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MEMS biosensor for detection of Hepatitis A and C viruses in serum

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ABSTRACT

Resonant microcantilever arrays are developed for the purpose of label-free and real-time analyte monitoring and biomolecule detection. MEMS cantilevers made of electroplated nickel are functionalized with Hepatitis antibodies. Hepatitis A and C antigens at different concentrations are introduced in undiluted bovine serum. All preparation and measurement steps are carried out in the liquid within a specifically designed flowcell without ever drying the cantilevers throughout the experiment. Both actuation and sensing are done remotely and therefore the MEMS cantilevers have no electrical connections, allowing for easily disposable sensor chips. Actuation is achieved using an electromagnet and the interferometric optical sensing is achieved using laser illumination and embedded diffraction gratings at the tip of each cantilever. Resonant frequency of the cantilevers in dynamic motion is monitored using a self-sustaining closed-loop control circuit and a frequency counter. Specificity is demonstrated by detecting both Hepatitis A and Hepatitis C antigens and their negative controls. This is the first report of Hepatitis antigen detection by resonant cantilevers exposed to undiluted serum. A dynamic range in excess of 1000 and with a minimum detectable concentration limit of 0.1 ng/ml (1.66 pM) is achieved for both Hepatitis A and C. This result is comparable to labeled detection methods such as ELISA.

1. Introduction

Detection of pathogens in liquid medium has significant applications in medicine (for the monitoring of biomarkers in body fluids) and in public health (for the monitoring of disease agents in water). For point-of-care (POC) diagnostics applications, compact and label-free sensors that can operate directly with body fluids without requiring expertise are desired.

There are many methods for biosensing (Arlett et al., 2011). MEMS cantilever sensors are promising candidates with good performance and rapid measurement times. Dynamic cantilever operation is based on detecting the frequency change in response to accreted mass on the cantilever surface (Gupta et al., 2006). Although mass detection limits on the order zeptograms can be achieved in vacuum (Yang et al., 2006), damping due to the liquid environment is a major drawback of the dynamic-mode operation (Hansen and Thundat, 2005). The associated decrease in the quality factor of the resonator turns liquid-phase measurements into a challenge. Usual practice, the so-called "dip & dry" method, has been to measure samples in air after the cantilevers are treated with target molecules. This leads to (i) loss of ability to work with activated microbes (Nugaeva et al., 2005), (ii) decreased appeal for POC applications (Skottrup et al., 2008), (iii) loss of insight into actual kinetics of biochemical surface reactions (Kwon et al., 2009), (iv) wetting or change in stiffness due to surface adhesion or stress and (v) decrease in affinity due to drying (Nugaeva et al., 2005). Despite the low quality factor, commercial AFM cantilevers have been used in the detection of *Vibrio cholera* O1 in solution (Sungkanak et al., 2010). Manalis (Lee et al., 2010) addressed the liquid operation challenge by operating the cantilever in air and building the liquid channels inside the cantilever. This method works well but is able to work with only very small sample volumes in small channels. Damping effect in liquid environment can be reduced by operating the micro-cantilevers at higher order vibration modes (Timurdogan et al., 2010b). Millimeter-size piezoelectric cantilevers operated in higher-order modes have also been used to overcome damping issues in liquid environment. Although going from micron to millimeter-scale inherently reduces natural frequency and thereby worsens the detection limits, utilizing higher-order modes allowed detection of 50–100 cells/ml concentrations of *Escherichia coli* O157:H7 in broth and beef samples (Campbell et al., 2007). Detection limits can be further decreased to 10 cells/ml if measurements are carried out in PBS buffer (Maraldo et al., 2007). Similar performance in solution is demonstrated on *Cryptosprodium parvum* oocyst and *Giardia lamblia* parasite (Campbell and Mutharasan, 2008; Xu and Mutharasan, 2010). Instead of the higher order vibra-

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Table 1
Sample preparation protocol for functionalization and measurements.

Biosensor Chip 1		Biosensor Chip 2	
Process	Concentration	Process	Concentration
DSP cross linker	2 mM	DSP cross linker	2 mM
Hepatitis A antibody	~1000 ng/ml	Hepatitis C antibody	~1000 ng/ml
Bovine serum	Undiluted	Bovine serum	Undiluted
	0.04		0.04
Negative control	0.1	*Negative control*	0.1
Hepatitis C antigen (ng/ml)	1	Hepatitis A antigen (ng/ml)	1
	10		10
	100		100
	0.04		0.04
Positive control	0.1	*Positive control*	0.1
Hepatitis A antigen (ng/ml)	1	Hepatitis C antigen (ng/ml)	1
	10		10
	100		100

tion mode, piezoelectric cantilevers were demonstrated at their first longitudinal extension mode to reduce the effects of damping (Capobianco et al., 2009).

Hepatitis is an inflammation of the liver that can be caused by different viruses including Hepatitis A, B and C (HAV, HBV, and HCV). It is important to detect the presence of viruses in serum and also monitor patients who acquired these viruses during prognosis. Currently, the detection of these viruses is carried out either by serological tests or by PCR where viral nucleic acid is detected in hospitals in a laboratory setting. Although molecular Nucleic Acid Testing (NAT) is preferable in terms of its sensitivity (Kuhns and Busch, 2006) for blood screening and diagnosis, NAT may miss some positive samples in some cases of occult HBV (Raimondo et al., 2007). It is also shown that NAT sensitivity is affected by viral load and pool size and it is too costly to test individual samples by NAT (Sato et al., 2001). Even by using individual HBV NAT, alternative screening tests may be necessary to prevent some HBV transmission through blood transfusion. Sensitivity of serological tests, like ELISA, is dependent on the commercial brand and the type of antibody been used (Yazdani et al., 2010). Their sensitivity varies between 0.2 ng/ml and 10 ng/ml. Those tests require expertise and advanced sample preparation involving labels. They are not suitable for portable application.

Only a few Hepatitis studies exist with label-free cantilever biosensors. Piezoelectric cantilevers are usually used for this purpose (Skladal et al., 2004; Backmann et al., 2005). Frequency sweep or chirped excitation signal instead of self-excitation with feedback is employed to detect frequency shifts in liquid. One of the reported works is based on DNA hybridization using HBV virus DNA precore/core region (243-mer) as the target (Cha et al., 2009). Detection limits on the order of picomolar concentrations can be achieved. This can be further reduced to femtomolar concentrations, if the mass effect is amplified by the use of silica nanoparticles attached to target DNA. In another study, same group used RNA aptamer as the receptor for HCV virus helicase (Hwang et al., 2007). The reported detection limit for the helicase is 0.1 ng/ml. These studies are important contributions to Hepatitis literature. However, those measurements are in buffer solution and not using biological samples like serum or blood. Furthermore, selectivity or specificity is not reported either.

In our prior work, detection of His-tagged κ-opioid (HKor) protein with 6 ng/ml minimum detection limit in buffer solution was reported (Timurdogan et al., 2010b). The custom flowcell design was presented recently in Timurdogan et al. (2010a) and Urey et al. (2011). Eliminating the drawbacks of dip & dry method, the use of flowcell is found to greatly improve sensitivity and selectivity. Using the same approach we report here for the first time highly specific and sensitive detection of HAV and HCV using dynamic mode microcantilevers without any labels or pre-amplification methods. Mechanical resonators especially microcantilevers are proved to be best candidate for biosensing due to their high dynamic range and sensitivity (Arlett et al., 2011). Unlike most cantilever-based sensors, all measurements are performed in liquid media and the antigens are introduced within undiluted (100%) fetal bovine serum (FBS). Although actuation and readout efficiency is decreased due to low quality factor (Q) and optical scattering in liquid, this method is replicating *in vivo* environment by placing undiluted serum and markers in a free flowing temperature stabilized flowcell. Liquid phase generally increases affinity based on biological molecular recognition (Backmann et al., 2005; von Muhlen et al., 2010; Waggoner et al., 2009). The sensor chip has no electrical connections and immune to vibration noise (Timurdogan et al., 2010b) making it attractive for portable diagnostics instrument applications with an easily disposable cartridge.

2. Materials and methods

In this work, electroplated nickel microcantilevers with magnetic actuation and optical detection using laser illumination are employed. Both the actuation and sensing methods are remote; therefore, no electrical connections are necessary. Fabrication process and optical detection based on embedded diffraction gratings were reported earlier (Ozturk et al., 2008). This is a robust optical readout method and unlike typical interferometric detection methods, it has good immunity to environmental vibrations (Timurdogan et al., 2010b). Dynamic-mode operation in liquid medium is made possible through the use of a self-starting feedback control loop that includes a broadband phase shifter and coil amplifier. The readout signal is obtained using a frequency counter with 10 s integration time. Each sample reading is achieved within 20 s. This operation leads to optimal measurement conditions, even if the first bending mode of the cantilevers is utilized.

2.1. Experimental procedure

Two biosensor chips were used in the experiments for testing HAV and HCV. Protocol is presented in Table 1 for the functionalization and other treatments. Briefly, for both biosensor chips, immobilization of antibodies (functionalization) utilized Dithiobis succinimidyl propionate (DSP) (Biochemika) for the gold-coated surface of the cantilever. DSP was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mM just before use, because DSP is moisture-sensitive. Cantilevers were immersed into DSP solution for half an hour at room temperature, and then washed with DMSO in the flow cell to remove unbound DSP with a syringe. For Biosensor Chip 1 HAV virus VP3 protein antibody (2 mg/ml) (Abcam) was diluted in 0.05% Tween 20/TBS pH 7.4 to a final concentration of 0.2 mg/mL. The cantilevers (with DSP on the gold surface) were

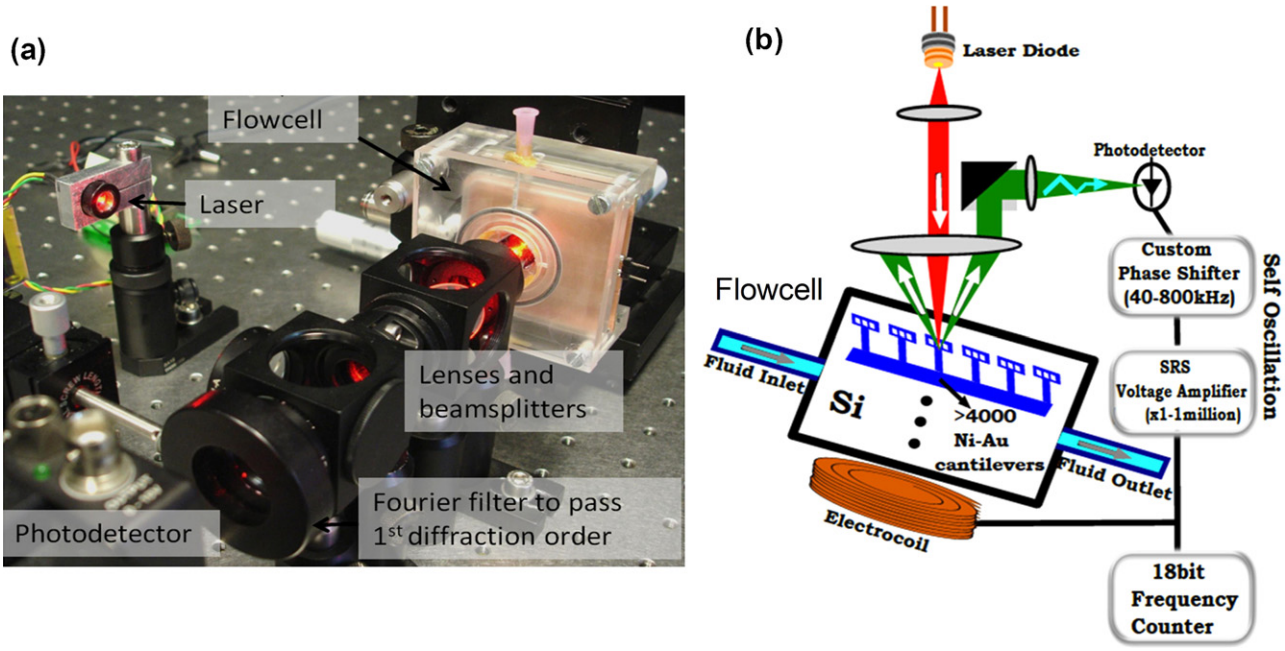

Fig. 1. (a) Flowcell developed for biosensing experiments. (b) Schematic view of the closed-loop controlled broadband magnetic actuator and optical readout with integrated diffraction grating.

coated with anti HAV antibodies solution for 1 h. The cantilevers were then rinsed with 0.05% Tween 20/TBS 3 times for 5 min. Then, both HAV and HCV antigen (Abcam) were serially diluted directly in the fetal bovine serum (FBS) (concentration of both antigen ranged from 0.04 ng/mL to 100 ng/mL). First, Biosensor chip 1 functionalized with anti-HAV was incubated in FBS that contain HCV antigen in serum for each specified concentration one after another and then washed 3 times with PBS. Measurements were performed after the wash cycles for each concentration in the PBS buffer.

After finishing the measurement with negative control, a similar experiment was carried out using FBS that contain HAV antigen. Biosensor chip 2 is functionalized with HCV antibody with the method described above. This chip was initially treated with negative controls with FBS that contain HAV antigen. Then the chip was washed with PBS and measurement was performed within the PBS buffer. Subsequently each chip was subjected to the HCV antigens at increasing concentrations up to the vicinity of the saturation level. Measurements were taken at different concentration levels and using 10–16 different cantilevers for each chip. Each set of experiments for the addressed cantilevers are finished in 30 min.

All treatment was performed in the flow cell and in liquid phase. Since cantilever performance is enhanced optically and mechanically in PBS buffer compared to serum (due to minimized scattering and low viscosity), we performed all measurement in the PBS. This also enables to take reference in PBS buffer before the exposure of serological complex. Measurement in PBS without drying the samples proved to be important in achieving excellent sensitivity and selectivity.

3. Results and discussion

3.1. Compact custom biosensing platform and experimental setup

Fig. 1a shows a picture of the experimental setup including (i) custom flowcell with <1 ml sample volume, (ii) MEMS chip with about 4000 cantilevers in 10 mm × 10 mm area. Optical detection is carried out using a 5 mW laser diode, diffraction gratings embedded at the tip of each cantilever, a photodetector, and lenses. The entire readout electronics can be miniaturized and made portable. A camera is also placed in the setup for imaging the cantilevers and to help align the laser. Fig. 1b shows the details of the optical measurement setup and the electronics. Laser is focused on the cantilevers through a transparent window attached on the flowcell and different cantilevers can be addressed by scanning the laser across the sensor die without requiring refocusing. Silicon substrate surface formed after KOH etch serves as the reference surface for the diffraction grating interferometer, whereas Ni surface on the grating platform is the moving surface of the interferometer. Since the reference surface and the moving surface are on the same die, the interferometric optical sensor is immune to environmental vibrations that are at much lower frequencies compared to >20 kHz operation frequencies (Timurdogan et al., 2010b). An aperture to serve as a Fourier filter in front of the detector is used to block the undesired diffraction orders. Either 0th or 1st diffraction order is passed onto the low noise silicon photodetector. Resonating cantilever produces a sinusoidal AC signal riding on a large DC bias. Thanks to the AC-coupled detection method, even nanometer-scale deflections can be detected with ease. A frequency counter is used to average several million cycles within a few seconds and allows sub-Hz frequency sensitivity even in liquids. The feedback control loop keeps the system at resonance by locking to the 90° phase difference between the input and the output. The control system is self-starting by using thermal vibrations and allows measurement results within 10–20 s. Local heating of the cantilever and induced frequency shift due to laser radiation are minimized by switching off the laser at 20 s intervals. Overall temperature of the flowcell was in the range 25–27 °C during the measurement. Compensator electronics cancel the inductive effects for a broad range of frequencies, which increased the coil current more than 30 times. Sensitivity and selectivity results are improved significantly using a flowcell and proved superior compared to “dip & dry” method, which is time consuming and unreliable. Fig. 2 shows the open-loop frequency response of the cantilevers in air and in liquid obtained using a frequency sweep. Liquid operation reduces the damped resonant frequency, the deflection, and the Q-factor from >100 to a

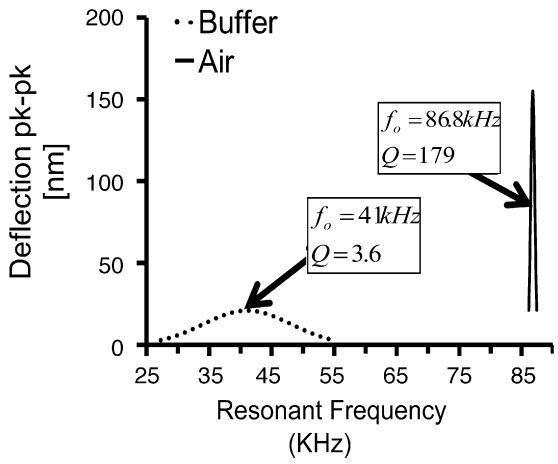

Fig. 2. Frequency response of one of the microcantilevers in air and in liquid. Deflection is measured with a laser Doppler vibrometer (LDV).

level of 4–10, which is still sufficient to start the self-sustaining oscillations with the closed-loop electronics.

Fig. 3a shows a close-up microscope picture of four cantilevers and the diffraction gratings fabricated at the tip of each cantilever. The slits that form the grating are 2–3 μm wide.

With the optical readout method described above, it is possible to perform readout of multiple sensors simultaneously using an array of optical detectors that are placed at an optical conjugate plane with the MEMS sensors. Since the photodetector pitch and the MEMS cantilever pitch are both lithographically defined, alignment of the photodetector array to the MEMS chip would be relatively straightforward.

3.2. Microfabrication of cantilevers

As reported in Ozturk et al. (2008), microfabrication is a simple one-mask process. Cantilevers are made of electroplated nickel of around 1 μm thickness with a thin Au layer underneath. Au has a thickness of 100 nm and serves as (i) seed layer during electroplating, and (ii) functionalization platform for biosensing. Timed etch of Au is critical to ensure the presence of Au layer underneath the cantilevers. This is also verified by SEM by removing the selected cantilevers from the chip and checking their backside for the presence of Au. Electroplating is followed by a final release in KOH to a depth of around 10 μm. A detailed fabrication flow was shown earlier (Timurdogan et al., 2010b).

3.3. Functionalization of cantilever surface

Both anti-HAV and anti-HCV are attached to the Au surface through a DSP linker. Then SEM is performed to see first the functionalization of Au surface with anti-HCV to its antigen. Fig. 3c shows a typical Au surface prior to functionalization. After functionalization, only an energy-dispersive X-ray spectroscopy (EDS) analysis can demonstrate a uniform distribution of anti-HCV proteins on the Au surface (Fig. 3d), where visually there is no difference. After exposure to HCV antigen, surface morphology changes drastically and complex antibody and antigen can be seen in Fig. 3e.

3.4. Detection of Hepatitis A and C

Undiluted serum is used for the experiments. One can obtain the serum from the whole blood using simple filtration, lab-on-chip sample preparation chips, or centrifugation (Strychalski et al., 2009). Separate cases are considered, and frequency measurements are taken using a series of 10–16 cantilevers in each case. Cantilevers functionalized with HAV antibody are exposed to either HAV (Case 1) or HCV (Case 2) antigens in increasing concentrations in undiluted serum, which contains many other proteins at various concentrations. Similarly, cantilevers functionalized with HCV antibody are exposed to either HAV (Case 3) or HCV (Case 4) antigens. This approach allows one to check for selectivity of the sensor in addition to standard performance criteria such as the minimum detection limit, sensitivity and linear range. Furthermore, in order to demonstrate repeatability, two sets of data are presented regard-

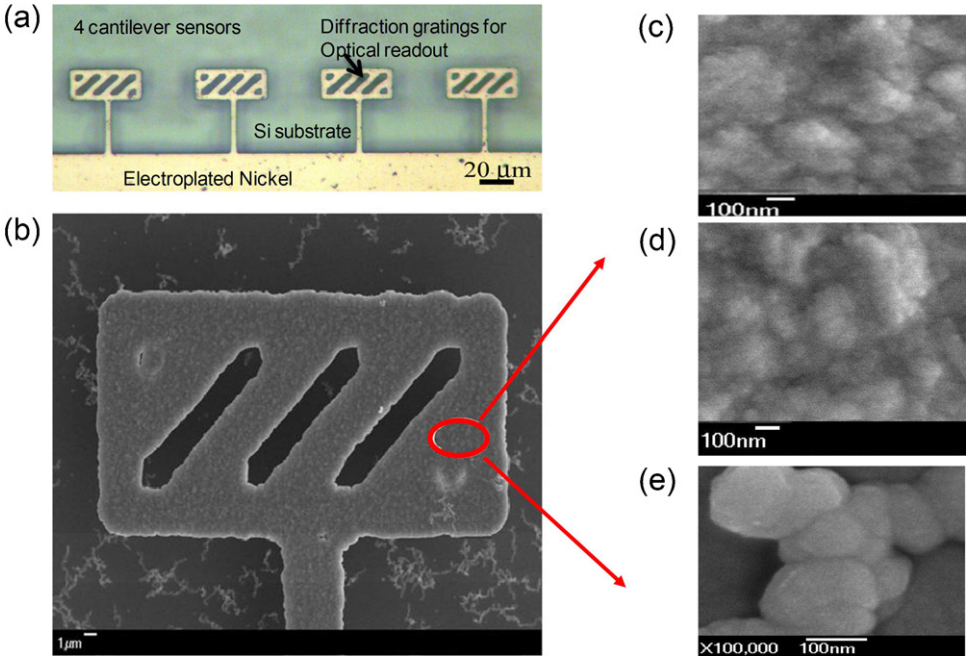

Fig. 3. (a) Close-up microscope picture of four cantilever. SEM pictures of a cantilever. (b) Full view of a cantilever and diffraction grating, (c) before functionalization, (d) after functionalization with HAV antibody, and (e) after exposed to HAV antigen. SEM pictures are taken from back side of the cantilevers.

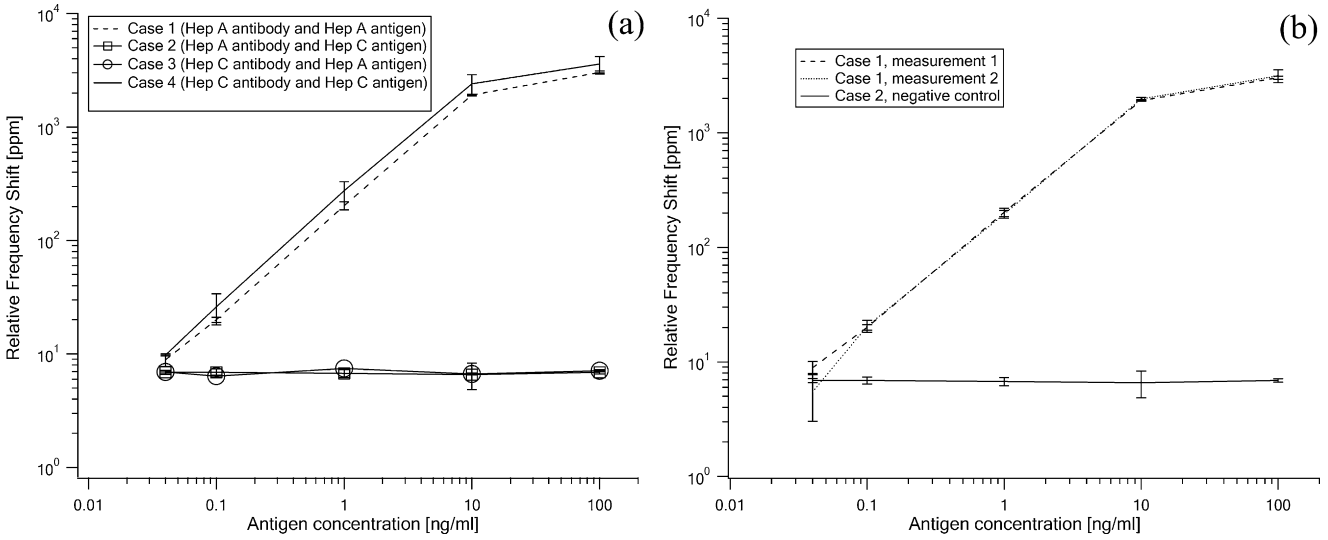

Fig. 4. (a) Hepatitis detection measurement results using two biosensor chips. Measurements are taken at concentrations indicated in Table 1 for negative controls and positive controls. Different HAV and HCV concentrations are introduced in fetal bovine serum. Minimum detection limit of 0.1 ng/ml is confirmed in both experiments. Negative controls demonstrate excellent selectivity and dynamic range. (b) Repeatability study for Case 1 in (a) using the same protocol but different MEMS chips. Both dynamic ranges, sensitivity, and selectivity are very similar in two experiments. Number of cantilevers measured in two experiments are 11 and 15 as indicated in Table 2. Figure shows only averages and error bars in normalized scale.

ing Case 1 (HAV antibody against HAV antigen) (Fig. 4a and b). In all cases reference cantilever measurements are taken as an offset to be removed from actual measurements. In order to improve sensor performance and have a sensitive reference data, measurements are taken in PBS after serum and before and after HAV and/or HCV treatment, which is first time in literature to our best knowledge. This is done to remove the actuation and readout drawbacks from serum treatment and to get rid of nonspecific binding that comes from the serum (which contains many different proteins). Initial results with HAV and HCV were previously reported in Urey et al. (2011) but the detailed protocol and the statistical data in Tables 1 and 2 are reported here for the first time along with the proof of selectivity.

Fig. 4 summarizes measured frequency shifts as a function of the utilized antigen concentration up to 100 ng/ml in all of the four cases. A detailed analysis of data can also be found in Table 2. It is clear that both Cases 1 and 3 exhibit very similar sensitivities around 190 ppm/(ng/ml) based on the slope of the curve for low concentrations. On the other hand, Cases 2 and 4 are negative controls performed for the testing of unspecific binding and the verification of high selectivity. Furthermore, measurements on Cases 1 and 3 reveal the same minimum detectable concentration of 0.1 ng/ml (1.66 pM – during the calculation molecular weight of each antigen of 60 kDa were used), which is comparable to the best results obtained through labeled sensing methods previously reported by ELISA (Yazdani et al., 2010). Linearity of the sensor response is also preserved up to 100 ng/ml (~1.66 nM), beyond which saturation takes place. Using the experimental results, dynamic range of this biosensing platform is found to be in excess of 1000:1 for the specific type of the Hepatitis antibody.

As far as repeatability is concerned, both runs in Case 1 result in very similar behavior as evident in Fig. 4b. In addition to chip-to-chip variations, variations among different cantilevers in an array on the same chip are also considered and indicated by using error bars on both Fig. 4a and b. It is to be noted that the standard deviation is <5% of the mean for all detectable concentrations for the HAV_Ab&Ag_Exp1 case in Table 2 (<10% for most other cases) and

Table 2
The mean value (μ) and standard deviation (σ) of relative resonant frequency shift in units of parts-per-million (ppm) for 11 cantilevers (15 cantilevers for exp 1 only) monitored during the experiments as a function of concentration. Actual resonant frequencies of the cantilevers are in 50–100 kHz range. 'Ab' represents antibody and 'Ag' represents antigen.

Negative control experiments for selectivity

Concentration [ng/ml]	Hep A Ab & Hep C Ag μ [ppm]	σ [ppm]	Hep C Ab & Hep A Ag μ [ppm]	σ [ppm]
0.04	6.90	0.29	6.93	0.13
0.10	6.89	0.49	6.37	0.14
1	6.74	0.55	7.45	0.12
10	6.58	1.73	6.67	0.13
100	6.89	0.23	7.11	0.15

Control experiments for sensitivity and repeatability

Concentration [ng/ml]	Hep A Ab & Ag Exp 1 μ [ppm]	σ [ppm]	Hep A Ab &: Ag Exp 2 μ [ppm]	σ [ppm]	Hep C Ab & Ag μ [ppm]	σ [ppm]
0.04	8.89	1.16	5.47	2.45	9.80	0.15
0.10	20.01	1.08	20.52	2.45	26.01	7.95
1	202.86	16.71	195.5	14.92	275.0	55.80
10	1918	37.82	1976	58.20	2410	489.0
100	3029	94.42	3147	406.4	3600	590.0

the peak-to-peak variation is confided to <10% for most of the data points illustrated in Fig. 4a and b.

4. Conclusion

The detection of Hepatitis viruses in serum is a critical issue that is currently carried out through serological tests or PCR. Use of nanomechanical resonators has recently found wide applications in biosensors. With their superior sensitivity and potential for multiplexing they emerge as an alternative to existing detection techniques of pathogens. This work is the first report that demonstrates the use of nanomechanical resonators in the case of Hepatitis in serum and provides a thorough evaluation of their performance in terms of specificity, sensitivity and resolution. The following conclusions are drawn

- Specificity can be achieved through the proposed technique. Simultaneous detection of both HAV and HCV is demonstrated.
- A minimum detection limit of 0.1 ng/ml (1.66 pM) is obtained in HAV and HCV.
- A nearly linear dynamic range of 1000:1 demonstrated in the experiments, which allows reporting of quantitative results.
- A negligible variation in measurements is demonstrated leading to a satisfactory level of repeatability.
- The following improvements on previous resonance-based Hepatitis studies are achieved:
  - the use of undiluted FBS for exposure,
  - a truly label-free technique with no mass-amplification, and
  - a clear demonstration of selectivity.

In addition to aforementioned points, the proposed system also comprises a set of valuable properties that are necessary for the commercial success of a portable hand-held device. These include low power consumption, robustness (superior noise performance), the possibility of multiplexed readout, and low cost due to the simplicity of the sensors. Raising the possibility of a completely disposable sensor, the lack of any electrical connections to the sensor is also a major advantage.

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References

Arlett, J.L., Myers, E.B., Roukes, M.L., 2011. Nat. Nanotechnol. 6 (4), 203–215.
Backmann, N., Zahnd, C., Huber, F., Bietsch, A., Pluckthun, A., Lang, H.P., Guntherodt, H.J., Hegner, M., Gerber, C., 2005. Proc. Natl. Acad. Sci. USA 102 (41), 14587–14592.
Campbell, G.A., Mutharasan, R., 2008. Biosens. Bioelectron 23 (7), 1039–1045.
Campbell, G.A., Uknalis, J., Tu, S.I., Mutharasan, R., 2007. Biosens. Bioelectron 22 (7), 1296–1302.
Capobianco, J.A., Shih, W.H., Leu, J.H., Lo, G.C., Shih, W.Y., 2009. Biosens. Bioelectron 26 (3), 964–969.
Cha, B.H., Lee, S.M., Park, J.C., Hwang, K.S., Kim, S.K., Lee, Y.S., Ju, B.K., Kim, T.S., 2009. Biosens. Bioelectron 25 (1), 130–135.
Gupta, A.K., Nair, P.R., Akin, D., Ladisch, M.R., Broyles, S., Alam, M.A., Bashir, R., 2006. Proc. Natl. Acad. Sci. USA 103 (36), 13362–13367.
Hansen, K.M., Thundat, T., 2005. Methods 37 (1), 57–64.
Hwang, K.S., Lee, S.M., Eom, K., Lee, J.H., Lee, Y.S., Park, J.H., Yoon, D.S., Kim, T.S., 2007. Biosens. Bioelectron 23 (4), 459–465.
Kuhns, M.C., Busch, M.P., 2006. Mol. Diagn. Ther. 10 (2), 77–91.
Kwon, T., Park, J., Yang, J., Yoon, D.S., Na, S., Kim, C.W., Suh, J.S., Huh, Y.M., Haam, S., Eom, K., 2009. PLoS One 4 (7), e6248.
Lee, J., Chunara, R., Shen, W., Payer, K., Babcock, K., Burg, T.P., Manalis, S.R., 2010. Lab Chip 11 (4), 645–651.
Maraldo, D., Rijal, K., Campbell, G., Mutharasan, R., 2007. Anal. Chem. 79 (7), 2762–2770.
Nugaeva, N., Gfeller, K.Y., Backmann, N., Lang, H.P., Duggelin, M., Hegner, M., 2005. Biosens. Bioelectron 21 (6), 849–856.
Ozturk, A., Ocakli, H.I., Ozber, N., Urey, H., Kavakli, I.H., Alaca, B.E., 2008. IEEE Photon. Technol. Lett. 20 (21-24), 1905–1907.
Raimondo, G., Pollicino, T., Cacciola, I., Squadrito, G., 2007. J. Hepatol. 46 (1), 160–170.
Sato, S., Ohhashi, W., Ihara, H., Sakaya, S., Kato, T., Ikeda, H., 2001. Transfusion 41 (9), 1107–1113.
Skladal, P., dos Santos Riccardi, C., Yamanaka, H., da Costa, P.I., 2004. J. Virol. Methods 117 (2), 145–151.
Skottrup, P.D., Nicolaisen, M., Justesen, A.F., 2008. Biosens. Bioelectron 24 (3), 339–348.
Strychalski, E.A., Henry, A.C., Ross, D., 2009. Anal. Chem. 81 (24), 10201–10207.
Sungkanak, U., Sappat, A., Wisitsoraat, A., Promptmas, C., Tuantranont, A., 2010. Biosens. Bioelectron 26 (2), 784–789.
Timurdogan, E., Nargul, S., Kavakli, I.H., Alaca, B.E., Urey, H., 2010a. Proceedings of the International Conference on Optical MEMS and Nanophotonics , Sapporo, Japan, pp. 9–10.
Timurdogan, E., Ozber, N., Nargul, S., Yavuz, S., Kilic, M.S., Kavakli, I.H., Urey, H., Alaca, B.E., 2010b. Biosens. Bioelectron 26 (1), 195–201.
Urey, H., Timurdogan, E., Ermek, E., Kavakli, I.H., Alaca, B.E., 2011. Proceedings of the 24th IEEE International Conference on Micro Electro Mechanical Systems , Cancun, Mexico, pp. 920–923.
von Muhlen, M.G., Brault, N.D., Knudsen, S.M., Jiang, S.Y., Manalis, S.R., 2010. Anal. Chem. 82 (5), 1905–1910.
Waggoner, P.S., Varshney, M., Craighead, H.G., 2009. Lab on a Chip 9 (21), 3095–3099.
Xu, S., Mutharasan, R., 2010. Environ. Sci. Technol. 44 (5), 1736–1741.
Yang, Y.T., Callegari, C., Feng, X.L., Ekinci, K.L., Roukes, M.L., 2006. Nano Lett. 6 (4), 583–586.
Yazdani, Y., Roohi, A., Khoshnoodi, J., Shokri, F., 2010. Avicenna J. Med. Biotech. 2, 207–214.