



The Concept of Making On-Chip Thermal Cycler for RT-PCR Using Conjugate Heat Transfer in Diverging Microchannel

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Abstract

Covid-19 is pandemic to which the world is fighting. Various precautionary measures are being imposed all over the world which is affecting the routine life of an individual and also the economy worldwide. Although, a definite vaccine is still not known to medical science but they are able to distinguish Covid-19 from the other types of flu. Presently this is being done by detecting the SARS-CoV-2 virus using RT-PCR technique as recommended by the World Health Organization (WHO) (WHO, Geneva, 2020). Reverse Transcription Polymerase Chain Reaction (RT-PCR) is a nucleic acid amplification test that converts the RNA into DNA and subsequently amplifies the specific DNA targets. This method was already being employed to detect the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) (Emery et al. in *Emerg Infect Dis* 10(2):311, 2004). The entire process of DNA amplification takes place in three steps: denaturation, annealing, and extension for which the sample is required to be maintained at constant temperatures of 95, 55 and 72 °C, respectively. This article introduces the technology to achieve a constant temperature which can be tweaked to develop on-chip RT-PCR.

Keywords RT-PCR · Diverging microchannel · Conjugate heat transfer · Constant wall temperature

What is the Technology?

This technology demonstrated a way to maintain the constant wall temperature on a microdevice. It employs the concept of conjugate heat transfer in the diverging microchannel. It is based on the idea of redistribution of heat flux at the solid–fluid interface. When constant heat flux is applied at the outer wall of the diverging microchannel with solid to fluid thickness ratio greater than two, then redistribution of heat flux occurs at the inner wall of the microchannel.

Figure 1c shows that the redistribution of heat flux at the solid–fluid interface is more in diverging microchannel compare to that in uniform cross-section. This leads to a more uniform temperature in diverging microchannel compare to that in uniform cross-section (see Fig. 1d). The effect of

various geometrical and flow parameters along with the thermophysical properties on constant wall temperature conditions were studied in detail and presented earlier (Duryodhan et al. 2016, 2017). This study also proposed the analytical model which can help in designing the different diverging microchannels leading to different constant temperatures as per requirement.

Novelty in the Technology and for What was it Developed?

In several pathological processes, biological samples required to maintain above the room temperature (Hung et al. 2005; Wan et al. 2013; Regan et al. 2008). At conventional scale, this can be done by keeping the samples in an incubator, thermal cycler or employing phase change materials etc. However, large sample size, loss of samples, large reaction time are some of the disadvantages associated with the conventional methods which creates the hindrances in diagnosis. On the contrary, working with the small sample size provides the advantage of quick diagnosis at minimum cost. Microdevices are used to harness the advantages of using small sample volume. Integration of these portable

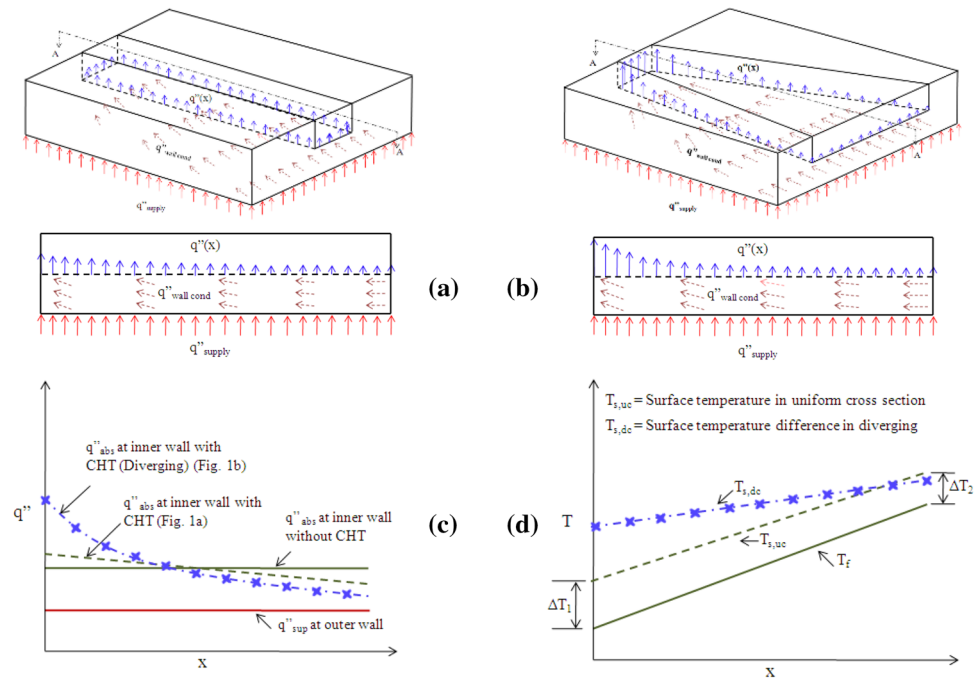
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Fig. 1 Schematic explaining the concept of achieving constant wall temperature in **b** diverging microchannel with reference to **a** uniform cross section microchannel, **c** heat flux redistribution, **d** temperature variation along flow direction



microdevices with conventional heating systems is cumbersome. Therefore, heating of microdevices can be achieved by using microheater (Selva et al. 2010). Fundamentally, microheater-based heating is equivalent to provide constant heat flux and therefore, non-uniformity in the temperature distribution is inherently associated with this method. Various designs of microheaters have been explored by the research community in order to achieve the constant temperature as summarized by Miralles et al. (2013). Novelty of this design lies in the fact that a constant temperature boundary condition is achieved while constant heat flux is supplied at the bottom wall. Otherwise, this would require the outer chamber with phase change material and a mechanism to liquefy/solidify the material in order to use the same in closed loop. This method was developed and proposed to use for heating biological samples where constant temperature requirement is the matter of utmost important. A proof of concept for of this technology have been presented earlier using numerical simulation (Duryodhan et al. 2016).

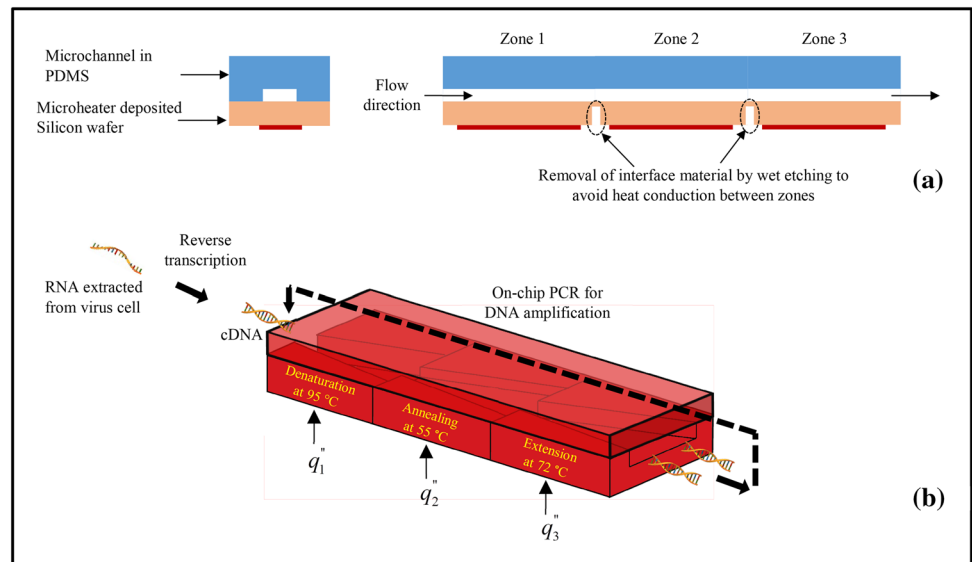
How the Technology Can Be Tweaked to Make it Relevant to Covid-19?

This technology can easily be employed to develop a micro-device for RT-PCR process which is a proven technique used to detect SARS-CoV-2 (Emery et al. 2004; Corman et al. 2020). RT-PCR is a cyclic process of DNA amplification where each cycle consists of three steps namely denaturation, annealing and extension for which the sample is required to be maintained at constant temperatures of 95,

55 and 72 °C respectively. The technology discussed above can be used to design diverging microchannels of different geometrical configuration so that each will provide different constant temperatures as mentioned earlier. One can use the analytical model proposed by Duryodhan et al. (2016) to decide on geometrical and flow parameters. It can be fabricated on thermally conducting substrate such as silicon, glass or quartz wafer. We propose to fabricate the channel in PDMS using soft lithography which can be covered with microheater deposited silicon wafer. Care should be taken to insulate one zone from the other by etching the silicon from the interface region of two subsequent zones as shown in Fig. 2a. This will reduce heat conduction from one zone to other thereby maintaining the constant temperature in each zone.

These diverging microchannels can be connected in series and supplied with different constant heat flux as shown in Fig. 2b. Collected clinical sample will be used to extract the RNA from the SARS-CoV-2 virus cell which is further converted to complimentary DNA (cDNA) using reverse transcription. A limited number of DNA along with primer and reagents will then pumped through the microdevice consist of three zones of constant temperatures as shown in Fig. 2b. The sample needs to recirculate through zone 1 as indicated by dashed line in Fig. 2b. Number of cycles will be decided based on the amount of amplification required for the further processing of the sample. A diagnosis methodology recently proposed by Corman et al. (2020) can be employed for further processing of sample. Conventional methods will require separate post processing to provide the qualitative results and also costly, whereas microdevice based RT-PCR

Fig. 2 Schematic of **a** micro-channel assembly and **b** application of microdevice in RT-PCR



method will have the advantages of less sample size, speed, portability and cost-effectiveness (Walker-Daniels 2012).

Timeline and Resource Envisaged for this Conversion

Steps involved in the conversion of this technology for RT-PCR application can primarily be divided into two parts: fabrication of microdevice and lab testing for polymerization of DNA sample. Around 3 months will be required to accomplish the first task, i.e. to design and fabricate the microdevice. Whereas, next 3 months will be spent for second task which includes testing of microdevice for amplification rate and subsequent optimization of parameters and comparing the results with that of conventional thermal cyclers. Therefore, around 6 months will be required to tweak this technology to make it relevant to Covid-19.

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