NANOLITER-SIZED SUPERHEATED BIOREACTOR

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ABSTRACT

We have designed, fabricated and tested a nanoliter-sized reactor capable of warming up water-based sample to 155 °C well above water boiling point. Its performance was simulated by finite element analysis method and experimentally verified using temperature sensitivity of fluorescence amplitude from fluorescein.

KEYWORDS: superheated, nanoliter, bioreactor

INTRODUCTION

Biochemical reactions are typically performed in water environment. Due to water boiling temperature, reactions are typically conducted in the temperature below 100 °C. It limits the applications as some reactions such as breaking spores to release the DNA to identify the pathogens require prolonging time for the reaction to be completed. Conventionally the systems operating above boiling point (at ambient pressure) are based on autoclaves to increase the pressure and thus boiling point operating in high pressure vessel. In the absence of nucleation sites however, the temperature of the liquid can exceed the boiling temperature in a phenomena called superheating, at the basis of the water explosions using microwave ovens for example. In this contribution we demonstrate application of superheated reactor for molecular biology applications to break spores in order to release DNA for its subsequent detection. Forming a device operating at temperature above boiling point without high pressure will find application in molecular biology as well as in chemistry.

Detection of DNA by polymerase chain reaction (PCR) is well established technique. In order to perform them the DNA has to be first released by breaking the cell or spore wall. Thermal lysis is a simple method which can be easily implemented in LOC (Lab-on-a-Chip) applications. However, if the spore lysis at temperature below 100 °C is not sufficient.

EXPERIMENTAL

Here, we report technical aspects of a nanoliter-sized microfluidic device capable of rapidly heating an aqueous sample to temperatures of up to 154 °C. It can serve as the front-end module for of an integrated LOCs system for general application as well as to detect anthrax, where the rapid breaking of spore to release DNA is required.

Previously [1] we have described system called 'virtual reaction chamber' (VRC) consisting of a free sample droplet encapsulated by oil on a glass slip. The heater and temperature sensor was underneath the glass. The original system was redesigned to increase heating and cooling rate (see Figure 1) for the cell breaking applications.

A 200nL sample with 1μL/Mol concentration of fluorescein was covered with 600nL of mineral oil (M5904, Sigma, Inc.). The heater temperature was then raised from room temperature to the 154 °C for a period of 10s. Temperature within the sample was simulated by ANSYS (see Figure 2) software. It was compared with the measurement based on the amplitude of a fluorescence signal [2] (see Figure 3). Achieved temperature was 54 °C above the water boiling point. Boiling was suppressed by eliminating bubble nucleation sites.

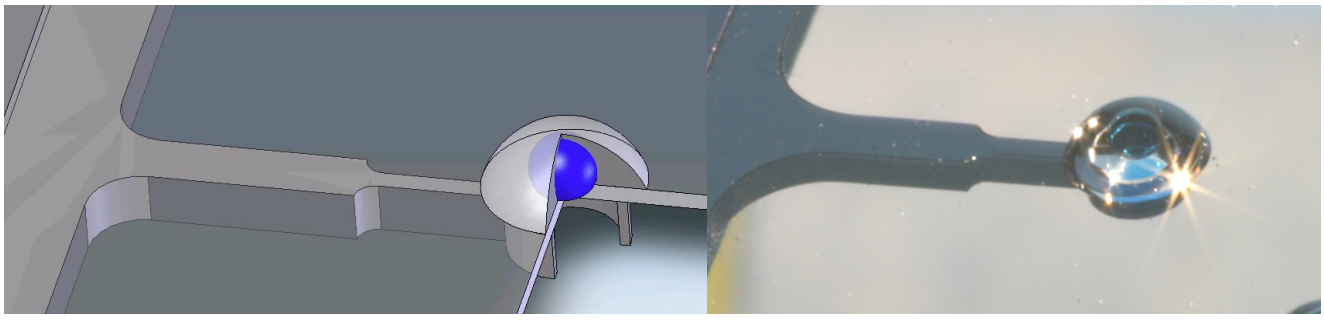

Figure 1: (A) Schematic drawing of the virtual reaction chamber (VRC) with a miniaturized 'hotplate' underneath. It consists of a ring-shaped silicon support with an integrated heater/temperature sensor made of gold. A cantilever connects the ring to a frame. The sample (represented by the blue sphere) is covered by mineral oil and placed on a thin teflon coated glass substrate. (B) Photograph of a fabricated structure with 200nL sample (blue color) covered with 600nL of mineral oil. The oil contact angle was 77° and water 113°.

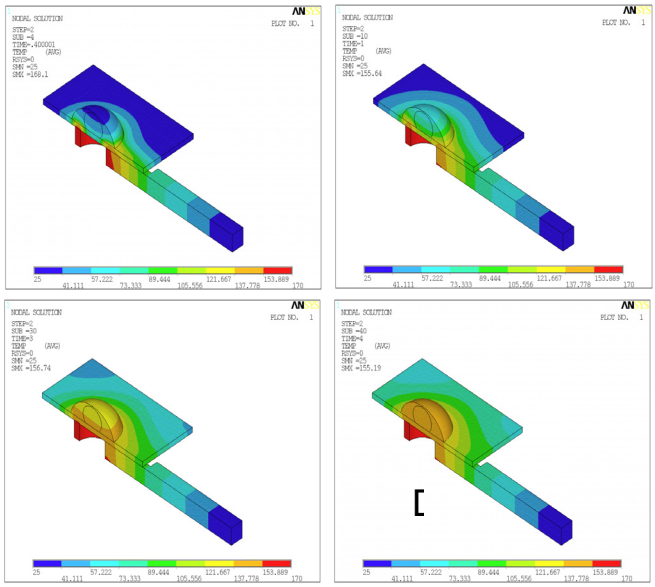

Figure 2: The transient thermal analysis of the VRC performed and temperature distribution at 0.4s (A), 1s (B), 3s (C) and 4s (D) are shown. The heat transfer from the heater to the sample is demonstrated. Extracted average sample temperature as function of time is plotted at Fig. 3.

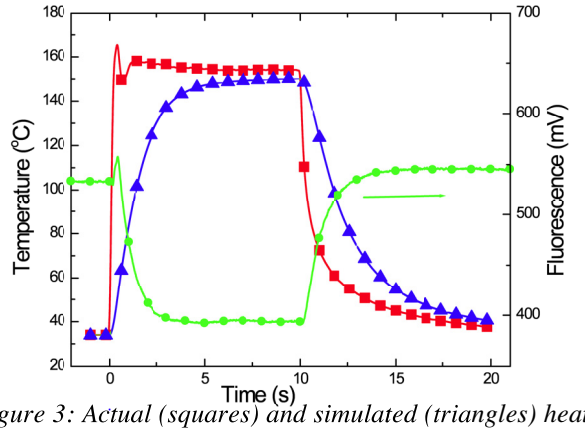

Figure 3: Actual (squares) and simulated (triangles) heater temperature using FEA and measured fluorescence signal (circles) as function of time. 154°C temperature of the heater was achieved in 0.2s corresponding to heating rate of 650°C/s. The sample temperature was delayed by 2.5s due to the heat transfer via glass and oil. Amplitude of the fluorescent signal is proportional to the temperature. Comparing the fluorescence with the ANSYS simulation suggests that the droplet cools faster than predicted by the simulation. At 15s fluorescence was already stabilized (green) while simulated droplet temperature (blue) was still decreasing.

CONCLUSION

Overall, we have proposed and tested a method to heat up the water-based above water boiling point. We have demonstrated that it is possible to reach 154 °C in 0.2s and keep the water droplet at this temperature without boiling. Thermal lysis as reported in this could be a simple option to disrupt Bacillus Anthracisis spores to release and detect their DNA. Due to its simplicity, it could become the method of choice as a front-end component of a Lab-on-a-Chip device for Anthrax detection..

REFERENCES

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