MONITORING BIOCHEMICAL REACTIONS USING MICROSPHERICAL GLASS SHELL WHISPERING GALLERY MODE RESONATORS

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ABSTRACT

Chip-scale microspherical glass shell (MSG) whispering gallery mode (WGM) resonators offer a unique platform to configure a high sensitivity calorimetric biosensor. The MSG optical resonator is realized using the chip-scale glass blowing technique. The backside silicon is thinned to 50 µm and attached to a 3D printed reaction chamber. The bio-reaction occurs directly on the thinned silicon surface for highly effective coupling of heat from the reaction chamber to the MSG shell to result in a sensitive thermal sensor. We present the latest results to quantify the concentration-dependent oxidation of glucose-mediated by immobilized glucose oxidase on superparamagnetic nanoparticles. The volume of the samples tested was ~10 µl.

KEYWORDS

Calorimetric biosensor, whispering gallery, optical resonator, magnetic nanoparticles urea-urease, glucose-glucose oxidase.

INTRODUCTION

In this paper, we use chip-scale blown-glass microspherical shell structures as WGM resonators. The high-Q (10^6 – 10^7) resonators are highly sensitive to small changes in size or the refractive index of the microspherical shells and therefore exhibit a high-temperature sensitivity. The resonator can be configured as a highly sensitive calorimetric biosensor by inducing enzymatic reactions on the backside silicon surface of the resonators. The bioreactor is formed using a 3D printed reaction chamber which is epoxy glued onto the backside of the MSG resonator chip and glucose oxidase enzyme immobilized superparamagnetic nanoparticles are used to catalyze the exothermic reactions to glucose.

DESIGN & METHOD

The MSG shell resonators were fabricated using the chip-scale glass blowing process as described in [1]. Circular features of 250 µm diameter were etched to a depth of 250 µm on a 400 µm thick four-inch silicon using the DRIE process. 100 µm thick borosilicate glass was anodically bonded to the silicon at a pressure of 1026 Torr at 400 °C to form sealed cavities. The wafer was diced to individual devices and the glass was etched using 49% HF to 50 µm. Finally, the device is heated on a silicon nitride ceramic heater to a temperature of 775 °C in a vacuum chamber maintained at 100 Torr for 45 secs. This softens the borosilicate glass and expands the nitrogen within the cavity to create the spherical shell following which it is immediately cooled down to ambient temperature. Finally, the silicon substrate was thinned down to 300 µm using 25% TMAH at 90 °C leaving a 50 µm silicon below the etched cavity of the MSG resonator as shown in Fig. 1.

A tunable 1550 nm laser (Newport TLB-6728) was used as the excitation source for inducing optical resonance in the glass microspherical shells. The laser was tuned using a triangle wave at 10 Hz by 17 nm (or 2.12 THz) about the center wavelength of 1550 nm. The light was evanescently coupled to the resonator via a tapered optical fiber. The fiber tapering was performed using an in-lab-built ceramic heater. The bare fiber is heated to 1250 °C allowing the silica fiber to soften into a viscoelastic state. The heated cavity attains thermal equilibrium in about 120 s and thereafter the

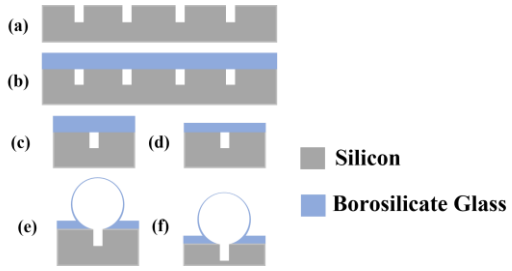

Figure 1: (a) Cylindrical Etch cavity of depth 250 µm in a 400 µm thick 4" silicon wafer. (b) 100 µm thick borosilicate wafer was anodically bonded to the wafer. (c) Devices are diced (d) 49% HF was used to thin the borosilicate glass to 50 µm. (e) The device was heated in a vacuum at 750 °C for 45 sec to create the MSG resonator. (f) Backside silicon was thinned using 25% TMAH to 50 µm in the region below the etched cavity.

translation stages were moved outwards at a constant pulling rate of 50 µm/s, while the transmission through the fiber was monitored on a photodetector. As the fiber is pulled, we observe multimode oscillations and finally with single mode at correct taper width. After about 18 mm of fiber elongation, the stages were stopped and the microheater was slowly allowed to cool down, to room temperature before removing the fiber from the heating source cavity.

A small change in size or refractive index can cause a significant shift in the resonance frequency. Both these quantities depend on temperature due to thermal expansion and thermo-optic effects respectively. This shift in resonance frequency of the resonator as a function of temperature was calibrated using a polyimide heater. Temperature sensitivity of 1.16 GHz/K was obtained. The system can resolve ΔT of up to 100 µK.

Fig. 2 shows the optical images of the biosensor. The spherical shell resonator with thinned backside silicon was attached to a 3D printed biochemical reaction chamber using thermal epoxy. The light was coupled to the resonator using a tapered fiber and resonance peaks are observed whenever the shell's equatorial circumference equals an integral number of the optical wavelengths. The excited resonance is seen as a dip in the transmission spectrum on the oscilloscope. The experimental setup to test the biochemical sensor is shown in Fig. 3. We use Pound-Drever-Hall (PDH) adaptive locking of the laser frequency at a fixed point on the slope

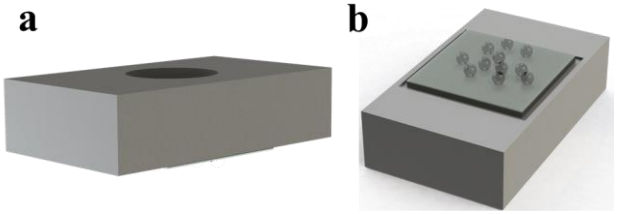

Figure 2: (a) Front side with circular cavity acting as the biochemical chamber. (b) Backside (inverted) with device stuck on the bioreactor using thermal epoxy. The backside etched silicon acts as the reaction surface for heat transfer from bioreaction to the MSG resonator.

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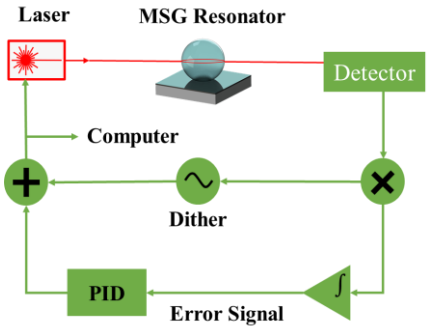

Figure 3: Schematic of adaptive locking sensing control system. A small high-frequency dither was used to modulate the driving laser frequency. When multiplied by the MSG resonator output and time-averaged, this dither signal generates an error signal whose amplitude is proportional to the difference between the current laser frequency and resonant frequency. This error signal is sent to a PID controller whose output is used to set the laser frequency, thus completing the feedback loop.

of the resonance curve [2] to track the shift in resonance frequency.

Chemical coprecipitation method was used to create superparamagnetic nanoparticles [3]. 2.36g of $FeCl_3.6H_2O$ and 0.86 $FeCl_2.4H_2O$ were dissolved in 40 ml of deionized water under a nitrogen atmosphere. This mixture was heated at 80 °C with nitrogen bubbling through it. 5 ml of ammonium hydroxide was added to this solution at a constant rate of 0.16 ml/min using a micropump.1 g of Fe_3O_4 nanoparticles are obtained after 30 mins and these superparamagnetic nanoparticles are separated using a neodymium magnet. These particles were further processed with a Sol-gel method to coat the superparamagnetic particles with Silica. 100 mg of the ferric oxide nanoparticles were dispersed in 50 ml of water with ultrasound for 10 min. A mixture of 1.0 ml of ethanol, 1.78 ml of TEOS (0.008 mol), and 1.0 ml of HCl (0.0024 M) was heated at 80 °C and stirred for 20 mins. Once the solution was clear and cooled, 0.5 ml of MPTMS was added. After 20 mins of stirring, a clear solution was obtained. This solution was added to the suspended nanoparticles in aliquots of 0.5 ml every 15 mins for two hours. The suspension was further stirred for another 30 mins to create silica over the ferric oxide nanoparticles with silane. These particles can be stored as dried nanoparticles or suspended in DI water. 50 mL of water containing Na_2HPO_4 (0.8 g) and 1 g of water-soluble carbodiimide (WSC) were used to dissolve 1 g of Ellman's reagent. 50 mg of the superparamagnetic nanoparticles were added to this mixture and stirred at 4 °C for 20 hours. The precipitate was separated magnetically and repeatedly washed with water. These nanoparticles are then mixed with a mixture of WSC (34.8 g), Glucose Oxidase (16.15 mg), and urea (2 M) dissolved in 10 mL (pH 5.5) of 0.1 M borate buffered solution. The mixture was stirred for 20 hours at 4 °C. The process ends with ultra-filtration to remove urea followed by magnetic separation of the nanoparticles. These nanoparticles with immobilized enzymes are stored at 4 °C.

RESULTS

Oxidation of glucose to gluconic acid in the presence of glucose oxidase has an associated enthalpy change of -80 kJ/mol. A precisely measured suspended nanoparticle solution with immobilized enzyme was first dropped over the silicon surface into the cylindrical reaction chamber. A neodymium magnet was placed below the device. This allows the particles to settle on the silicon surface and the phosphate buffer can be extracted using a micropipette. The laser is locked at the middle of the positive slope of the resonance frequency (1550 nm). As the glucose of various concentrations is dropped on the silicon surface with the immobilized enzyme on the nanoparticles, the resonance frequency shifts due to the exothermic nature of the reaction which results in a thermal expansion of the MSG shell. The change in responses is shown in Fig. 4 which represents the shift in resonance as the glucose molecules are catalyzed by the immobilized glucose oxidase and as the reaction completes, the signal drops to return to the base resonance frequency.

We studied the relative activity of the calibration sample at various pH values about the standard pH of 7.4. The pH of typical body fluids like plasma and serum is in the range of 7.35 – 7.45 [4]. We see the highest response for glucose at a pH of 7.4. The response drops to 20% as the pH is lowered to 4 and increased to 10. Following this, blood sample from a healthy patient is used to extract Serum. We observe a frequency shift of 110 MHz in the resonance frequency which from Fig.4 can be calculated as 4.72± 0.25 mM of Glucose.

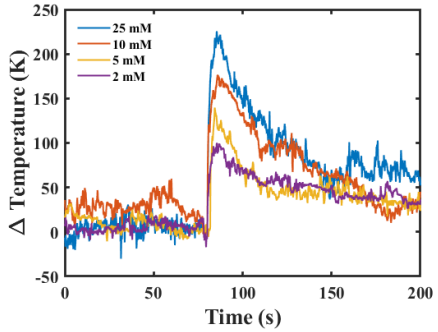

Figure 4: Time dependence of the output of the Microspherical Shell resonator for different concentrations of glucose solutions for liquid batch testing. Since a finite quantity of the analyte was dispensed in this experiment, the output increases initially, reaches a maximum, and finally decays with time.

CONCLUSION

In this paper, we configure chip-scale blown-glass microspherical shell WGM resonators as a highly sensitive glucose sensor. We present the shift in resonance as the glucose molecules are catalyzed by the immobilized glucose oxidase up to 2 mM concentrations. The volume of samples tested was ~10 µl. We also studied the relative activity for various pHs and observed the highest response at 7.4 pH. Finally, we see a response of 110 MHz shift in resonance frequency for serum processed from a healthy patient's blood sample. This matches 4.72± 0.25 mM of glucose which is in the normal range of 3.6 mM to 5.8 mM in healthy humans.

REFERENCES

[1] Zhang, Chenchen, et al. "On-chip glass microspherical shell whispering gallery mode resonators." *Scientific Reports* 7.1 (2017): 1-11.

[2] Drever, R. W. P., et al. "Laser phase and frequency stabilization using an optical resonator." *Applied Physics B* 31.2 (1983): 97-105.

[3] Ma, Zhiya, Yueping Guan, and Huizhou Liu. "Superparamagnetic silica nanoparticles with immobilized metal affinity ligands for protein adsorption." *Journal of Magnetism and Magnetic Materials* 301.2 (2006): 469-477.

[4] Boyanton Jr, Bobby L., and Kenneth E. Blick. "Stability studies of twenty-four analytes in human plasma and serum." *Clinical chemistry* 48.12 (2002): 2242-2247.

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