Application of Microfluidic Device to Malaria Diagnosis

By

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ABSTRACT

Of many diagnostic devices and technology developed, microfluidics could be superior in terms of ease of fabrication, cost, portability, speed and sensitivity. The application of diagnosis of malaria infection by microfluidics is studied. Malaria infected red blood cells will cause a cell stiffening, and the different behaviors of iRBCs could be detected by microfluidics. The malaria market and various business model is analyzed, and a suitable business model could be chosen to commercialize this device. However, limitations exist at current stage.

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Table of Contents

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List of Figures

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Chapter 1 Introduction and Overview

Malaria is one of the most common infectious diseases, which is generally associated with poverty. It is also a cause of poverty and a major hindrance to economical development. Malaria is widely spread in tropical and subtropical regions of Americas, Asia and Africa [1]. Most affected countries are poor countries, and they are hard to afford the cost of controlling disease as well. Annually, there are 350 to 500 million infections in human and 1 to 3 million deaths; mostly are young children in Sub-Saharan Africa [2]. Thus, malaria is an enormous public-health problem.

Human malaria is caused by protozoan parasites of the genus *Plasmodium* [3]. There are four species of *Plasmodium*, and the most serious form of malaria is caused by *Plasmodium falciparum.* Those parasites are transmitted by female *Anopheles* mosquitoes, and they multiply within red blood cells, causing symptoms of anemia, and others symptoms such as fever, nausea, chills, and even coma and death [3].

Currently, there is no vaccine available for malaria. Instead, there are various preventative drugs and antimalarial drug treatments. However, those drugs are simply too expensive for most people living in endemic areas. Drug resistance increases as well if misuse of drugs continues. Thus, in the meantime, efforts must be put in new drugs researches and vaccine development. In the development of drugs and vaccines, as well as in malaria diagnosis, *in vitro* test is needed.

In vitro test of parasite infected red blood cells (iRBCs) is allowed in microfluidics. Microfluidic device is resulted from the impact of microelectronics fabrication impacting on microbiology. The physical dimensions of microfluidic devices are within 1 to 100μm at least in one dimension, which agree well with microorganisms. In this thesis, a microfluidic device, which is fabricated in poly(dimethylsiloxane) (PDMS), is studied. Various designs of microfluidic channels, with dimensions varying from 2 to 8μm, are fabricated to mimic the blood vessels and capillaries. *In vitro* test condition could be satisfied by testing the iRBCs' behaviors in capillaries at physiological pressure and temperature. Effective and quantitative results could be obtained, with an affordable price level for those who need it.

The idea of the microfluidic device is simple. By applying pressure difference, iRBCs can be forced to travel through microchannels, and their behaviors can be recorded by high speed camera. Several parameters could be measured, such as recovery and deformation time, deformation, speed, etc.. By applying well established mechanical models for RBCs, mechanical properties could be calculated. It is known that at different stage of *Plasmodium* life cycle, the RBCs exhibit different mechanical properties [4]. Thus the disease progression could be studied, and the effect on drug treatment could be tested in *vitro* condition.

Besides, another potential market for microfluidic device is malaria diagnosis. Currently the most economical and reliable diagnosis of malaria is microscopic examination of blood films. There are other diagnosis such as antigen detection tests, polymerase chain

reaction, and flow cytometry. Each testing method has its own advantage and drawbacks. Microfluidics is unique in its low cost, high sensitivity and portable features in order to have a market share.

Besides technical issues, the development and manufacturing of those devices must be supported by a market. Malaria research totals around 300 million US dollars per year worldwide, and most of this money is used for new drug development and education development in endemic areas [1]. Microfluidic device, as a complimentary technology for drug development, as well as a potential diagnostic device, currently needs to be funded by government and various malaria research organizations. There is still a great deal of development in order to access the malaria market.

In order to commercialize this technology, several business models could be used depends on the amount of investment available. And it is wise to focus on the development of functional commercialized device first, with outsourcing manufacturers.

Thus, the objective of this thesis is to develop a portable and automated diagnostic device which could provide *in vitro* test, to conduct market analysis for malaria diagnostic device, and to establish business model and IP analysis for this device.

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Chapter 2 Technology

Various researches on the direct and indirect effect of *Plasmodium falciparum* on the RBCs have been done [23], and the mechanical property changes of iRBCs and induced malarial anemia have been studied [4, 12, 26]. Different mechanical models have been proposed as well [5, 27]. In Shelby's work [6], they showed a microfluidic model of single-cell cappilary obstruction by iRBCs. And in Antia's work [13], the interaction of iRBCs to host cell ligands (ICAM-1 and CD36) in microfluidic channel mimiked microvasculature, and the effect of branched microfluidic channels was studied. The microfluidic device used for iRBCs testing is developed upon this background.

2.1 Technology Development

Microfluidic device is an integrated system designed to manipulate small (10^{-9} to 10^{-18} liters) amounts of fluids, using channels with the dimensions between one to hundreds of micrometers [21, 22].

Microfluidics is developed from the development of molecular analysis, biodefence, molecular biology and microelectronics. Microanalytical methods provide ways of high sensitivity and high resolution analysis using small amount of samples. The explosion of genomics in 1980s and followed by DNA sequencing, requires higher sensitivity and resolution which could be offered by microfluidics. The development of microelectronics offers ways of fabricating microfluidic devices, such as soft lithography by using PDMS,

glass and silicon. Soft lithography is a main fabricating route used currently. In 1990s, in order to counter the threats posed by chemical and biological weapons, the Defense Advanced Research Projects Agency (DARPA) of the US Department of Defense supported a series of programs, and those programs aimed at developing field-deployable microfluidic systems, which were the main stimulus for the rapid growth of academic microfluidic technology [7, 21].

Microfluidics has lots of advantages, such as the ability of using small quantity of testing sample, high resolution, high sensitivity, low cost, short time to analyze and portable device[7, 20, 22]. However, it is not widely used yet and still in laboratory stage. Design and fabrication of microfluidic channels is not a issue today, however, issues on packaging, integration with user interface and fabrication of a portable device are still technology barriers to economize this device.

2.2 Materials

Microfluidic device fabrication is originated from the microelectronic device fabrication. Photolithography and associated technology was originally used. Thus silicon and glass were used in early works of microfluidic device.

However, silicon is opaque to visible and ultraviolet light, and it is not suitable for optical detection, which is widely used in biological diagnosis. Besides, it is expensive. Thus it is replaced by polymers soon. Poly(dimethylsiloxane) (PDMS), in terms of its optical

property, mechanical property, ease of fabrication, low cost, nontoxicity and biocompatibility, is widely used in microfluidic channel fabrication [8]. And an *in vitro* test condition could be provided to study the single iRBC property in a capillary-like microenvironment.

This paper will present a microfluidic device design by using PDMS and cover glass, silicon wafer and SU-8 photoresists are used during fabrication.

2.3 Fabrication

Soft lithography was used to fabricate test channels by PDMS. Firstly, a high-resolution (2μm) chrome mask was generated from CAD file and etched by electron beam etching. Channel deign on the mask was negative, and the mask was used in contact photolithography with SU-8 photoresist. Thus a negative master, which has the channel design feature of SU-8 on a silicon wafer, was fabricated. PDMS channels were molded from the master, and the features could be duplicated by cured PDMS. Then they were sealed irreversibly to a borosilicate glass coverslip after plasma oxidation treatment. The ends of the channels were perforated with plastic tubing (PE20) to allow flow to and from channels. Tubes were connected to a 3-ml syringe through which fluid could be introduced into the channel, and pressure difference could be generated [25]. The fabrication process is shown as in Figure 1

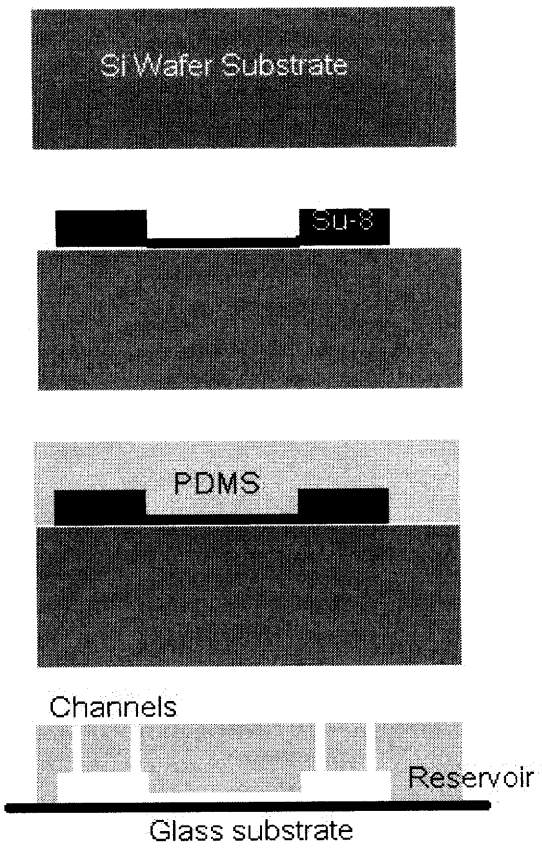

Figure 1 Illustration of microfluidics fabrication

2.4 Components

2.4.1 Microfluidic channels

Microfluidic channels as fabricated above are the key part of the device. It provides a *in vitro* test environment for single cell study.

Human capillary could be modeled as a cylindrical shape with radius of 2 to 8μm. It is hard to fabricate a circular intersection of microfluidic channels with radius larger than 1μm, thus rectangular channels were designed with intersection dimensions of 2x2, 2x4, 2x6 and 2x8μm. The length of the channel was designed to be around 3 to 5 times its width. And the depth of all channels was restricted to 2 μm in order to prevent the disk-shaped erythrocytes from turning on their sides and traversing the constriction.

The basic design of the channel and reservoir is shown in Figure 2 below,

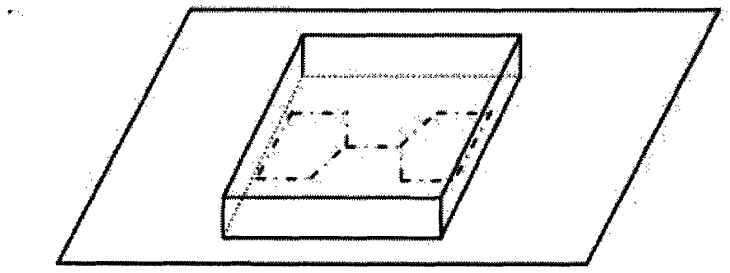

Figure 2 Schematic drawing of microfluidic channel design

Cells are injected into reservoir and forced to travel through the channels under pressure difference.

2.4.2 Pressure system

2 holes were punched at each side. One was used as cell inlet or outlet, and the other one was connected to pressure system. Pressure is provided by potential energy difference of PBS solution, since $p = \rho gh$, pressure difference could be calculated from height

difference. The pressure difference of PBS solution was set to simulate the human normal blood pressure, which varies from 80 to 120mmHg.

2.4.3 Cell visualization

Cell visualization could be achieved by a Nikon microscope with a Nikon x100 Superfluar objective for bright-field, differential interference contrast, and fluorescence imaging. A high speed camera could be coupled to record cell behavior.

2.4.4 Temperature control

The testing sample was mounted onto a hot plate. And it was set to be at 37°C, and 42°C, in order to simulate the normal human body temperature and fever temperature.

Test could also be done at room temperature without hot plate, which was presented in Shleby *et al*'s work.

2.4.5 *Plasmodium falciparum*-infected erythrocytes

P. falciparum parasites were maintained under standard condition [6] in a 2% suspension of human A+ erythrocytes in complete medium. Mixed-stage parasite cultures were synchronized by two consecutive sorbitol treatment [6] and harvested for analysis at the ring stage (0-6 h postsync), early trophozoite stage (16-21 h postsync), late trophozoite

stage (21-24 h postsync), and schizont stage (36-42 h postsync). Giemsa staining of thin smears showed >95% purity of the synchronized cultures. Each cell culture sample contained around 1% of infected erythrocytes [6].

2.5 Testing Results

Various behaviors of iRBCs in microfluidic channels were recorded and compared with normal RBCs, and possibly the similar behavior *in vivo* could be predicted. The microfluidic device could help providing useful information in study of how the iRBCs interact with environment.

2.5.1 Behavior of normal erythrocytes in Shelby's work

In all tests, there is no or little adherence between RBCs and channel walls observed. Furthermore, cells could easily pass through all channels, regardless of the channel size. Large deformation was observed while cells passing through microfluidic channels [6].

2.5.2 Behavior of infected erythrocytes in Shelby's work

Infected RBCs at different stages (ring-form stage, early trophozoite, late trophozoite, and schizont) were passed through microchannels of different sized, and their behaviors were studied individually.

As shown in Figure 3, in ring-form stage infection, RBCs could pass through channels with all size constriction; the behavior was similar to normal RBCs, but at a slower group velocity. In contrast, iRBCs at early trophozoite stage had difficulty when passing through channels with size less than 4μm, which indicates the lack of deformability at this stage. At late trophozoite stage, cells blocked the 4 and 2μm channels, which simulates the late trophozoites blockage in human capillary. And at schizont stage, RBCs exhibit a remarkedly increased rigidity, and they had difficulties in passing through channels with width less than 6μm.

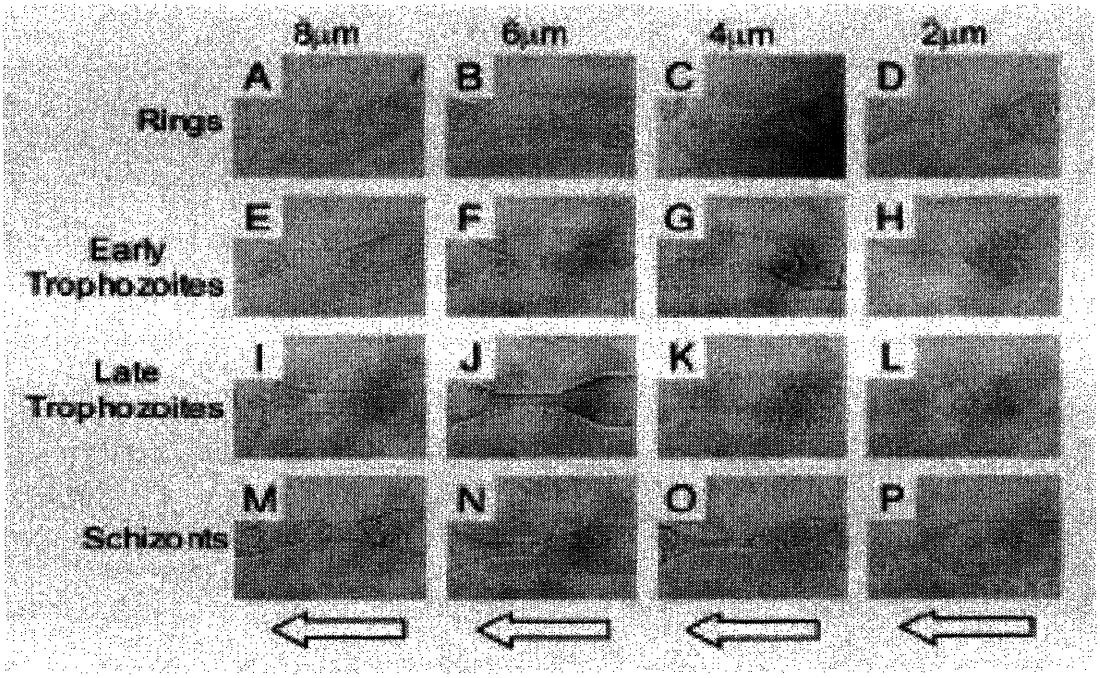

Figure 3 Microscopic image illustrate iRBCs induced channel blockage at different stages for different channel dimensions [6]

Besides, pitting was observed as iRBCs passing through the restricted portion of the channel. During pitting, the intracellular parasite is physically pushed back and eventually dissociated from the normal portion of the cell as the infected cell passing through the tiny blood vessels in the spleen [6]. It is believed similar process could be observed as iRBCs travels in human capillary.

Currently, studies only qualitatively illustrate the channel blockage caused by *p. falciparum* infected cells at different stage at room temperature. And further studies should be carried to study the cell behavior at different temperatures (37°C and 42°C). Besides, single cell behavior should be studied for *p. vivax, p. ovale,* and *p. malariae* infected RBCs, although they are rare compared with *p. falciparum* infected RBCs.

2.5.3 Interaction of iRBCs with ICAM-1

ICAM-1 is particularly important for mediating cytoadhesion in the brain, and adhsion of iRBCs to ICAM-1 is important for malaria pathogenesis *in vivo* [14]. In Antia *et al*'s work, it shows that ICAM-1 alone may be able to mediate the stable adhesion of iRBCs in micrlofluidic channels.

The experiment was carried at in a 50μm wide x 29μm tall microfluidic channels pre-coated with ICAM-1. Physiological shear stress (0.2 -2.5 Pa) [15] was applied. About 86% of iRBCs showed a rolling behavior on purified ICAM-1 coated channels rather than stationary binded to the channel or detaching. The trajectories of individual iRBCs

showed that the rolling was in a stepwise manner with periodic changes in velocity, as shown in Figure 4

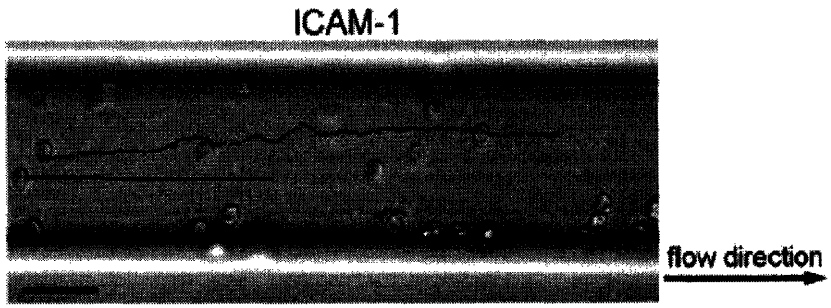

Figure 4 Trajectory of iRBCs rolling on ICAM-1

The variation in rolling velocity was studied as well. It was expected that without other mechanisms, most of the cells would increase the rolling speed while increasing the shear stress, untill finally detach from the channel. On ICAM-1, experiments revealed that population of iRBCs showed significant difference in variance of rolling velocities under different pressures, cells rolled in different fashions. At low pressure, the increase in rolling velocity was not obvious as increasing the shear stress, while at high pressure, the rolling velocity increased with increasing pressure.

The similar distribution of rolling velocities of iRBCs with the stability of leukocyte rolling velocities on selectins showed a possibility that it may be attributed to a shear-dependent increase in the number of receptor-ligand bonds per rolling step [16].

2.5.4 Adhesion of iRBCs in branched channels

In Antia's work, the adhesion and accummulation of iRBCs in branched channels were studied as well.

Branched capillaries are common in circulatory system, and in which the blood flow pattern changes, the wall shear stress changes as well [17]. The microfluidic branched channels were fabricated and used in this experiment to mimic the branching capillaries, and the cell behavior was recorded.

There were two different rolling behaviors of iRBCs on ICAM-1 at the sites of shear stress changes. Some cells did not show significant increasing in rolling velocity while approaching to the bifurcating point, while others displayed an significant increasing in velocity. By comparing with the channels coated with CD36, another ligand, these studies showed how changing shear stress due to the shape of a capillary could be critical in determining where cytoadhesion would likely occur.

2.6 Limitations and Further Developments

As shown in various works, the microfluidic devices could provide *in vitro* test, could manipulate single cells, and a portable device is hopefully to be made based on the microelectronics fabrication technology. However, limitations at current stage still exist.

In Shelby and Antia's work, a way to differentiate iRBCs and healthy RBCs is provided. However, improvements are needed.

Firstly, only qualitative behaviors are obtained so far, and it is still hard to determine whether a cell is infected or not, or at which stage the disease progress, if only one single cell is manipulated. The boundaries between infected and non-infected, as well as between different stages are still not clear. Quantitative results are needed in further work.

Secondly, it should be aware that not all malaria parasites will induce cell stiffening, and not all stiffening should be caused by malaria parasites. In sickle-cell anemia, the mechanical property of cells will change as well, and in this case, misdiagnosis should be avoided. Thus, this technology should be a complimentary diagnostic technology with many other diagnosis and disease analysis.

Lastly, in order to make and commercialize a portable device based on microfluidics, there are various issues on packaging and user interface. Those issues are not considered in works done so far, but they are unavoidable in the future.

Chapter 3 IP Strategy

Currently this technology is still at laboratory research stage, thus, the intellectual property (IP) of this device should be carefully managed due to long term development issues. Besides, it is hard to find a position in malaria market in near future to support research and development of diagnostic device, thus the resistance to enter the malaria market is quite high at current stage. Thus it is a concern for the duration of the patent protection of the microfluidic channel design. However, it is not quite a problem for the packaging such as user interface, casing, etc.. With good planning, it is possible to hold IP for this technology for a few years.

The first patent for the microfluidic device should be able to prevent the channel design to be copied. However, currently there are many microfluidic devices exist, although they are not purposely designed for malaria diagnosis, the channel designs should be similar to our technology. Thus in order to avoid this, in the patent for the final device, the actual dimension of the channels and the purpose of the device should be specified. Although the specified patent will not allow the expansion of the design into other microfluidics field, it could effectively prevent other technology entering this field. As a first patent, it should last for the entire lifetime of the technology, and the worst thing is that, it may expire even before the technology is fully utilized, and a careful plan is needed. Patent on packaging design should be followed and hopefully this patent could last for the needed time for malaria diagnosis.

Several factors should be considered before entering malaria market, e.g., current stage of drug and vaccine development, and the prevalence of the disease. Malaria mainly exists in third word countries, and if it does not expand into developed countries, there is no need to pursue the IP protection in those countries. If drug treatment still occupies a large share in malaria market, and if improper use of drugs exists, in order to prevent the increase of the drug resistance in endemic countries, the need for accurate diagnosis of malaria should increase.

However, the appearance of effective vaccune will be the ultimate killer of all malaria drug treatment and malaria diagnostic device. According to current research state, vaccines will not appear so fast although. The antigens and antibodies involved in *plasmodium falciparum* and/or other parasites are rather complex [12], which increase the difficulties of vaccine development. However, it should be no surprising that vaccines should appear ultimately, possibly in 20 to 30 years. Thus awareness should also be focused on the development of vaccines, and countries which will be affected by vaccines first. All judgments on these factors need a long term view.

As a conclusion, patent should be done carefully so that the diagnostic device could fully perform its task. The right time to release this device is important. If the device is released early, its economical value may not be fully utilized before the patent expire, and if it is released late, too many competitors may already exists, or even worse, malaria market may not exist due to appearance of vaccine. However, even then, by careful plan, patent on package could be used in other medical research field.

Chapter 4 Market Analysis

4.1 Malaria market

Human has been infected by malaria since 50,000 years before. Throughout recorded history, periodic fevers of malaria could be found, beginning in 2700 BC in China during Xia Dynasty [28]. The term malaria originates from Medieval Italian "*mala aria*", which means "bad air".

In 1880, the first significant advance of scientific studies on malaria was made, when a French army doctor, names Charles Louis Alphonse Laveran, observed parasites inside red blood cells of blood samples from malaria infected people. Then it was proposed that malaria was caused by this protozoan [29]. It was the first time that protozoan were identified as the causing malaria. The protozoan was called *plasmodium* by Italian scientists Ettore Marchiafava and Angelo Celli [30]. Then, a Britain, Sir Ronald Ross, who worked in Indian, finally proved in 1898 that malaria is transmitted by certain species of mosquito [31].

Now it is known malaria is caused by protozoan parasites of the genus *Plasmodium*, and the most serious forms of the disease are caused by *Plasmodium falciparum* and *Plasmodium vivax*. And there are some other related species, *Plasmodium ovale, Plasmodium malariae*, and *Plasmodium knowlesi* can also infect humans. This group of human pathogenic *plasmodium* species is usually called as *malaria parasites.*

Malaria parasites could be transmitted by female A*nopheles* mosquitoes. And those parasites, once injected into human blood stream, could multiply in red blood cells, causing several symptoms including fever, chills nausea, and even death.

The first effective treatment for malaria was from the bark of cinchona tree, which contains quinine. This natural product was used firstly by inhabitants of Peru, and then it was introduced to Europe during 1640s. And in 1820, the active ingredient quinine could be extracted from the bark as the widely accepted treatment [32].

Although malaria life cycle and blood stage were established about 100 years ago, currently there is still no effective vaccine available for malaria due to the plethora of various antigens present without mutation, and preventative drugs, such as prophylactic drug treatment, are simply too expensive for people living in endemic areas. Besides, most adults infected have a degree of long-term recurrent infection and partial resistance, thus those adults may be susceptible to severe malaria. Also, drug resistance is increasing.

Annually, malaria causes about 350-500 million infections and 1-3 million deaths in human, about one death every 30 seconds. Children under age of 5 years, and pregnant women, are especially vulnerable to malaria. Although efforts have been taken to reduce transmission of malaria and increase treatment, there has been little changes in which areas this disease prevalence. In fact, if the prevalence of malaria stays on its present upwards course, the death rate could double in next 20 years [1]. Since many cases of

malaria occur in rural areas, where people could not afford health care, precise statistics about malaria are unknown, thus it is believed the majority of cases are undocumented.

Besides, co-infection of malaria and HIV exists. Although it does not cause increased mortality, HIV and malaria do contribute to each other's spread. Malaria infection increases viral load, and HIV infection increases human's susceptibility to malaria infection.

Most malaria endemic areas are tropical and subtropical regions, in most developing countries of the Americas, Asia, and Africa. Malaria is more common in rural areas rather than in cities, which is contrast to dengue fever. Countries where malaria is endemic are shown in Figure 5.

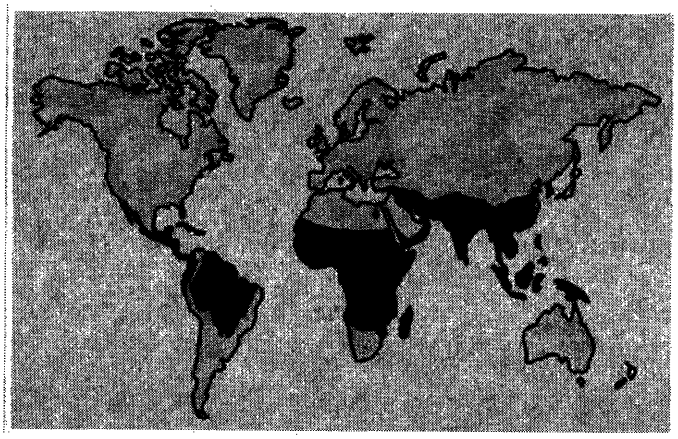

Figure 5 Areas affected by malaria, regions in dark indicate where malaria is common [11]

Besides health problem, malaria also induces a huge impact on economical development in malaria affected countries. A comparison of average per capita GDP in 1995, between malarious and non-malarious countries, demonstrates a fivefold difference (US$1,526 vs. US$8,268). Besides, in malarious countries, average per capita GDP rises only 0.4% per year from 1965 to 1990, compared with 2.4% per year risen in other countries [9]. These economical impacts are estimated including cost of health problem, labor lost due to thickness, decreased productivity due to brain damage from cerebral malaria, loss in investment and tourism.

Thus, as a social, economical, political and medical problem, there is a huge market existing for malaria. Since effective and cheap drug treatments, as well as vaccines are still under research, precise malaria diagnosis and drug testing tools are important, which could provide microfluidic device a potential market. In this document, market analysis in malaria diagnosis will be done first, including competitors existing in this market, and market size.

4.2 Existing technologies

Currently several testing methods have been developed over years for malaria diagnosis. Most widely used methods are optical microscopy for blood samples, polymerase chain reaction (PCR) looking for specific DNA sequence, rapid dipstick test (RDT) and enzyme-linked immunosorbent assay (ELISA) for particular protein characteristic of

malaria [10, 24]. Those testing methods have been developed with various degrees of success and disadvantages.

4.2.1 Optical microscope

Optical microscope is still the most economic, preferred and reliable diagnostic method for malaria. Under optical microscope, four major parasite species could be distinguished. Two sorts of blood films, with different film thickness, are traditionally used. Thin films are used for species identification and thick films allow the microscopist to screen a larger sample volume, thus increased the sensitivity of the test. However, microscopic diagnosis is difficult for low level or mixed infection, since parasites are hard to be differentiated in this condition, and personnel with high skills are required [10].

4.2.2 PCR

Polymerase chain reaction (PCR) is used to look for particular chain of DNA. In this test, samples could be any cell contains the particular DNA strand of malaria parasite. The concentration of the DNA strand could be magnisfied via chemical means, and then the magnification runs through electrophoresis in order to make visible result. Besides, fluorescent dyes could be incorporated to mark positive matches in the test.

However, the equipment used in PCR is quite expensive, and it is not affordable by most affected countries.

Recently, works have been down on making PCR into a lab-on-a-chip implementation. Small sample size is needed in this implementation and hence lower sensitivity. The effetiveness of it is still under research [10].

4.2.3 RDT

Rapid dipstick test was quickly accepted since it came into the market, due to its fast testing speed, low price and effectiveness. In this test, different species of malaria and/or malaria in general could be tested depends on the cost of the test. However, it is not as effective as its advertisement. In this test, *P.falciparum* could only be screened within 28 days as infected, which means that it is useless after months of infection.

The sensitivity of the test strongly depends on the environmental conditions, which lead to an increasing in testing cost. However, the effectiveness of this test is not improved as much as expected. Thus, although RDT is generally used now, despite its low cost, fast speed and certain level of sensitivity, there is not much academic research on it [10].

4.2.4 ELISA

Enzyme-linked immunosorbent assay (ELISA) is currently used for HIV detection, and it could also be used in malaria diagnosis. In this test, particular antigen or antibody, which is specific to the infection, is binded to a substrate. Then another enzyme is binded to the antigen or antibody. After all unbound materials are washed off, a third material is added

to bind the enzyme, and a significant change is produced, usually a color change. Through the color change infection and concentration of antibody could be determined. In this method, the concentration of antibody could be magnified by enzyme, since one enzyme bounded on one antibody could bind with several color-changing material, thus the sensitivity of this test is increased.

However, several hours of work and wet chemistry are involved in this test, which disqualify its use for malaria detection. And recently, lab-on-a-chip device based on this testing method is under research [10].

In Table 1, advantages and disadvantages, and costs, are compared for various malaria diagnostic methods.

Table 1 Overview of malaria diagnostic methods [10]

	Cost/test	Advantage	Disadvantage
Microscopy	US$0.30	Economical, easy and reliable	Operator dependent
PCR	US$15.00	Effective	Slow and expensive
RDT	US$1.00	Fast and economical	Low effectiveness
ELISA	US$10.00	Effective	Slow, expensive, lab required

4.3 Market Analysis

Malaria affected countries are tropical and subtropical regions. However, not all countries would like to spend on malaria diagnostic devices, yet could afford the diagnosis cost. The potential market for malaria treatment could be estimated from WHO statistics of malaria cases of each year and GDP of each country. It could be assumed that 30% of actual infection among all malaria diagnosis, thus the total number of test required could be calculated. Countries need little number of tests could be removed from the list. Then, in order to estimate whether the country could afford the test, it could be assumed that 4% of an endemic country's GDP is spend on healthcare, and 40% of which is spend on malaria. Among the money spend on malaria, 1% is actually spend on malaria diagnosis. Thus, whether a country could afford malaria diagnosis, and number of tests it needs could be viewed.

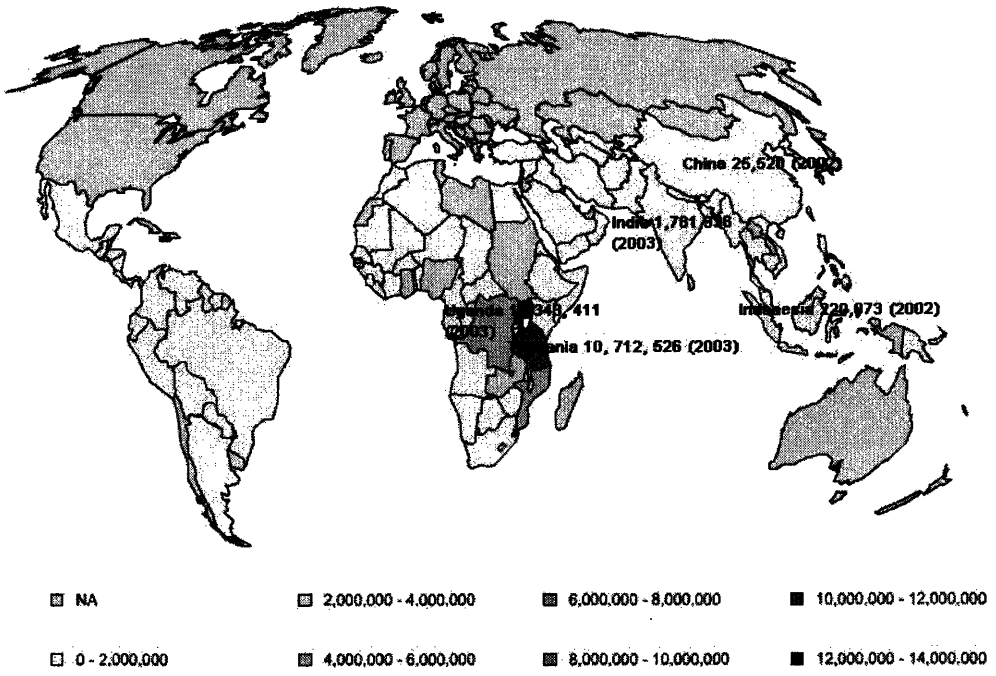

Figure 6 Malaria cases (Data from recent year available) [18]

Microfluidic device, as new technology, could not be widely accepted at the beginning. However, there may still be markets in military and foreign aid organizations, which are willing to support new technology research. Also, countries like India, Indonesia and China could be the starting market for microfluidic device since they have the capacity to afford the device. However, larger markets are expected then. The potential market is in African.

Table 2 Comparison of 5 countries with malaria cases each year and GDP [18,19]

Countries	Malaria cases	GDP
China	25,520	7,262
India	1,781,336	3,319
Indonesia	220,073	827.4
Tanzania	10,712,526	23.71
Uganda	12,343,411	39.39

As analyzed, there are several competitors existing in malaria diaganosis market, and they have achieved various degree of success, yet improvements are needed. By comparison, microfluidic device could provide fast, economical and sensitive detection. However, the effectiveness of this technology is still under research. Current research has only been done on *P.falciparum*, and it has been proved that different stages of life cycle of this parasite could be differentiated by using microfluidic device, further researches are still needed for other species of malaria parasite.

Vaccines, pesticides, drug treatment, and education, on the other hand, are competitors for all malaria diagnostic device. Although there are plethora of different antigens presented, which increases the difficulty of vaccine development. It should be no surprising that vaccines will appear in next 20 to 30 years, and then there is no malaria market.

Chapter 5 Business model

Various options for commercialization of this microfluidic device for malaria diagnosis will be presented, and those business models are based on the assumption that a testing device has been made or will be made soon. Currently, it is still too early to commercialize this device, as elaborated in the previous chapter, more tests are needed and limitations at current stage exist. However, based on the assumption, the business model could be analyzed.

The business is assumed to be started in China, in which the potential market of diagnosis of malaria is 85,067 cases per year (according to the data obtained in 2002, and under the assumption that 30% of people who go to clinics are diagnosed as malaria infected). Fundings could be obtained from government, and various foundations which have a strong concern on the public health, especially HIV, TB, and malaria. Foundations such as Bill & Mellinda Gates foundation, Welcome Trust, and Interfaith Center on Corporate Responsibility could be approached.

Under these assumptions, there are various natural ways to extract market values for this device.

5.1 IP Company

In this model, the main source of revenue is the licensing of this technology, thus, continuous development in IP is quite critical and it is the main expense for this business model. Licenses must be carefully controlled, a wide license will impede further license in other field where it may be more lucrative, and a narrow license could not effectively block other technology into this field. Besides, it is better to license the technology to a manufacturer which performs a high value step in order to get a higher royalties return. For example, in order to license the microfluidic device, it is better to license it to an entire device manufacturer rather than a wafer manufacturer, since 1% return of the price of the device is definitely higher than 1% return of just a wafer.

To estimate the cost involves in this business model. Firstly, fees for patent filing and maintenance are one of the major expenses. Then it could be assumed that the company is just a small team of 3 to 5 engineers at US$18,000 per year for salaries and benefits. Besides, 10 to 15 support personnel, such as technicians and administrative assistants, at US$3,000 per year for each should be needed. Thus, it brings personnel costs to US$21,000 per year. The materials and facilities costs are assumed to be about US$206,000 per year. Thus for a small development company, total cost per year is about US$217,000.

It is possible that there is no actual licensing for operating for a few years; it is also possible that the cost increases as the prototypes end up requiring a particular rare and

hard-to-be-realized technique to be manufactured. The market for malaria detection is not so big and relatively hard to access now. Thus a business model using this strategy is probably struggle.

5.2 Device Manufacturer

In this model, a plant is built to make the device and sell it. It is a time consuming business model; however, all assembly levels are under control by the manufacturer. In the process of fabrication of such device, various steps, such as wafer fabrication and soft lithography, have been commoditized already. Thus, it is reasonable to assume that it would take about 5 years to build the plant and train staffs to produce devices at the price competitive with existing technologies. However, if it takes about 5 years to build the plant, then there are about only 10 years left for the device under patent protection, if there is no new IP resets the IP protection time. It would also assume that within the 5-year time, there is no new competing technology appears in the market. However, this assumption is unreasonable. Thus, at this stage, there is no meaning to estimate the cost for this business model.

5.3 Service Company

In this model, diagnostic devices are sold to labs, drug manufacturers and clinics for malaria detection. Manufacturers are outsourced, but development of the device, as well as quality control, is important. Thus, at least two additional people are needed for device

testing and supplying, at an annual pay of US$3,000 and US$2,500. Thus the total cost on personnel is US$63,000

It is estimated that the malaria market in China is 85,067 cases per year. And the production volume is set to be at 80,000 pieces of device per year. The total cost, including raw material, land, power and personnel is estimated to be US$533,916 per year. And the cost per piece is US$6.67. In order to breakeven, the price per device should be higher than this value. By comparison with the prices of existing technologies (Table 1), the price should be set between US$6 (breakeven in first year) and US$10 (Price of ELISA). Microfluidic technology advances in terms of effectiveness and accuracy by comparing with RDT, and it advances in terms of cost and portable by comparing with ELISA and PCR.

However, whether there is enough market for this device, and will market actually support this device, are still waiting to be seen. And they all depend on the effectiveness of this device.

Chapter 6 Conclusions

A fast, portable and economical malaria diagnostic device could be developed from microfluidics, and it could open up the use of microfluidic technology in disease diagnosis. However, there are still many technical issues to overcome. So far only one of all four species of human malaria parasites has experimental data at room temperature. More tests should be carried out at body temperature and fever temperature and for other three species. Issues related to packaging and user interface, which transfers the mechanical response to other signals, e.g., electrical signal, as well as protecting, are under consideration. If all the technical issues are resolved, this device should be able to be economized for malaria diagnosis.

The timeframe of device development is hard to estimate, since the first prototype of this device has not been made yet. Thus IP should be carefully designed for long term protection. A good IP strategy could effectively protect the design of this device, yet fully utilize it.

The best business model adopted for this device is to establish a service company. Several business models are considered. It will be hard to establish an IP company due to the difficulty of new innovation; it will also be hard to establish a manufacturing company since it will take a long time to build all infrastructures. However, the effectiveness of service company need to be tested by the market.

The existing malaria market is not so large, and there are already many competitors in the market already. The microfluidic device has competence in the market in terms of portability, price, analysis speed and effectiveness. However, the quality of the device will be important and an industry who has experience in fabricating and packaging microfluidic devices will be a good partner.

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Appendix A. Statistics of Malaria Endemic Countries (Case/Year)

Rank	Country Name	Number	Year
	Global	**408,388,001**	**2002**
1	Uganda	12,343,411	2003
2	Tanzania (United Rep. of)	10,712,526	2003
3	Mozambique	5,087,865	2003
4	Congo (Dem. Republic of)	4,386,638	2003
5	Ghana	3,552,869	2003
6	Sudan	3,084,320	2003
7	Malawi	2,853,317	2002
8	Nigeria	2,608,479	2003
9	Madagascar	2,114,400	2003
10	Zambia	2,010,185	2001
11	Burundi	1,808,588	2002
12	India	1,781,336	2003
13	Burkina Faso	1,451,125	2002
14	Angola	1,409,328	2002
15	Zimbabwe	1,252,668	2002
16	Senegal	1,120,094	2000
17	Guinea	889,089	2000

18	Rwanda	856,233	2003
19	Mali	809,428	2003
20	Benin	779,041	2001
21	Liberia	777,754	1998
22	Myanmar	716,100	2003
23	Niger	681,707	2002
24	Cameroon	664,413	1998
25	Afghanistan	591,441	2003
26	Ethiopia	565,273	2003
27	Namibia	444,081	2003
28	Togo	431,826	2001
29	Sierra Leone	409,670	1999
30	Cote d'Ivoire	400,402	2001
31	Chad	386,197	2001
32	Brazil	379,551	2003
33	Yemen	265,023	2003
34	Indonesia	220,073	2002
35	Guinea-Bissau	194,976	2002
36	Mauritania	167,423	2002
37	Colombia	164,722	2003
38	Gambia	127,899	1999
39	Kenya	124,197	2002
40	Pakistan	122,560	2003

41	Central African Republic	95,644	2003
42	Solomon Islands	90,606	2003
43	Gabon	80,247	1998
44	Peru	79,473	2003
45	Eritrea	72,023	2003
46	Cambodia	71,258	2003
47	Papua New Guinea	70,226	2003
48	Sao Tome and Principe	63,199	2003
49	Bangladesh	56,879	2003
50	Ecuador	52,065	2003
51	Philippines	43,644	2003
52	Viet Nam	37,416	2003
53	Swaziland	36,664	2003
54	Thailand	35,076	2003
55	Timor Leste	31,819	2003
56	Venezuela	31,719	2003
57	Guatemala	31,127	2003
58	Guyana	27,627	2003
59	China	25,520	2002
60	Somalia	23,349	2003
61	Botswana	22,418	2003
62	Bolivia	20,343	2003
63	Lao People's Democratic Rep.	18,894	2003

64	Congo	17,122	1998
65	Iran (Islamic Republic of)	17,060	2003
66	Korea (Dem. Peo. Rep. of)	16,538	2003
67	Vanuatu	15,240	2003
68	Suriname	14,657	2003
69	South Africa	13,446	2003
70	Equatorial Guinea	12,530	1995
71	Sri Lanka	10,510	2003
72	Honduras	10,122	2003
73	Haiti	9,837	2003
74	Nepal	9,394	2003
75	Turkey	9,182	2003
76	Panama	9,000	2003
77	Nicaragua	6,812	2003
78	Malaysia	5,477	2003
79	Tajikistan	5,428	2003
80	Djibouti	5,036	2003
81	French Guiana	3,823	2003
82	Mexico	3,819	2003
83	Bhutan	3,806	2003
84	Comoros	3,718	2001
85	Paraguay	1,392	2003
86	Dominican Republic	1,296	2003

87	Korea (Republic of)	1,107	2003
88	Belize	928	2002
89	Costa Rica	718	2003
90	Saudi Arabia	596	2003
91	Azerbaijan	480	2003
92	Kyrgyzstan	465	2003
93	Georgia	308	2003
94	Iraq	303	2003
95	Cape Verde	143	2000
96	Argentina	122	2003
97	El Salvador	85	2003
98	Algeria	52	2002
99	Uzbekistan	33	2003
100	Mauritius	22	2002
101	Armenia	8	2003
102	Oman	6	2003
103	Morocco	4	2003
104	Syrian Arab Republic	2	2003
105	Turkmenistan	1	2003

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Appendix B. Costs of Land, Materials and Labor Hood

Item	Cost (US$)
High Speed Camera	US$166,100.00
8 Inch Wafer	US$332.00
Mask, Design and Drawing	US$1661.00
Wafer Dicing	US$33.22
NRE Cost	US$664.4
PDMS	US$160/500g kit
Coverslip	US$14.58/1000 pieces
Salary of PhD in China	US$375/month
Salary of Technician in China	US$250/month
Land cost in China	US$204.12/$m^2$
Fee of domestic patent filing in China	US$1046/year for first filing