A Lab-on-a-chip Device for Telomerase Activity Detection based on ATP Bioluminescence System

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Abstract

Lab-on-a-chip (LOC) devices are a subset of Micro Electro Mechanical Systems (MEMS) devices and also called as Micro Total Analysis Systems (μTAS). Telomerase is an unique enzyme which can keep the length and stability of telomeres in order to protect the chromosomes. Active telomerase can be detected in more than 85% tumor cells. The detection of telomerase activity is very important in cancer diagnoses and the study of telomerase inhibitors. In this poster, we proposed the design of a lab-on-a-chip device which can detect the telomerase activity in a convenient way with a short processing time and micro-amount of sample usage. When the extracted telomerase is mixed with telomerase substrate (TS) primer and the deoxyribonucleotide triphosphate (dNTP) to perform an extension reaction, pyrophosphate (PPi) is released. After the released PPi is converted to ATP, the level of ATP can be detected by the ATP bioluminescence system, which can reflect the activity of telomerase. This LOC device contains one sample inlet, six pre-stored reagents, two mixing zone and two reaction chambers connected by microchannel with diameter of 100μm. COMSOL simulation shows this LOC device works effectively with satisfying results.

Introduction

Telomere and telomerase are proven to have significant connection to human lifetime and genetic diseases. Telomere is the specific repeated structure at the ends of eukaryotic chromosomes. Because of the particular double-stranded DNA structure and the specific DNA semiconservative replication procedure, an unfilled gap remains during each DNA replication. As a result, the telomeres protect the gene-code by be frayed. However, the length of telomeres is not infinity. If the telomeres cannot be synthesized, it will finally disappear and damage will occur on the gene-code parts. Telomerase is a unique eukaryotic enzyme which can help synthesize the telomeres by reverse transcription. The unlucky thing is that the telomerase is inhibited in normal cells but reactivated in tumor cells. By using the connection between telomerase and tumor or other disease, the telomerase activity can be used as a biomarker to help scientists develop a series of new diagnostic methods and therapies for cancer and some inherited diseases. A great variety of telomerase activity detection methods in different body fluids and tissues have been developed in the past decade, such as telomere extension assay, PCR-based TRAP assay, hybridization protection assay and a lot of newly developing PCR-free assays. However, these detection methods need relatively long time to obtain the results. They involve multiple complex reaction steps with a large amount of sample and reagent usage. Furthermore, it is hard to achieve satisfactory sensitivity with the above methods.

Detection Method

In this poster, we proposed the design of a LOC device by taking the advantage of the well-developed ATP bioluminescence system for the telomerase activity detection. By using telomere extension reaction products to generate ATP, a highly sensitive and effective telomerase activity detection method has been reported. When telomerase extracted from tumor cells and the four deoxyribonucleotide triphosphate (dNTP) monomers (dATP, dCTP, dGTP, and dTTP) are present, the repeating of TTAGGG are added to the telomerase substrate primer (5′-AATCCGTCGAGCAGAGTT-3′) to initiate the telomere extension reaction. A large amount of pyrophosphate (PPi) is released during the absorption of the dNTP monomers into the substrate primer. The released PPi is then transformed into ATP with adenosine phosphosulfate (APS) and catalyzed by ATP sulfurylase (ATPase). The schematic of the method is shown in Figure 1.

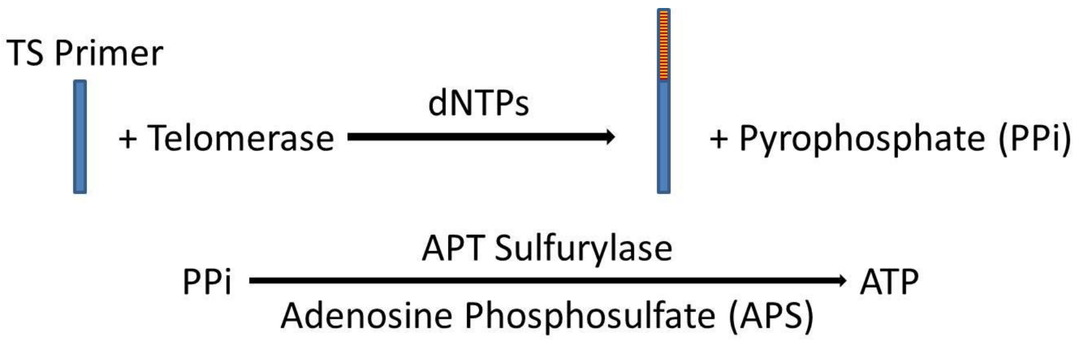

Figure 1. Schematic of the telomere extension reaction and ATP generation.

The ATP-based bioluminescence assay is an accurate, fast and high sensitive measuring method for ATP detection. D-luciferin-luciferase reagent is used to induce bioluminescence at 560 nm by a reaction with ATP in the presence of an Mg+2 buffer, as shown in Figure 2. The output light signal is increased quickly and reaches the maximum in a few minutes after ATP reacts with D-luciferin-luciferase reagent. The signal also last long enough for the recording and analysis of the detection results.

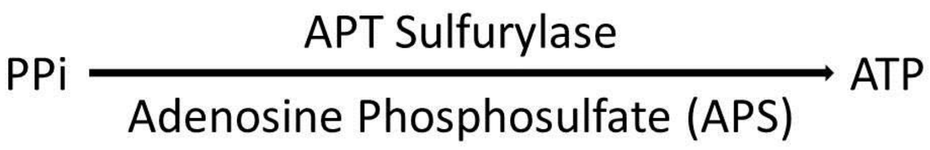

Figure 2. ATP bioluminescence system.

LOC Device Design

Figure 3 shows the design and components of this LOC device. This device consists of two parts which are used to carry out two steps reactions. The first part includes the sample inlet, four reagent inlets (dNTPs, TS primer, APS, ATPase), passive mixing zone and a reaction chamber which is used to generate ATP. The other part of this device is used to achieve the ATP bioluminescence reaction by importing the generated ATP and the D-luciferin-luciferase reagent with a Mg+2 buffer into the second reaction chamber, which is also used as a detection area by an ICCD camera. The two multi-curved shape mixing zone is essential for the reagents mix. The red little rectangles represent the micropumps and microvavles which are used to control the micro flow. The blue circles are the pre-stored reagents which are using the blister packs technology to pre-storage the reagents on the chip.

The length of sample inlet channel is 2000μm; the length of four reagent inlets is 1000μm; and the length of each mixing zone is 5cm. The diameter of the reaction chamber and detection area is 2000μm, and the whole chip size is 1.5cm×1.5cm.

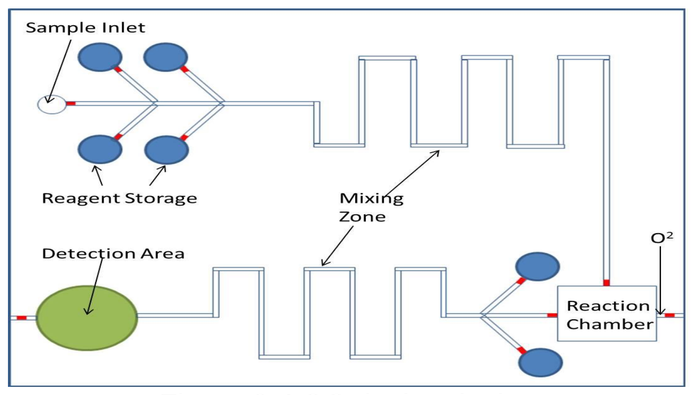

Figure 3. LOC device design.

COMSOL Simulation

Channel width	100μm
Channel depth	100μm
Inlet pressure	50Pa~150Pa
Outlet pressure	0Pa
Flow type	Laminar flow
Compressibility	Incompressible flow
Temperature	293.15K
Diffusion coefficient	$1\times10^{-11}m^2/s$

Table 1. COMSOL simulation Parameters

COMSOL Multiphysics is an effective simulation software based on Finite Element Method (FEM). In this poster, we usie the Microfluidics Module to simulate the microfluidics flow and study the velocity, pressure, and mixing efficiency of the LOC device. All the parameters for COMSOL simulation are given in Table 1. In the simulation, both 2D and 3D models are used, and the inlet pressure has been changed from 50Pa to 150Pa to study how the inlet pressure affects the microfluidics system.

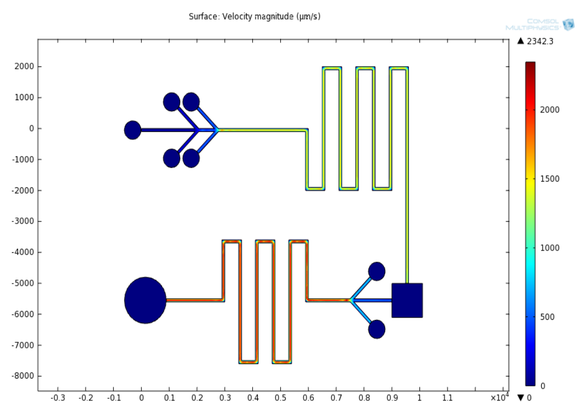

Figure 4. Velocity plot; Inlet P=100Pa.

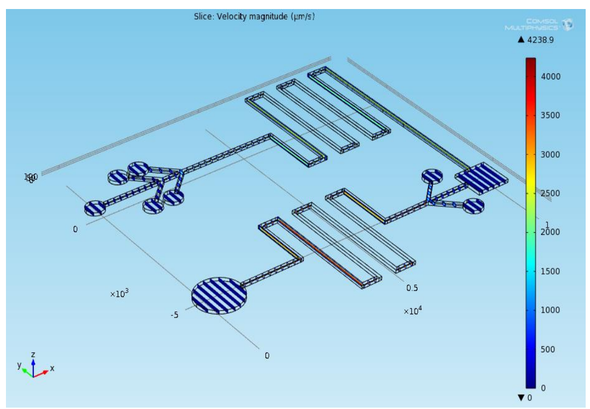

Figure 5. Velocity plot; Inlet P=150Pa.

When the inflow pressure is 100Pa for all the inlets, the max velocity is 2300μm/s as shown in Figure 4. The flow rate is 1.38μL/min, and the Reynolds number is about 0.23. To enhance the test speed, the inflow pressure is increased to 150Pa, the velocity plot (Figure 5) shows that the max velocity has reached 4200μm/s, and the flow rate goes to 2.5μL/min. The Reynolds number is changed to about 0.42.

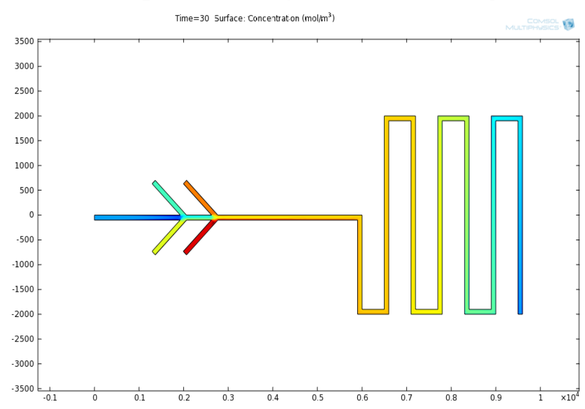

Figure 6. Concentration plot; Inlet P=100Pa.

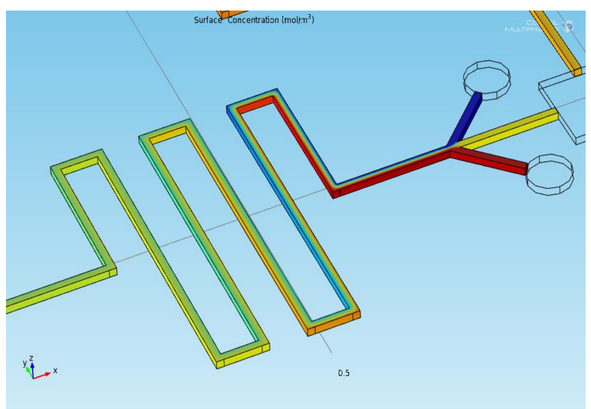

Figure 7. Concentration plot; Inlet P=100Pa.

To inspect the passive mixer efficiency, the Physics of Transport of Diluted Species has been added to the simulation. All the inflows have been given different concentrations. The concentration plots (Figure 6 and Figure 7) have shown the diffusion phenomenon of all the solutions which reflects how the inflows are mixed by the mixing zone. The concentration plot has clearly shown the inflows appear as separate laminar flows first. Then in the mixing zone, the flows begin to transform into a single flow. Finally, a fully developed flow is generated at the outlet channel, which indicates the inflows have been fully mixed.

Device Fabrication

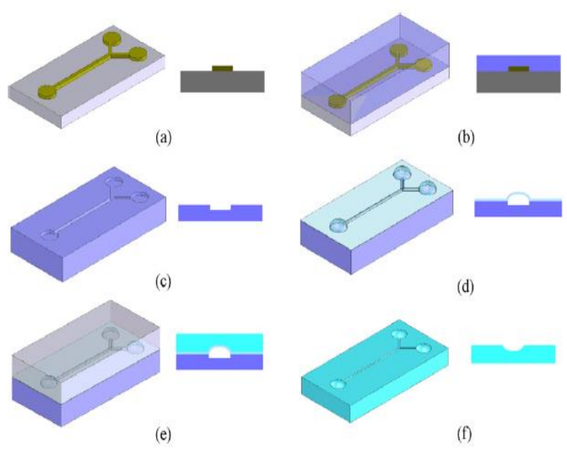

Figure 8. Soft fabrication procedure.

Figure 8 has demonstrated the soft fabrication procedure of this LOC device by using a pre-fabricated silicon molding. (a). Apply the cast molding procedure (b), Form the bottom section (c). Drill from bottom of the PMMA model at the storage structure and coat the pattern side of PMMA layer. (d). Cure the PMMA layer by constant air flow and cool it down to finalize the blister pack storage model (e). Finally bond the top section (f). LOC device is fabricated by second cast molding procedure with the undrilled bottom section.

Conclusions and Future Work

Telomerase activity is very important in human cancer diagnosis and treatment. In this poster, we designed a convenient, fast and highly sensitive lab-on-a-chip device for telomerase activity detection based on telomerase-ATP-bioluminescence assay with micro-amount of sample and reagent usage. The novel blister packs reagent storage technique has been used for the fabrication of this LOC device. In the future, if a rapid telomerase extraction method can be developed, it is possible to integrate the sample preparation procedure into the LOC device, which may lead to significant progress in the clinic cancer diagnosis and therapy development.