Experimental validation of numerical study on thermoelectric-based heating in an integrated centrifugal microfluidic platform for polymerase chain reaction amplification

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A comprehensive study involving numerical analysis and experimental validation of temperature transients within a microchamber was performed for thermocycling operation in an integrated centrifugal microfluidic platform for polymerase chain reaction (PCR) amplification. Controlled heating and cooling of biological samples are essential processes in many sample preparation and detection steps for micro-total analysis systems. Specifically, the PCR process relies on highly controllable and uniform heating of nucleic acid samples for successful and efficient amplification. In these miniaturized systems, the heating process is often performed more rapidly, making the temperature control more difficult, and adding complexity to the integrated hardware system. To gain further insight into the complex temperature profiles within the PCR microchamber, numerical simulations using computational fluid dynamics and computational heat transfer were performed. The designed integrated centrifugal microfluidics platform utilizes thermoelectrics for ice-valving and thermocycling for PCR amplification. Embedded micro-thermocouples were used to record the static and dynamic thermal responses in the experiments. The data collected was subsequently used for computational validation of the numerical predictions for the system response during thermocycling, and these simulations were found to be in agreement with the experimental data to within $\sim 97\%$. When thermal contact resistance values were incorporated in the simulations, the numerical predictions were found to be in agreement with the experimental data to within $\sim 99.9\%$. This in-depth numerical modeling and experimental validation of a complex single-sided heating platform provide insights into hardware and system design for multi-layered polymer microfluidic systems. In addition, the biological capability along with the practical feasibility of the integrated system is demonstrated by successfully performing PCR amplification of a *Group B Streptococcus* gene. © 2013 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4789756>]

I. INTRODUCTION

Polymerase chain reaction (PCR) is a common analytical technique used for the detection of biological samples, especially for samples with low target concentrations. PCR enables the detection of pathogenic species by amplifying the target DNA into quantities large enough to be detected by fluorescence or electrochemical methods.^{1,2} Micro-total analysis systems (μ -TAS) that include PCR amplification processes render such miniaturization benefits as

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reduced reagent use, improved heating rates, and the ability to integrate with sample preparation and detection steps.^{3–8}

The effectiveness of PCR as a diagnostic tool is measured by the fidelity, specificity, and efficiency of amplification. Temperature uniformity is particularly important for the specificity of a PCR assay when handling samples which contain competing targets. For instance, blood samples taken for sepsis detection have various competing nucleic acid targets resulting in a large amount of background noise. In the annealing step of PCR, going below the ideal annealing temperature, by as little as -0.5°C can cause errors in the primer annealing to the competing targets.⁹

In the denaturing step of PCR, reaching temperatures above 95°C for extended periods of time can damage the enzyme. Damage to the polymerase enzyme will not only cause a loss of assay sensitivity and negatively affect the reproducibility between tests. On a product development note, the polymerase enzyme is an essential and costly reagent needed for the PCR amplification reaction. Therefore, it is particularly important that the thermal aspects of microfluidic systems for nucleic acid analysis are well characterized.

A. Centrifugal microfluidic platforms

The centrifugal microfluidic platform has been utilized for many biomedical applications, including sample preparation, immunoassays, biochemical analysis, and PCR amplification.^{10–13} Centrifugal microfluidics often utilize intrinsic, non-contact pumping and valving mechanisms which are ideal for liquid handling.^{14–16} However, the handling of liquid/vapor PCR samples in microfluidic devices is not a simple task when confronted with the high temperatures and pressures created within the PCR chamber region.^{17–23} Thermoelectric-based ice-valves are an ideal valving method for a stationary integrated centrifugal microfluidic system.²⁴ These ice-valves are vapor-tight and strong enough to withstand the high temperatures and pressures within the PCR chamber. In addition, by implementing both thermoelectric heating and valving, the complexity of the hardware architecture and control scheme is minimized for the designed integrated system.

B. Numerical modeling of centrifugal microfluidics

The volume of fluid (VOF) method used to simulate the filling of a microchamber (in the presence and absence of centrifugal force) was successfully demonstrated in a previous publication.²⁵ In this study, when the thermocycling experiments are performed on the centrifugal microfluidics platform, the temperature gradient due to heat conduction is expected to be higher along the thickness of the centrifugal disc (CD), especially because of the small size of the PCR microchamber. Numerical analysis was performed for predicting the transient thermal response of the PCR microchamber in order to explore the effect of the various design parameters (e.g., microchamber dimensions, material properties, power input, or heater layout). The parametric analyses is a convenient tool for determining the system's optimum operating conditions (e.g., temperature ramp rate, holding period for denaturing and annealing steps, cooling rates, and the thermal response of the microchamber for an imposed heat flux or temperature boundary condition).^{24,26–30}

Typically, the materials used for the CD platform have low thermal conductivity values, which enables the temperature deviations to be smaller than the designed values.²⁶ However, low thermal conductivity materials result in delayed thermal response to the imposed boundary conditions.²⁹ Thus, in this study, different thicknesses of top and bottom layers are used to ensure the rapid thermal response and temperature uniformity within the PCR microchamber.

II. EXPERIMENTAL MATERIALS AND METHODS

A. CD fabrication

The CDs designed and tested here consist of multi-layer structures made of polycarbonate plastic sheets, polycarbonate thin films (McMaster-Carr, CA, USA), and pressure-sensitive adhesives (PSAs). A simple CNC machine (T-Tech, GA, USA-QuickCircuit 5000) was used to cut chamber and through-hole features into the thicker 1.016 mm polycarbonate sheets. A

computer-controlled cutter-plotter (Graphtec, Japan-Graphtec CE-2000) was used to cut channel features in the 101.6 μm thick PSA and 127 μm thick polycarbonate film layers.

The microfluidic CD platform presented here consists of 5 layers: (1) top polycarbonate CD with CNC-machined sample loading, sample removal, and air venting holes (sealed using a thin adhesive film during operation), (2) pressure-sensitive adhesive with channel features cut using a plotter, (3) middle polycarbonate CD with CNC chamber features, (4) thin film polycarbonate layers with PCR chamber features cut using a plotter, and (5) solid bottom polycarbonate CD (Fig. 1).

Once the appropriate pieces have been designed and machined, they are aligned, and then thermally welded in a hydraulic thermal press (Tetrahedron Associates, CA, USA). The bottom three layers, those layers which comprise the PCR chamber, were thermally bonded at 145 °C and 720 kPa for 30 min, then slowly cooled to room temperature at heightened pressure to prevent warping of the disc. Upon reaching room temperature, the pressure was released and the part removed from the hydraulic press. These thermally bonded layers were then aligned with disc layers 1 and 2 and run through an industrial press to complete the assembly process.

B. Experimental apparatus

The experimental apparatus included a temperature control module that consisted of three thermoelectric modules mounted on a heatsink and fan assembly.³¹ The temperature control apparatus was attached to a linear solenoid-based motion system, which when activated, presses the thermoelectric modules into contact with the bottom surface of the disposable CD. A servo motor and corresponding motor controller (Pacific Scientific, IL, USA) provided the rotational power (centrifugal pumping mechanism) for fluidic propulsion within the microfluidic disc.³²

The fluidic pumping apparatus (servo motor), temperature control apparatus, and the solenoid-based motion system were controlled using LABVIEW automation software (National Instruments, TX, USA). Experimental parameters for fluidic pumping and thermocycling (e.g., rotational speed, ramp rates/cooling rates, holding period for annealing/denaturing) were controlled through a LABVIEW user interface. The thermocycling process was performed while the disc was stationary and the reaction chamber was in close contact with the thermoelectric modules.

The placement of the central thermoelectric (TE) module, positioned directly below the PCR chamber region of the CD, is specifically designed for reproducible performance for thermocycling (Marlow Industries, TX, USA – XLT2422-02LS). Two smaller thermoelectric modules are positioned below the entrance and outlet microfluidic channels, as shown in Fig. 2. These smaller thermoelectric modules, functioning as active valves (i.e., as ice-valves), prevent the leakage of PCR fluid during thermocycling. These ice-valves form a liquid- and vapor-tight seal arising from the freezing of ice plugs in the narrow inlet and outlet microchannel regions.²⁴

FIG. 1. An exploded view of the multi-layered microfluidic disc for automated PCR amplification.

FIG. 2. (Left) Schematic of the microfluidic CD showing the thermoelectric heater and ice-valve locations with respect to the PCR microchambers. (Right) Schematic of the simplified geometry for the numerical simulations.

In order to ensure rapid and complete freezing, two-stage thermoelectric modules (Marlow Industries, TX, USA – NL2064T-11AB) were used to generate the ice-valves on both sides of the PCR microchamber (i.e., within the microchannels at the inlet and exit ports of the PCR microchamber).

The central TE module (for thermocycling) and the two smaller TE modules (for forming the ice valves) were mounted on a 50.8 mm-thick aluminum heat sink (Newark, NJ, USA – 15J9644) and fan assembly (Dynaflor, CA, USA) to achieve the temperature control and the microvalving capabilities. Thermal grease (Arctic Silver, CA, USA) was applied at the interface between the TE modules and the heat sink to reduce thermal contact resistance of the system (and enable faster thermal response for the microchamber apparatus). The thermoelectric modules were powered by a main-frame supply (Agilent Technologies, CA, USA-N6701A) that enabled automated USB-based control of power (magnitude and polarity) from a computer using the LABVIEW software. Copper sheets were attached to the top of the thermocycling module using adhesives to improve the homogeneity of the overall thermal profile (temperature and heat flux). A small groove was cut into the middle of this copper sheet and a t-type thermocouple (Omega, CT, USA – COCO-005) was glued into the groove using thermal compound. Temperature readings from this thermocouple were recorded by the LABVIEW code for implementing the PID control system to achieve precise temperature values by either heating or cooling the bottom surface of the microchamber using the central TE module.

Additional thermocouples were positioned at different locations for recording the thermal response, and the experimental apparatus was subsequently used for either calibration of the numerical model or validation of the numerical predictions. To monitor the fluid temperature in real-time during thermocycling, a thin film t-type thermocouple (RdF Corporation, NH, USA – 20102-2) was embedded directly into the PCR microchamber. Similar t-type thermocouples were placed directly above and below this thermocouple (i.e., for monitoring the fluid temperature within the microchamber). This enabled the recording of temperature data for the bottom and top surfaces of the CD, directly above the center of the PCR microchamber. Thermocouples were also placed on the bottom and top surfaces of the CD and were positioned (centered) at the location of the left and right ice-valves. All thermocouples were connected to the analog temperature input modules (National Instruments, TX, USA – SCC-TC02) within a signal-conditioning box (National Instruments, TX, USA – SC-2345) which allows the continuous monitoring and digitization of the analog signals using LABVIEW.

C. Biological validation

The *cfd* gene for group B *Streptococcus* (GBS) was used as the DNA sample for biological validation of the microfluidic CD system for PCR amplification. The specificity of the PCR assay was verified using purified genomic DNA from a *Streptococcus agalactiae* strain (ATCC

#27591). The target sequence for PCR amplification (5'-TTTCACCAGCTGTATTAGAAGTACATGCTGATCAAGTGACAACCTCCACAAGTGGTAAATCATGTAAATAGTAATAATCAAGCCCAGCAAATGGCTCAAAAGCTTGATCAAGATAGCATTGAGAAATATCAAA GATAATGTTCAGGGAAC-3'), forward primer (5'-TTTCACCAGCTGTATTAGAAGTA-3') and reverse primer (5'-GTTCCCTGAACATTATCTTTGAT-3') were synthesized as indicated by Ke *et al.*³³ The forward and reverse primers were added at a concentration of 0.4 mM, and the positive control template DNA starting concentration ranged from 10^4 copies down to 100 copies. In addition, a 20 μ l reaction volume included 200 mM dNTPs, 10 mM Tris-HCl, 49.8 μ M BSA, and 0.5U of TAQ polymerase (Promega, WI, USA) in the testing of the integrated CD system. Positive controls were amplified using a standard commercially available PCR thermocycler (MJ Research, MA, USA – PTC-100).

Gel electrophoresis was used to validate the amplification of the *cfb* gene of Group B Streptococcus. Precast agarose gels containing ethidium bromide dye were used for gel electrophoresis studies (Invitrogen, CA, USA – G501804). Amplified bands of PCR products were run along with a 10 bp DNA marker (Invitrogen, CA, USA – 10488019), and imaged using an AlphaImager UV Illuminator (AlphaInnotech, CA, USA).

III. NUMERICAL AND THEORETICAL MODELS

The 1-D theoretical model provides a convenient yet reasonably accurate approach for performing parametric design study (e.g., for exploring the optimum wall thickness). The predictions from the 1-D theoretical model can also be used for calibrating the thermo-fluidic simulations using 3-D transient computational fluid dynamics (CFD) and computational heat transfer (CHT). The 1-D theoretical model can also be used to perform feasibility studies before performing the actual experiments as well as for design of experiments. The incorporation of other extraneous experimental parameters in the theoretical model (e.g., thermal contact resistance or thermal inertia effect) is also discussed. For this purpose, the equivalent thickness of air gap for effect of thermal contact resistance and transient temperature boundary conditions for the thermocycling heating process are incorporated into 3-D numerical model using a commercial solver (i.e., *ESI CFD-ACE+*[®]).

A. Theoretical (1-D) model development

In this study, the 1-D model was developed and implemented to explore the temperature variation in the CD platform for different values of sample thickness with the intention of optimizing the design geometry and the experimental parameters. This model was developed by assuming a simplified geometry and by neglecting the insulating effect of the polycarbonate substrates. This three-layer model, shown in Fig. 3, corresponds with the experimental multi-

FIG. 3. Schematic showing the cross-section of the PCR microchamber, for the 1-D theoretical model that is used for calculating the thermal response during thermocycling.

layer disc design with polycarbonate layers of various thicknesses. While the disc design shown in Fig. 1 is composed of five layers, only three layers exist in the outer region of the disc where the thermocycling process occurs.

The governing equation for simulating the transient temperature response (1-D Model) as well as the initial condition and the boundary conditions are listed as follows:

$$\frac{\partial^2 T}{\partial z^2} = \frac{1}{\alpha} \frac{\partial T}{\partial t}, \quad (1)$$

$$T(z, 0) = T_0, \quad (2)$$

$$T|_{z=0} = T_i(t), \quad (3)$$

$$-k \frac{\partial T}{\partial z} \bigg|_{z=\delta} = h(T - T_\infty), \quad (4)$$

where T is the temperature [K], z is the coordinate in the vertical (depth) direction [m], t is the time [s], α is the thermal diffusivity [m^2/s], T_0 is the initial temperature [K], k is the thermal conductivity [$\text{W}/(\text{mK})$], h is the convective heat transfer coefficient (free convection in air: $5 \text{ W}/(\text{m}^2 \text{K})$), δ is the thickness of the chamber [m], and T_∞ is the room temperature (293.15 K). To measure the balance between the buoyant force associated with thermal expansion and the dissipation energy due to viscosity and thermal diffusion for a fluid-filled microchamber, where the fluid is confined between two parallel horizontal plates at a distance δ apart (as shown in Fig. 3), the Rayleigh (Ra) number is evaluated as Eq. (7)

$$Ra = \frac{g\beta(T_1 - T_2)\delta^3}{\alpha\nu}, \quad (5)$$

where Ra is the Rayleigh number, g is gravitational acceleration [m/s^2], β is the volumetric thermal expansion coefficient [K^{-1}], ν is the kinematic viscosity [m^2/s], T_1 and T_2 are bottom and top temperatures, respectively. For Rayleigh numbers less than a critical value of $Ra_c = 1708$, buoyancy forces cannot overcome the resistance imposed by viscous forces and there is no advection within the chamber filled with the liquid. Hence, heat transfer from the

FIG. 4. Comparison of thermal response of different values for the thickness of the PCR microchamber ($\Delta T_{\delta=125\mu\text{m}} = 0.08$ $^{\circ}\text{C} \rightarrow Ra = 0.0063$, $\Delta T_{\delta=250\mu\text{m}} = 0.16$ $^{\circ}\text{C} \rightarrow Ra = 0.1012$, $\Delta T_{\delta=500\mu\text{m}} = 4.05$ $^{\circ}\text{C} \rightarrow Ra = 20.137$, and $\Delta T_{\delta=1000\mu\text{m}} = 33.27$ $^{\circ}\text{C} \rightarrow Ra = 1144.3$).

bottom to the top surface is dominated by conduction without any induced fluid motion due to buoyancy effects.³⁴ Predicted temperatures of the fluid at the top inside the chamber are compared in Fig. 4, which was obtained by implementing the governing equations in a simple PDE solver in MATLAB[®].

The Ra values were calculated by using the temperature values obtained from 1-D modeling (as shown in Fig. 4), the liquid thickness, and the related thermo-physical properties were evaluated at the average (film) temperature for the thermal performance calculations. Since the Ra for all the cases shown in Fig. 4 were below the critical number (Ra_c), the thermocycling process can be characterized by pure conduction. Also, the thickness value (i.e., 125 μm) selected for the experiments provides a reasonably optimum value for temperature difference (i.e., 0.08 °C) between top and bottom surfaces for the PCR operation. These results also show that the fluid temperature within the PCR microchamber is fairly uniform with negligible temperature gradients being established within the control volume, thus ensuring the required sensitivity for PCR.

B. Numerical (3-D) model development

The thermal conductivity of the top and bottom polycarbonate substrates was also incorporated in the 3-D numerical models (CFD/CHT). The coupled momentum (Navier–Stokes) and energy equations that were implemented in the 3-D numerical models for an incompressible Newtonian fluid are

$$\frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \nabla) \vec{v} = -\frac{1}{\rho} \nabla p + \frac{\mu}{\rho} \nabla^2 \vec{v} + \vec{f}, \quad (6)$$

$$\frac{\partial T}{\partial t} + (\vec{v} \cdot \nabla) T = \alpha \nabla^2 T, \quad (7)$$

where ∇ is the spatial gradient (vector) operator, $\vec{v}$ is the three-dimensional velocity field [m/s], ρ is fluid density [kg/m^3], $\cdot$ is the vector dot product, $\vec{f}$ is the body force acting on the fluid (e.g., gravity force), p is pressure [Pa], μ is dynamic viscosity [Pa s], and α is thermal diffusivity [m^2/s]. The 3-D numerical model was implemented and solved by using a commercial solver (*ESI CFD-ACE+[®] v2009*). Two separate sets of numerical simulations were performed (1) static (i.e., steady state) and (2) dynamic (i.e., transient). The wall temperature boundary conditions (steady state or transient) were also implemented in the solver. Thermo-physical properties of water were used in the simulations for the fluid in the microchamber (from the database of *ESI CFD-ACE+[®]*).

C. Steady and transient boundary conditions

For the implementation of the numerical model, the bottom surface (of the bottom plate) is subjected to an isothermal heating condition. Also, natural convection (in air) is implemented as the boundary condition for the top surface (of the top plate). In addition, the side walls of the microchamber (except for the surfaces representing the ice-valve located at the both ends of the PCR chamber – which are isothermal surfaces) are implemented as adiabatic surface conditions. The adiabatic condition can be justified to be due to relatively lower temperature gradients in the horizontal direction compared to that in the vertical direction (as shown in Fig. 3) which arises from the slender shape and high aspect ratio of the PCR microchamber geometry. The list of boundary conditions that were used for the steady state simulations are summarized in Table I.

The isothermal boundary conditions for the left and right ice-valves (as well as for the bottom surface) were based on experimental data. The disparities between temperature of the left and right ice valves arise from the asymmetry of their spatial positioning on the heat sink and fan assembly (and are also implemented in the numerical simulations) (Fig. 5).

The value of convective heat transfer coefficient (h) in Table I is estimated under the assumption of pure conduction (i.e., Nusselt number equivalent of unity for pure conduction). Since the temperature difference between the top and bottom surfaces is less than the critical

TABLE I. Boundary conditions for each surface defined in the PCR chamber.

Wall boundary surface	Boundary conditions
Left ice-valve	Isothermal (-26.76°C)
Right ice-valve	Isothermal (-35.30°C)
Top	Convective ($h = 20 \text{ W}/(\text{m}^2 \text{ K})$)
Bottom	Isothermal (97.5°C)
Side	Adiabatic

value required for the fluid to become unstable (i.e., Rayleigh number is less than the criterion for instability), the conduction is dominant and Nusselt number is equal to 1

$$Nu = \frac{hL_c}{k} = 1, \quad (8)$$

where L_c (equals t in Fig. 3) is the characteristic length [m]. For a total thickness (t) of CD microfluidic system of 1.268 [mm], then the convective heat transfer coefficient is approximately 20 [$\text{W}/(\text{m}^2 \text{ K})$].³⁴

IV. RESULTS AND DISCUSSION

A. Steady state experiments and simulations

The initial simulations for the 3-D model were performed using the steady state boundary conditions. From the simulations, the temperature difference between the top and bottom surface of the PCR microchamber was predicted to be 0.103°C . However, the simulated value for the maximum temperature of the fluid (at the middle height) inside the microchamber was estimated to be 97.22°C . This value is marginally higher than the experimental data (i.e., 94.05°C). This is probably due to overestimation in the value of the heat transfer coefficient in air and underestimation of the thermal inertia of the CD platform.

By incorporating thermal contact resistance (TCR) at the interface between the polycarbonate substrate (bottom plate) and the TE module (along with thermal grease), the accuracy of the numerical model can be improved, since these were neglected in the initial set of simulations. Hence, incorporation of TCR in the numerical model provides more realistic values of temperature variations inside the PCR microchamber. TCR was implemented in the solver (*ESI CFD-ACE+[®]*) by using an equivalent thickness of air gap between TE module and CD system. The equivalent thickness of air gap was calculated from the 1-D heat transfer model by using an equivalent network thermal resistance network, as shown in Fig. 6.

The equivalent air gap (δ_a) corresponding to the TCR is obtained from Eq. (9) and the value used in the 3-D numerical model is $\sim 45 \text{ } \mu\text{m}$

FIG. 5. The temperature distribution at the mid-plane in the fluid volume for the 3-D numerical model. The central TE heater region is identified by the dotted line, and the inlet and outlet microchannels are bounded by the ice-valve regions.

FIG. 6. One-dimensional heat transfer through multi-layers and electrical analogy for calculation of air gap thickness.

$$q'' = \frac{T_i - T_\infty}{\frac{\delta_{pb}}{k_p} + \frac{\delta_w}{k_w} + \frac{\delta_{pt}}{k_p} + \frac{1}{h}} = \frac{T_i - T_e}{\frac{\delta_a}{k_a} + \frac{\delta_{pb}}{k_{pb}} + \frac{\delta_w/2}{k_w}}, \quad (9)$$

where T_e is the fluid temperature inside the microchamber obtained by experiments at steady state and subscripts p , w , h , a , pb , pt denote polycarbonate, water, surrounding air, air gap, bottom layer, and top layer, respectively. Fig. 7 shows the numerical simulation results when TCR effect is considered. The fluid temperature inside the chamber was estimated to be $94.16\text{ [}^\circ\text{C]}$ which is consistent with the experimental data for the steady state conditions. From the results, it was demonstrated that incorporation of TCR provided more accurate estimations. The temperature distribution within the PCR microchamber was found to be fairly uniform, while the temperature difference between top and bottom surfaces was small (e.g., $0.27\text{ [}^\circ\text{C]}$). Hence the numerical model designed in this study was successfully validated, as the numerical predictions for the steady state conditions matched the experimental data to within $\sim 97\%$.

FIG. 7. Comparison of temperature profile at the mid-plane (as shown in Fig. 5) at different heights within the microchamber. The simulation results incorporate TCR values that were estimated from the calibration of the numerical model using steady state experimental data. (Fluid temperature inside the PCR chamber is $94.16\text{ [}^\circ\text{C]}$ in simulation and $94.05\text{ [}^\circ\text{C]}$ in experiment).

FIG. 8. Comparison of predictions from transient numerical simulations with data from thermocycling experiments for (a) 97.5 °C—60 cycles for 45 [s] and (b) 97.5 °C—60 cycles for 60 [s].

B. Thermocycling experiments and transient simulations

The same surface temperature boundary condition that was applied in the 1-D model was applied to the bottom surface for the 3-D model, except that the value of the temperature changed with time. The boundary conditions for other surfaces were fixed to identical values, as given in Table I.

The transient simulation results and the data from the thermocycling experiments are plotted in Fig. 8 as a function of time. The relatively thick top layer of polycarbonate substrate (compared with the thinner bottom layer of the polycarbonate substrate) reduces the convective heat to the ambient (air). The thinner bottom layer enables faster thermal response to the imposed temperature changes in the thermocycling experiments (as well as for lower power inputs to the TE modules). The aspect ratio of the PCR microchamber (thin slender structure) enables better temperature uniformity to be achieved for the fluid (analyte of interest). As discussed earlier in Fig. 4, microchamber thicknesses of 250 μm or less result in very minor temperature gradients throughout the height of the microchamber. The designed microchamber

height (i.e., 127 μm) ensures uniform heating of the reaction fluid for improved specificity and efficiency of amplification.

The results plotted in Fig. 8 show that the numerical predictions are consistent with the experimental measurements for the imposed temperature profiles that were implemented as boundary condition in the numerical simulations (for both 45-s and 60-s cycle times in the thermocycling experiments). The numerical predictions for the transient simulations, with the incorporated TCR values, matched the target temperature data from the thermocycling experiments to within $\sim 99.9\%$ for the maximum temperature reached in each cycle. However, the transient profile for the thermal response (as predicted by the numerical model) was slightly faster than the data obtained from the thermocycling experiments. This is probably due to marginal underestimation in the thermal inertia of the microfluidic platform (the multi-layered CD substrate, shown schematically in Fig. 6), the TE apparatus (used for the automatic temperature control), and the solenoid motion control apparatus (for achieving better thermal contact at the interface between CD and TE module). Hence, the maximum error in the prediction of temperature was less than 5% during the temperature rise phase of the thermocycle and was less than 10% during the temperature decay phase.

C. Biological validation of microchamber PCR

The biological performance (i.e., the efficacy for pathogen detection) of the microfluidic CD apparatus was validated via PCR amplification of a *cfb* gene of *Group B Streptococcus*. The on-CD amplification was then compared to a traditional tube-based heating block (HB) system as a positive control. Shown in Fig. 9, the amplified bands in lanes 2 through 4 correspond to PCR runs in the CD system, and lanes 6 through 8. Lanes 1 and 5 contain the negative control samples for the CD and heating block, respectively. These negative control lanes show no amplified bands, suggesting that there was little to no contamination during the process run of the CD. A positive band in gel lane 4, corresponding to the 153-bp amplified region of the GBS gene, demonstrates the PCR sensitivity, or limit-of-detection, to be better or equal to 100 copies for the integrated CD system. The marker lanes, M, show a DNA ladder with a bright band at 100 bp, helping to confirm the size of the amplified bands in lanes 2 through 4 and 6 through 8. Throughout the amplification trials, there was some minor inconsistency with the amplified bands from the CD system. For example, the 1000 copy sample band is slightly weaker than the 100 copy band (lane 4). Although a concern, this is likely due to incomplete sample removal from the disc and not due to uniformity issues with the heating system. System

FIG. 9. DNA gel image comparing the PCR amplification of a GBS gene on the microfluidic CD system with a traditional HB system. (M) Molecular weight ladder, (1) CD—negative control (2) CD—10 000 copies positive control, (3) CD—1000 copies positive control, (4) CD—100 copies positive control, (5) HB—negative control, (6) HB—10 000 copies positive control, (7) HB—1000 copies positive control, (8) HB—100 copies positive control.

issues with sample removal are diminished if on-CD detection methods (i.e., real-time PCR) are integrated where the sample remains within the microchamber.

In microfluidic systems, where the surface area-to-volume ratio is quite large, PCR inhibition due to chemical leaching from the polymer material or reagent absorption to the surfaces is a major developmental concern. As stated earlier, BSA was added to the PCR reaction mix to minimize absorption of the polymerase or other PCR reagents to the inner walls of the PCR microchamber. This addition substantially improved the performance of the CD system and resulted in high performance similar to the commercial PCR tube heating block system. In addition, to further improve the performance of the CD device, utilizing PMMA compared to polycarbonate materials would minimize any inhibitory effects due to material choice.

D. Practical applications of experimental and theoretical/numerical models

The theoretical and numerical models described in this study were developed and validated experimentally to demonstrate the feasibility of this numerical/theoretical framework. The models are applicable beyond the realm of the centrifugal microfluidics platforms and can be extended (or generalized) for use in many other microfluidic systems which require analyses/predictions for heat transfer (both transient and steady-state situations). This numerical/theoretical framework is most commonly applicable for PCR amplification-based diagnostic platforms in microfluidic systems.

As the microfluidic CD is heated while stationary, a similar system with single-sided heating (and different geometric and operational parameters and configurations) can be characterized without much alterations to the theoretical and numerical models that were proposed and validated in this study. Using a similar modeling framework for this microfluidic platform, the thermo-physical characteristics (such as device geometry, material choice, contact mechanism) can be used as input parameters into these models for more in-depth exploration and more sophisticated analyses of the thermal performance characterization.

V. CONCLUSIONS

The numerical modeling strategy described and implemented as well as validated in this study consisted of three different techniques with increasing levels of sophistication. Initially, 1-D theoretical (analytical) modeling was performed to rapidly traverse the design space for optimizing thermal performance of the designed microfluidic platform. This 1-D model can also be used for initial calibration of numerical models (CFD/CHT).

Subsequently, 3-D numerical simulations were performed for enumerating the thermal behavior and steady state performance of the microfluidic platform. Experimental data were used to calibrate the steady-state model and enable the estimation of the values for the TCR at the interface between the CD substrate and the central TE module that was used for automated temperature control of the PCR microchamber. Calibration of the 3-D steady-state numerical model (and in combination with the 1-D theoretical model) showed that the equivalent air gap for the corresponding values of TCR is $\sim 45 \mu\text{m}$. These simulations also demonstrated that the temperature profile within the PCR microchamber was fairly uniform (to within $\sim 0.27^\circ\text{C}$). The numerical predictions matched the steady state experimental data to within $\sim 97\%$.

Transient simulations were performed for the same geometry (and by incorporating the value of TCR that were extracted from the calibration step for the 3-D steady state numerical simulations, which were then used as the boundary condition to the 3-D transient model). For computational validation, the predictions from the transient 3-D simulations were compared to the data obtained from the thermocycling experiments. The predictions were found to be in good agreement with the experimental data for the fluid temperature in the PCR microchamber (to within $\sim 99.9\%$).

Subsequently, PCR experiments were performed to demonstrate the biological capabilities of the designed platform as well as demonstrate the feasibility of the experimental platform for diagnostic pathogen detection. The results from the gel imaging experiments were consistent with the expected performance of the CD microfluidic platform.

Future research activities will include further optimization of the numerical as well theoretical models and computational validation (for the uniformity of heat flux and temperature distribution within the PCR microchamber) by using infrared imaging of the CD microfluidic platform. Better strategies for estimating the thermal inertia of the total experimental apparatus will also be explored to improve the predictions for the steady state performance of the CD microfluidic platform.

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