubic vessels in the right channel's wall could not get separate control lines for each and two pressure lines for controlling these vessels have been considered; as you can see from the right image in Fig.2 we can control three vessels by two control lines, by encapsulation of the third one between two adjacent separate input pressure lines.

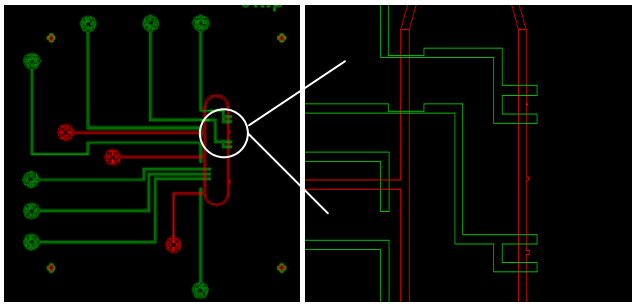

**Fig.2. Design of the initial microfluidic system with two layers of control line (green) and flow line (red). The right images shows the picoliter cells designed in the right wall.**

## MOTIVATION AND PROS

Fig. 3 shows the schematic of the system with magnetic beads in cubic wells in the gate-controlled PDMS micro-channels. As noted in the introduction, there are two special characteristics for this system:

**A.** The proposed system the material for the channel is PDMS (instead of fiber optics in the 454 platform (10)) to be able to gate-control the flow lines by pressurizing control lines. [16].

**B.** Magnetic beads are suggested and used in the system instead of using conventional glass, polycrystalline or Sepharose beads (e.g. Sepharose is a bead-form of agarose, a polysaccharide polymer material extracted from seaweed, which are used widely in the current biotechnology industry (10, 32, 33)).

The effects of the first modification permit these gate-controlled wells for an excellent isolation. It effectively reduced cross-talk between adjacent picoliter chambers. Therefore one of the main issues of miniaturization in such a microfluidic sensing technology could be eliminated.

The use of magnetic beads has three significant advantages: Firstly it gives an extra control for allocation of the beads in the channel and flow process. (to test this ability in the experiment all the control lines, inlet, outlets and rotary pump in the design of Fig. 2 are drawn in the left side of the wall with vessels in the right side), and after that wall in the right side, there is a thin layer of PDMS, after cutting the extra part, where the external magnet could be placed (e.g. cubic usual magnet or a magnet MEMS actuator) This magnet could be used for positioning of beads inside the channel. Secondly, holding the beads with the extra magnet would dramatically affect the washing steps since by beads can be held with this external magnetic force and injection with much higher pressure is possible. Thirdly, by the extra degree of freedom due to magnetic field and vibrating the beads in wells, mixing would be done more effectively. This magnetic property of the beads can help on mixing issues and bringing the enzymes and nucleotides to the site of DNA strands better.

In addition, by shrinking the size of the beads from a typical diameter of ~30-40 micrometer in conventional pyrosequencing technology to magnetic beads of a few microns (~1-2.8 um for usual range, we used 2.8 um M270 DYNAL iron beads with original concentration of about hundred millions of bead /ul for our experiments) provides for a better signal to noise ratio by providing for enhanced detection sensitivity.

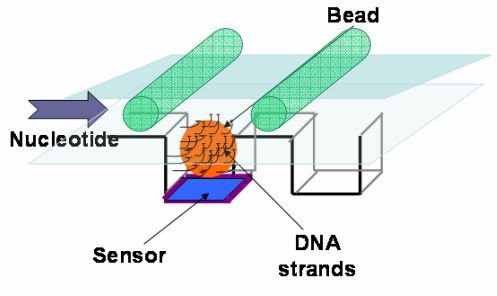


**Fig.3. Schematics of the Gate-controlled System with magnetic beads**

## EXPERIMENTS AND RESULTS

In Fig.4, a micrograph of the fabricated microfluidic chip is shown. The chip was placed in a machined attachment of the translation stage of an inverted Olympus IX50 microscope, and the micrograph was taken with the help of an additional 10X lens. The beads, which have been used for the experiments of the initial 100 µm wide and 10 µm high channel platform, are 2.8 um DYNAL magnetic beads with original concentration of 1E8 beads/µl which have been diluted after injection into the channels. The control gates have been manually tested by pressurizing the control valves, with a set-up where pressure can be switched between control lines and between flow lines in the presence of the beads floating in the channel. Having a continuous-flow air supply, a set-up could be built where the pressure supply (in the range of 0-30 psi) to several independent manifolds could be regulated. Each manifold allows us to manually control the supply pressure (ON/OFF) to several independent control lines or flow lines.

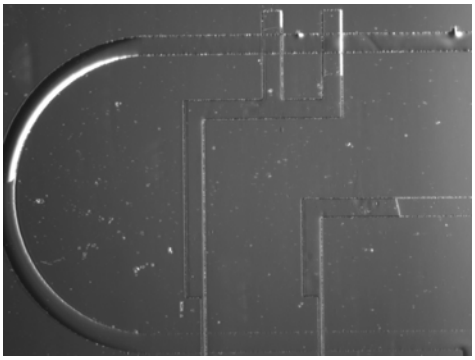

**Fig. 4 A Micrograph of the PDMS channel with Gate-Controlled Valves**

In Fig. 5 the micrograph of 2.8 μm magnetic beads floating in the same channel (e.g. 100μm wide and 10 μm high) is shown. These magnetic beads can be coated with a layer of biotin and then single-strand-DNAs are attached to this interface layer to bind to the bead surface. The process of immobilization of DNA to surface of beads could be done after PCR multiplication of single-stranded DNA. Effect of the magnet on the motion of the beads inside of the channel during different phases of ON/OFF controlling and incubation of beads in the wells has been further tested.

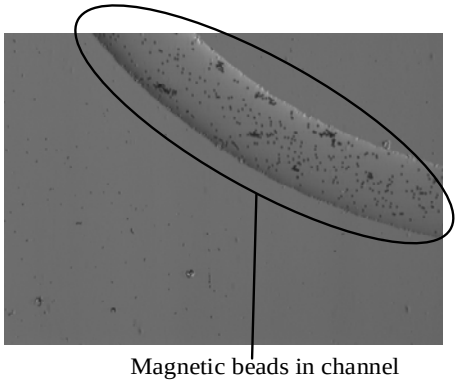


**Fig.5. Magnetic Beads in the channel**

Fig. 6 shows two phases of close and open control valves for one of the cross sections in the system, when the beads can flow after the pressure is released in the control line. The left micrograph in this figure shows the control line under pressure of DI-water, when the right images is just after removing the pressure and releasing the flow in the channel. In addition, leaky valves, which can be used for trapping beads right under the cross section junction of flow and control lines, have also been tested which confirm the theoretical expectation.

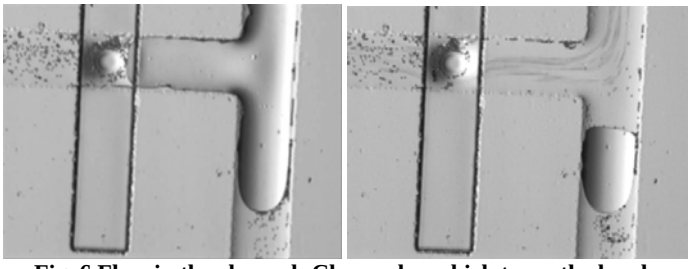

**Fig. 6 Flow in the channel: Close valve which traps the beads behind the gate (left), Open valve (right)**

## DISCUSSION AND CONCULSION

In this paper we introduce a novel platform for DNA sequencing-by-synthesis technology,. There are two major changes in the proposed system rather than the conventional pyrosequencing one (e.g. 454 Life Science Corp. technology): First, this platform is based on PDMS gate-controlled microfluidic channels. This leads to encapsulation of the beads in picoliter wells as detection volumes and can significantly improve the signal to noise ratio of the system by preventing cross-talk between adjacent wells. The other modification is the use of magnetic beads instead of conventional Sepharose or glass beads, gives another degree of freedom on controlling the allocation of the beads by an external magnetic force and makes more efficient washing and injection phases possible, and helps on reducing flow process issues.

This novel DNA sequencing-by-synthesis technology is subsequently used to explore the impact of the design's shape and dimensions of the micro-channels, fluidics parameters, leaky valve trapping mode combined with the role of biotinylated DNA-immobilized iron beads in the PDMS microfluidic system. The resultant potential tradeoffs were discussed which show the advantages of this method in practical applications.

Depends on applications and design schemes, the width and height of the channels have to be modified accordingly for optimizing performance (e.g. for encapsulation of just one bead per well, the width of channel should be rational to the diameter size of the bead while usually the height of the channel is restricted to allow one bead per time from each cross section in the vertical axis). One of the challenges in the ongoing research on valve controllable PDMS microfluidic systems is the diffusion of air bubbles to the channel through the thin membrane between control and flow lines; this case is more likely to happen when the control line is pressurized with air, therefore pressurizing control lines with liquid such as water is preferred to air.

The proposed picoliter micofluidic system could have a very effective role for a number of other bio-species detection and sorting templates such as pathogen detections, antibody-antigen interactions, or real-time PCR detections (9, 18, 19, and 20).

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