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Thermal Performance Simulation of an LTCC Micro-Reactor for RT-PCR in Detection of SARS-Cov-2 Detection

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Abstract
In present paper, thermal simulation of LTCC based micro-chamber has been performed which is a key part of RT-PCR device. The RT-PCR device plays an important role in SARS-CoV-2 testing. The rRT-PCR system requires three different thermal cycles for DNA amplification which takes part in detection of SARS-CoV-2. The thermal cycle can be equipped using a heater structure in the chamber. A new LTCC based technique to develop micro-chamber has been designed and simulation has been performed using COMSOL to optimize thermal properties. Temperature distribution for a micro-chamber at three different voltages has been simulated. The temperature distribution is more uniform in micro-chamber with a buried metallic layer in comparision to micro-chamber without a metallic layer. The heater and temperature sensor were located outside the reaction chamber. A platinum based pattern as PTC temperature sensor is used in temperature measurement.

I. INTRODUCTION

The aim of the research is to develop a faster, low power and miniaturize reaction chamber to diagnose severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). Mainly, seven types of human corona viruses (HCoVs) are known in which commonly detected are four HCoVs such as OC43, 229E, NL63 and HKU1 while three other are SARS-CoV, MERS-CoV, and SARS-CoV-2 as indicated in Fig. 1 [1]. These three viruses increased the death rates in human beings.

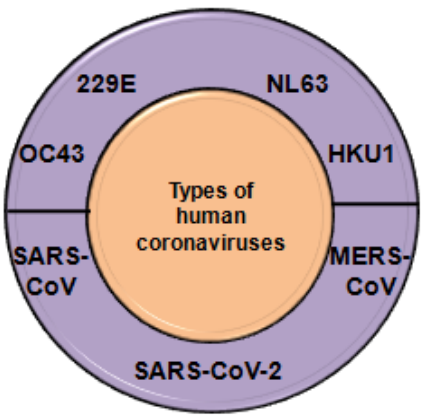


Figure 1: Types of human corona-viruses

SARS-CoV-2 has been identified in Wuhan, China and which has now spread in many countries as global epidemic [2]. In the present situation, the disease has spread in many countries and America is bearing the main center of mortality compared to other countries. The virus was first discovered in December 2019 and named COVID-19 or 2019-nCoV by World Health Organization (WHO) [3, 4]. International Committee of Taxonomy of Viruses (ICTV) has decided the name SARS-CoV-2 originates as a sister to SARS-CoVs in the documentation [5]. WHO decides that the symptoms for patients are severe acute respiratory infection (fever, dyspnea, and cough) and need admission to hospital [6, 7]. It is a person-to-person transmissible virus and transmitted through respiratory droplets from coughs or sneezes as shown in Fig. 2.

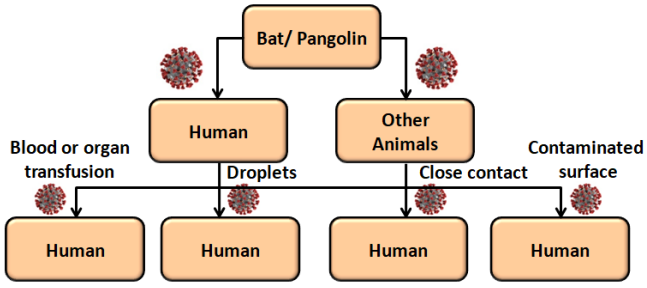


Figure 2: Transmission of SARS-CoV-2

Structure of virus

The COVID-19 is developed from four main proteins i.e. S, M, E, and N [8, 9, 10, 11]. The structure of SARS-CoV-2 is illustrated in Fig. 3.

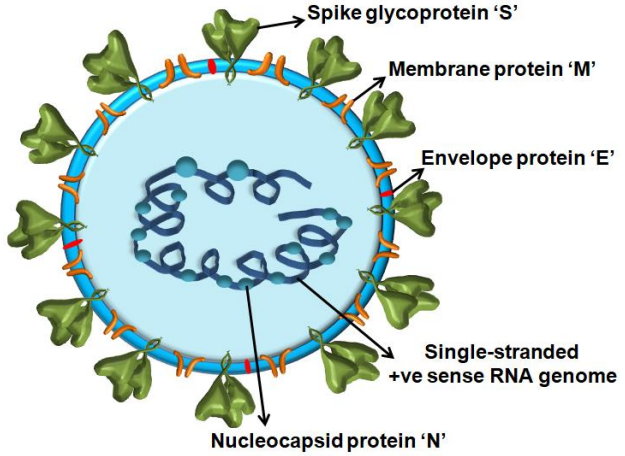


Figure 3: Structure of SARS-CoV-2

The spike protein 'S' developed big structured spikes on surface. The protein 'N' is connected with ribonucleic acid and creates nucleocapsid inside the envelope. The 'N' protein is mostly involved in replication cycle of COVID 19 infection. The nucleocapsid, which holds viral nucleic acids, is made up of protein shell. It also wrapped RNA (ribonucleic acid) as a virus envelop. The viruses are surrounded by wrapper which contains viral nucleocapsid and the nucleocapsids are helical in structure [12]. The diameter of Coronavirus is approximately 120 nm and it looks like coronal shape due to Club-shaped glycoprotein spikes 'S'. The envelope structure depends on membrane protein 'M' which is found in large numbers. The protein 'E' is small in structure and it is also helpful in formation of envelope of the virus. Chunbao Xie has reported the different characteristics of 19 suspect cases [13]. In the given report, the CT scans and nucleic acid amplification tests (NAAT) results are used to identify the virus.

II. DIAGNOSIS METHODS FOR COVID-19

The most important challenge for hospitals and health centers is a fast and easy testing of COVID-19. The lack of laboratory facilities and health infrastructure makes it complex in prediction when the epidemic will be on its peak.

There are three different testing that are popular for COVID 19 testing i.e antibody (serology) testing [14], antigen testing [15] and rRT-PCR [16]. Antigen is faster and inexpensive than others but it has limitations. The test can be performed through the proteins from virus. It shows less accuracy and therefore it may not be proper solution to diagnose COVID 19. The next is antibody test disclose if someone has previously been showing an infection, by detecting antibodies in their blood or serum. This test is performed on antibodies while the antibodies will be occurs after 5-10 days of infection, so it cannot be used to identify current disease. While in comparison, the Nucleic acid amplification testing (NAAT) i.e. rRT-PCR has reported better results. It is a better diagnostic tool to recognize and quantify any critical pathogenic agents [16, 17]. The researchers are working for fast and accurate detection kits for 2019-nCoV.

III. DIAGNOSIS PROCESS OF RT-PCR AND EFFECT OF THERMAL CYCLE

The throat swabs, cough, blood and respiratory tract secretions are used in detection of nucleic acids of 2019-nCoV [18]. In working process of real time RT-PCR, first the sample is collected using BLS or EPS method. The swap is a mixture of cells, virus and other microbes. It is passed through chemical process to extract RNA from sample. The extracted RNA is reverse transcribed to DNA then insert fragments of viral DNA. In presence of virus, the fragments attached to the viral DNA. This mixture is taken for testing in RT-PCR machine. The amplification for specific fragments of DNA is performed using RT-PCR. The PCR takes three temperature cycles to identify COVID 19 patient, N gene play an important role which is carries direction for making nucleocapsid protein. The RT-PCR amplifies many copies of segment of the N gene in 35-40 thermal cycles [19]. A successful polymerase chain reaction is based on the temperature to provide DNA amplification process. PCR can generate numbers of DNA replicates on different temperatures at fixed time interval. The heating and cooling rate in micro-chamber proposed by PCR is ~ 3˚C/s and ~ 2.5˚C/s respectively. The initial denaturation process takes ~5 min at 95°C, then other 30 to 40 cycles proceed for 30 sec time periods on different temperature ranges i.e 95˚C, 55˚C and 72˚C. The final extension cycle takes 5 min to proceed (Micro-fabricated PCR-electrochemical device for simultaneous DNA amplification and detection). The first process is denaturation in which proceed at high temperature i.e 90 – 95˚C. Here, hydrogen bonds are broken due to high temperature. The second step in Annealing of primers which is mainly depends on length of nuceleotide and temperature provide to bond. The annealing temperature should be 5˚C less then DNA melting temperature. Normally its range is 50 to 70˚C. The process of thermal cycle is shown in Fig. 4 [20].

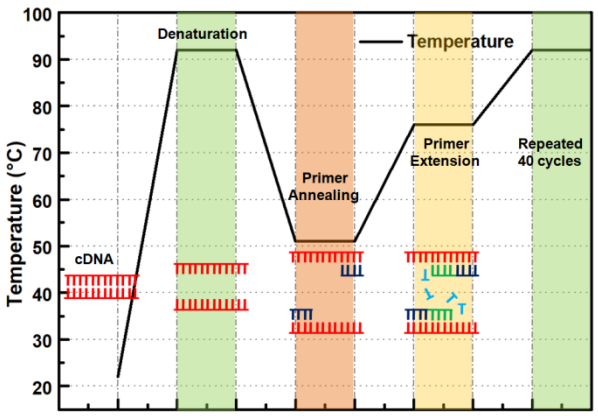


Figure 4: Thermal cycle graph for SARS-CoV-2

The different methods which provide the required temperature for nucleic acid amplification are Infrared heating [21], Thermoelectric [22] and Microwave [23]. The RT-PCR becomes a quickly identify and quantify system for dangerous pathogenic viruses [24 -26]. This is highly sensitive, faster and precise technique for detection of the COVID-19. It provides fast and accurate results if its heat transfer rate is enhanced. By rising heat transfer rate and reducing thermal mass the speed of PCR test can be improved [27, 28]. Numbers of techniques with different substrate materials are used to develop PCR micro-chambers or micro-channels such as glass [29, 30], polydimethylsiloxane

(PDMS) [31, 32], perfluoroalkoxy-modified polytetrafluoroethylene (PFA) [33] and stainless steel [34].

IV. DESIGN OF LTCC BASED MICRO-CHAMBER

The temperature distribution in RT-PCR micro-chamber has been simulated using COMSOL Multiphysics software. To develop better heating and cooling rate in micro-chamber, LTCC based substrate with platinum as heating material is selected for simulation [35]. The LTCC is composition of alumina and glass materials and it shows better resistance in respect of chemical solvents. This feature is valuable for chemical and biochemical reactors. The micro-chambers or micro-fluidics developed using LTCC are useful in simplify essential biological operations such as DNA amplification, cell capture, pumping, etc. The LTCC is a stable technology due to its easy surface-modification in green stage, heating and cooling properties [36,37]. The platinum has high thermal conductivity, and high temperature stability which is suitable for heating purpose. The different temperatures are provided through micro-heaters. The micro-heaters work on joule heating principle by applied voltage. The LTCC based micro-heaters provide stable temperature with applied voltage. The stability of LTCC based micro-heater has been characterized in Kulhari et. al. 2020.

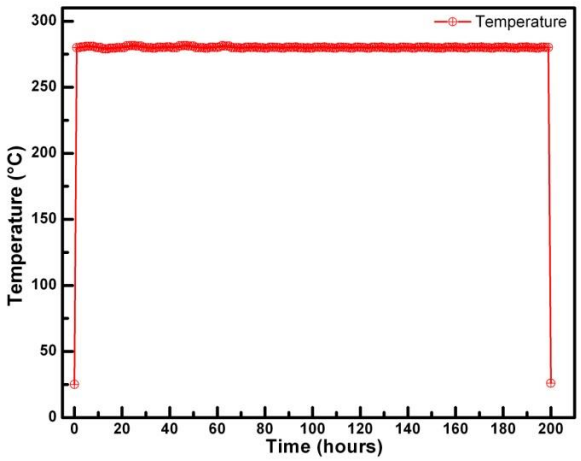


Figure 5: Stability test of LTCC based micro-heater for 200 hrs at 280 °C [38]

By increasing heat transfer rate and reducing thermal mass, the PCR test can be performed faster. The advent of LTCC is helpful in reducing thermal mass, power consumption and increasing heating/cooling rates. Northrup et al., 1993 was initiated the first stationary chamber type PCR module [39]. Here, the solution of PCR/ droplet is stationary and chamber's temperature is varied sequentially with respect to required time. Due to enhanced properties of LTCC, the schematic diagram of LTCC based micro-chamber is shown in Fig. 6.

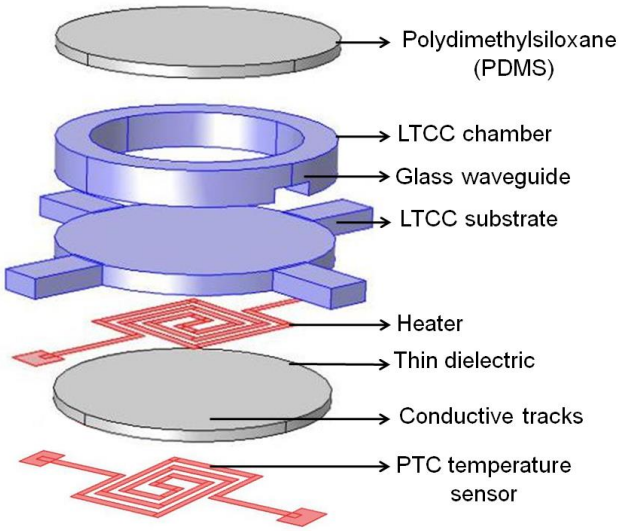


Figure 6: schematic diagram of LTCC based micro-chamber

The platinum heater is sandwiched between LTCC tapes below the reaction chamber and a dielectric thin layer. The sandwiched heater takes low losses as compared to surface heater. A PTC temperature sensor of platinum is patterned on dielectric layer to measure and control the temperature of chamber. The platinum pattern shows linear variation in resistance with temperature [40 - 42]. The polydimethylsiloxane (PDMS) is selected as a covering material due to its biocompatible, non-toxic, non-flammable and transparent properties. The PDMS sheet of required dimension can be bonded with LTCC [43, 44]. The reaction chamber is designed in circular shape in order to smooth the movement of the liquid and to avoid residual in the chamber. The micro-hotplate distributed the temperature uniformly and a PCR needs stable temperature cycles. Therefore, the measurement and control of temperature for micro-hotplate is a very important task. The temperature sensor built with micro-heater observes the temperature and control the temperature of reaction chamber using a programmable control system.

V. MATHEMATICAL ANALYSIS

The heat losses and joule heating are illustrated by the given equations:

$$\sigma(T)(\nabla V)^2 = P_{cond} + P_{conv} + P_{rad} \quad (1)$$

where: P_{cond} = conduction
P_{conv} = convection
P_{rad} = radiation power losses
$\sigma(T)$ = electrical conductivity

The power losses through the structure can be defined as:

$$P_{cond} = -\nabla.(k\nabla T) \quad (2)$$

where: k = thermal conductivity
∇T = change in Temperature

The convection losses are defined as:

$$P_{conv} = \frac{\left\{\frac{[0.104(T - T_a) + 4.129\,x\,10^{-4}(T - T_a)^2]}{5x\,10^{-7}}\right\}}{t} \quad (3)$$

where: T = temperature
T_a = ambient temperature
t = thickness of substrate.

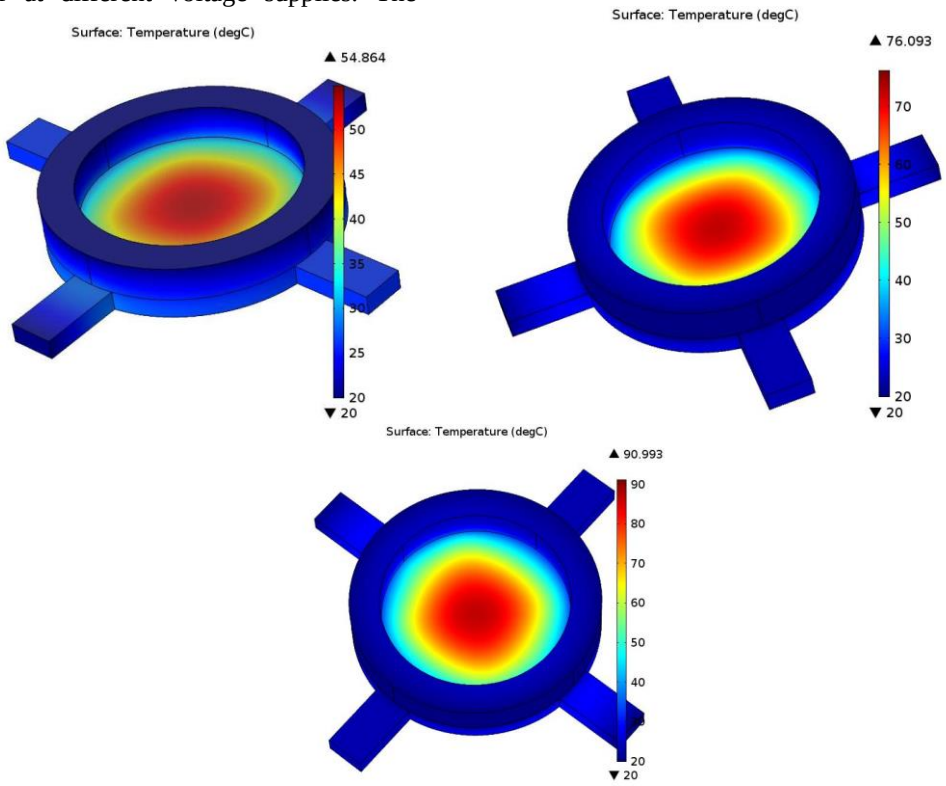
Power loss through radiation is described as follows:

$$P_{rad} = \frac{2\sigma\varepsilon(T^4 - T_a^4)}{t} \quad (4)$$

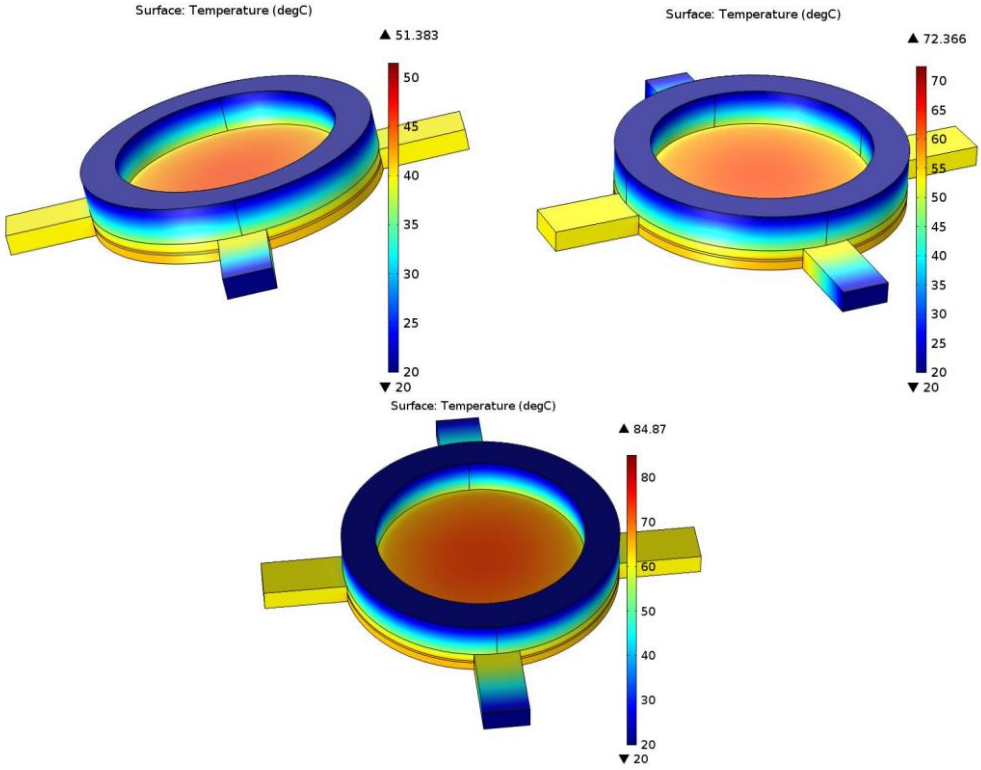
where emissivity ε is 1 and Stefan-Boltzmann constant σ is 5.67×10^{-8} W/m^2/K^4.

VI. SIMULATED RESULTS OF LTCC BASED MICRO-CHAMBER

The thermal simulation has been performed for LTCC based micro-chamber at different voltage supplies. The thermal simulation is performed for the designed micro-chamber (without metallic layer) is shown in Fig. 7 (a) on applied voltages of 1.5 V, 2.5 V and 3V. To improve the thermal distribution in micro-chamber, simulation is performed with a metallic layer sandwiched between LTCC tapes below the reaction chamber. The simulation results are shown in Fig. 7 (b) with applied voltages of 1.5 V, 2.5 V and 3V. The Fig. 7 indicates that temperature distribution is more uniform in micro-chamber with metallic layer while it consumes some more power as compared to without metallic layer micro-chamber. Therefore, the temperature uniformity can be improved by printing a buried metallic layer between two LTCC layers.



(a)



(b)

Figure 7: Temperature distribution for a micro-chamber at three different voltages (a) without a metallic layer (b) with a buried metallic layer

The temperature vs applied voltages graph in Fig. 8 indicates the required temperature for micro-chamber that can be achieved through applied voltage variation.

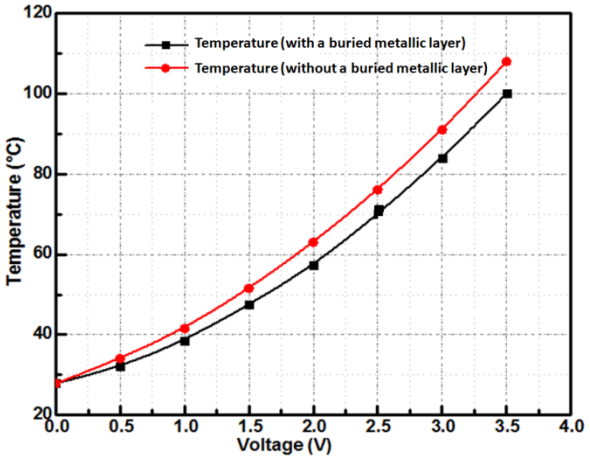


Figure 8: Temperature variation on non buried metal layer and buried metal layer

The temperature control for micro-chamber is very important. Therefore, a Platnum based pattern is printed on dielectric layer which covers the heater pattern. The resistance of the pattern will vary with variation in temperature which will be controlled by temperature control circuit. The calibration of different temperatures in micro-chamber with resistance variation is an important part to control the temperature of micro-chamber.

VII. FABRICATION METHODOLOGY

The fabrication of micro-channel structure was carried out by following LTCC fabrication steps. Vias were created on LTCC green tapes using via punching system. LTCC tapes with desired thickness were stacked, tagged and bag sealed. Subsequently, lamination and firing processes were carried out. Screen printing for heater pattern (platinum) was carried out to fabricate a micro-hotplate. Firing was performed after each printing process [38].

VIII. CONCLUSION

The results of simulation proved that LTCC based micro-chamber is a very suitable device for RT-PCR. The temperature distribution is more uniform in micro-chamber with a buried metallic layer in comparision to micro-chamber without a metallic layer. But the micro-chamber with a buried metallic layer consumes some more power. The LTCC based micro-chamber is an enhanced micro-chamber due to bio-compatible properties, low power consumption and miniaturization.

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