Gravimetric biosensor based on a 1.3 GHz AlN shear-mode solidly mounted resonator

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ABSTRACT

We investigate the performance of solidly mounted resonators based on Ir/tilted-AlN/Ir piezoelectric stacks as biosensors. These films are deposited by varying the pressure, the cathode power and the temperature of a two-step process based on depositing (00.2)-tilted AlN active layers over an (10.3)-oriented AlN seed layer. To minimize the influence of the temperature coefficient of frequency on the stability of the biosensor, we use insulating acoustic mirrors made of layers of SiO₂ and amorphous TaO_x with non- $\lambda/4$ thicknesses, which enables to reduce the TCF to -14 ppm/°C. The mass loading of the resonators with SiO₂ thin films results in a sensitivity of 1800 kHz/pg·cm². Surface functionalization consists on the binding of silane groups on plasma oxidized SiO₂ surfaces. After a glutaraldehyde link, streptavidin is bonded to the surface to receive biotinylated receptors for several species. We test thrombin-binding aptamer (TBA29 against thrombin, and IgG antibody against immunoglobulin). The sensors response to species of different molecular weight like TBA-29 (9.75 kDa) or IgG antibody (150 kDa) is monitored. Finally, we assess the response of the biosensors to different thrombin concentrations (ranging from 4 nM to 270 nM) on surfaces functionalized with the TBA29 aptamer.

Keywords:

Gravimetric biosensor
Shear mode AlN resonators
Surface functionalization
Sensor sensitivity

1. Introduction

In the last decade, there has been an increasing need for developing highly sensitive biosensors suitable for real-time detection of all type of species, like proteins, DNA chains, toxins, virus, or bacteria. Among the different types of biosensors, electroacoustic resonators based on piezoelectric materials combine the advantages of having ultra-high sensitivity, small size and electronic signal output, in addition to being label-free, easy-to-use, and low-cost. Their operation is based on the frequency shift when a mass binds to their surface; specific binding methods for targeted species allow for selectivity. Since the sensitivity of resonant sensors increases with the squared resonant frequency [1], high frequency resonators are preferred. Quartz crystal microbalances are well-established gravimetric biosensors used since 1972 [2]. However, their detection limit lies around few nanograms, not enough for the detection of small molecules (<50 kDa) in low concentrations (<100 nM).

To lower the detection limit to the picograms range, higher frequencies are needed, which has boosted the development of bulk acoustic wave (BAW) resonators based on piezoelectric thin films operating in the GHz range. AlN and ZnO-based resonators have been developed some ten years ago [3,4] for in-liquid operation. The technologies for fabricating AlN-based BAW resonators, either as solidly mounted resonators (SMRs) or as free-standing film bulk acoustic resonators (FBARs), are mature and already in production for electronic applications [5,6]. Besides, thin-film-based resonators have lower fabrication costs and offer the possibility of integration with CMOS technologies and fabrication of arrays of sensors for parallel or multi-detection [7]. The main challenge for these devices is the excitation of the shear mode, capable of maintaining a good quality factor during in-liquid operation, because this mode hardly propagates in liquids, as opposed to the longitudinal mode. Excitation of such mode requires the application of electric fields perpendicular to the crystallographic c-axis of the piezoelectric film. Unfortunately, shear mode excitation through coplanar electrodes in c-axis oriented films is too weak for the foreseen applications [8]. A more effective excitation is achieved through the transverse excitation of AlN microcrystalline films with c-axis tilted with respect to the normal of the surface. The use of textured surfaces together with off-axis sputter deposition allows

us to grow this kind of films in which the shear mode is efficiently excited using conventional sandwiched structures [9].

In-liquid operation of resonator-based sensors usually requires the isolation of the electrical contacts from the ion-containing solutions. In-wafer integrated microfluidic systems are frequently used [10], although discrete fluidic systems are preferred to reduce costs and allow for more flexible designs [11]. Isolation techniques require extending the electrical contact pads to take them away from the wet zone. These extensions need a careful design to minimize electrical parasites, which could alter the measurements of the electrical response of the resonator by reducing both the electromechanical coupling factor (k^2) and the quality factor (Q) [12].

Good thermal stability of the resonant frequency is also essential for reliable sensing. Typical values of temperature coefficients of frequency (TCF) in AlN and ZnO-based devices range around $-25 \text{ ppm}/^\circ\text{C}$ and $-40 \text{ ppm}/^\circ\text{C}$ respectively, which limits the detection reliability. The TCF mainly arises from the temperature dependence of the elastic constants. A common technique to reduce the TCF is to compensate the negative temperature coefficients of AlN and ZnO elastic constants by adding a film with positive temperature coefficient, such as silicon dioxide or elinvar [13]. Both the thickness of the compensating layer and its position relative to the piezoelectric layer are essential to achieve good compensation. Whereas in FBARs the place of compensation layers is limited to a few positions [14], SMRs are complex structures involving a large number of layers, each of which contribute to a greater or lesser extent to the TCF, thus offering many possibilities for the design of the compensation structure. Since compensation inevitably degrades the performance of the devices, a tradeoff between compensation and performance must be achieved.

The functionalization process is an essential step in the fabrication of a biosensor, which is achieved by binding receptors for a targeted species to the active area of the device. Both the bond between the receptor and the surface of the resonator and that between the targeted species and the receptor must be strong enough to lead to changes in the resonant frequency on account of the overall device-mass increase. In other words, the binding must be acoustically effective. A receptor firmly bonded to the active area allows for label free, specific, and selective detection of the targeted molecule.

Sensor calibration has to tackle both the mass sensitivity, defined as the shift of the resonant frequency per unit of bonded mass, and the sensor sensitivity to the concentration of the targeted species. Mass sensitivity can be easily measured by weighting the resonator with a known mass of an inorganic rigid material and adjusting the frequency response of the device using well known models, such as Mason's model [15] or finite element modelling (FEM). The sensitivity to concentration of a particular species in the liquid is strongly influenced by the nature of the targeted species, the efficiency of their affinity reaction with the receptor, the changes in viscosity and density near the sensor surface and the dynamic of the liquid feeding process. Therefore, a biosensor has to be calibrated for each targeted species. A calibration curve depicting the quantitative output of the sensor as a function of the concentration of the targeted species provides the sensor sensitivity (slope of the curve) and the limit of detection (minimum detectable concentration).

In this paper, we describe the way to achieve gravimetric biosensors based on AlN thin films optimized to operate in liquid media. AlN films specifically grown with the c -axis tilted allows for an effective excitation of the shear mode. Overall device TCF is reduced by designing asymmetric acoustic reflectors that partially compensate the variations of the AlN sound velocity with temperature. A careful design of the extension of the electrical contacts enables using a fluidic system to feed the liquid to the active area of the sensor while keeping the electrical pads separated from the wet

zone. The performance of the biosensors is assessed by calibrating their mass sensitivity, investigating their response to species of different molecular weights and analyzing their response to a particular species (thrombin) as a function of its concentration in the fluid.

2. Material and methods

The core of the sensing technology is the piezoelectric stack formed by a thin AlN film containing tilted grains sandwiched between two iridium electrodes, which allows an effective excitation of both shear and longitudinal modes. The AlN active layers were sputtered with their wurtzite microcrystals tilted around 25° with respect to the normal through a two-stage sputtering process [9]. The method consists in the off-axis sputter process of the AlN active layer on a thin (10-3)-oriented AlN seed layer. The samples are separated 3 to 5 cm from the sputtering target axis to obtain a preferred directional atom flux. A high purity 150 mm Al target was sputtered in an Ar/N₂ (40:60) mixture using a pulsed-DC source (MKS ENI 235) operated at 50 kHz. The samples were deposited in an ultra-high-vacuum system pumped to a base pressure below 9×10^{-7} Pa. The 100 nm-thick AlN seed layers were deposited at high pressure (0.67 Pa), room temperature and low power (600 W) to promote the growth of the (10-3) preferred orientation by controlling the energy supplied to the adatoms reaching the substrate surface. The active AlN film was deposited at lower pressure (0.23 Pa), substrate temperature of 400°C and higher power (1200 W) to a thickness of around 700 nm, which sets the resonant frequency of the sensor to about 1.3 GHz. Both top and bottom electrodes were made of iridium, a metal that offers the advantage of having a very large acoustic impedance [16] and being chemically inert. To simplify the fabrication process, SMRs were excited from the top by capacitive coupling to avoid the etching of the piezoelectric layer. The bottom electrode was defined by Ar ion milling using a Mo layer as etching-mask, while the top electrode was defined by lift-off. Top electrode tracks to the connection pads were regrown with a $2 \mu\text{m}$ -thick Mo layer to reduce the series resistance. The acoustic isolation of the piezoelectric stack was achieved using a fully insulating acoustic reflector. The choice of insulating materials (porous SiO₂ [17] as low acoustic impedance material alternated with Ta₂O₅ [18] as high acoustic impedance material) allowed reducing the electrical parasitic capacitances [12]. The thickness of the different layers composing the whole structure were optimized to reduce the TCF of the device [19].

After fabrication, the devices were covered with a SiO₂ layer (for functionalization purposes) and diced into $9 \text{ mm} \times 9 \text{ mm}$ chips. The active surface was functionalized using a slightly modified standard APTES ((3-Aminopropyl) triethoxysilane)-GA (glutaraldehyde) functionalization protocol [20,21]. The reagents used were APTES 2% (Sigma-Aldrich) in ethanol (Merk), Glutaraldehyde (GA) (Sigma-Aldrich), streptavidin $10 \mu\text{g}/\text{ml}$ (Sigma-Aldrich) in NaCl 50 mM , thrombin-binding aptamer (TBA29) (Sigma-Aldrich).

Sensors were characterized by measuring their electrical impedance from 10 MHz to 10 GHz with an Agilent N5230A network analyzer using calibrated RF probes for on-wafer contacting. A fluidic system made of a laser-cut methacrylate and sealed with a nitrile O-ring was used for in-liquid measurements. A peristaltic pump enabled feeding the liquid inside the sensing chamber of $30 \mu\text{l}$ of capacity. For accurately tracking the resonant frequency during the bio-detection process, the maximum of the real part of the admittance was fitted to an eighth degree polynomial in a narrow frequency interval. The roots of its first derivative were calculated and the resonant frequency identified. This procedure was implemented in a LabView® application allowing measuring the frequency with 1 kHz accuracy each 3 s.

Fig. 1. The real part of the admittance spectra of a representative resonator working in air (blue dashed line) and in-liquid (red solid line). Note the major reduction in quality factor of the longitudinal mode relative to the shear one.

3. Results and discussion

3.1. Design of sensors

The fabrication of sensors for the detection of biological targets required paying attention to several issues. First, the sensor was intended to operate in liquid media (buffers), which usually display ionic conductivity. As mentioned above, the need to operate in fluids imposes the use of the shear mode to prevent excessive acoustic energy losses into the medium [22]. Fig. 1 displays the electrical response of an AlN resonator operating in air and in liquid. The two-stage method developed to obtain AlN films with tilted grains [9] enabled the fabrication of shear-mode resonators with effective coupling factors close to 3% and quality factor in air close to 250, dropping to 190 when operating in liquid.

Handling liquid samples was also a challenge for the design of the contact pads, as they had to be isolated from the sensing area. To take the contact pads away from the fluid, we investigated the effect of extending them to lengths ranging from 0.5 mm to 2 mm. These extensions behaved as a track of a waveguide and, thus, had to be carefully designed to avoid signal reflection that could prevent the sensor from being excited. We designed these tracks by using an electromagnetic simulator with finite element modelling (FEM) based on Ansys-HFSS platform and set the characteristic impedance of the line to 49.5Ω . Since the extensions were always significantly shorter than the wavelength of the 1.3 GHz electrical signal, their effect in the performance of the device was similar to that of a series inductance. The combination of this series inductance with the static capacitance and the series resistance of the resonator formed a leaky-LC tank circuit with a resonant angular frequency of $(LC)^{-1/2}$. Fig. 2 displays the effect of the electrical extensions on the frequency response of the devices, clearly showing that the parasitic resonance induced by the electrical extensions could seriously distort the shape of the resonant frequencies of the resonator. Experimental results show that the electrical extensions had to be shorter than 2 mm to keep the shear mode resonance away from the influence of the parasitic resonance, whereas a maximum extension of 0.5 mm was allowed before the longitudinal mode started to degrade. A detailed study of the effects of electrical extensions on the resonator behavior can be found in [12].

Fig. 2. Modulus of the electrical impedance spectra of some representative resonators with electrical extensions of different lengths. Both longitudinal and shear modes are visible, as well as the parasitic LC resonance arising from the parasitic inductance of the electrical extensions and the static capacitance of the resonator.

A second important issue related to the extensions of the contacts was the conductive character of substrates lying below the piezoelectric stacks. Common Bragg mirrors usually alternate SiO_2 films and metallic layers of high density (W, Mo, Ir) because this combination displays the largest acoustic impedance mismatch. However, extending the contact pads over substrates containing metallic layers introduced an additional parasitic capacitor in parallel with the resonator that seriously damaged its response. To eliminate this effect all the structure lying below the piezoelectric stack (acoustic reflector and substrate) had to be entirely insulating. Although insulating materials of high acoustic impedance are not commonly used in Bragg reflectors, the use of AlN [23], Si_3N_4 [24], or Ta_2O_5 [25] films has been previously reported. To preserve the electrical response of the devices we substituted the high impedance metallic layers by amorphous TaO_x films [18] and used highly resistive substrates (high resistivity silicon or glass).

The acoustic reflectors were also engineered to reduce the resultant TCF of the whole device [19]. The thickness of the uppermost SiO_2 film of a standard $\lambda/4$ Bragg reflector was increased to $\lambda/2$ and the thickness of the uppermost TaO_x layer was reduced nearly to $\lambda/8$. This design led to a reduction of the TCF from $-30 \text{ ppm}/^\circ\text{C}$ to $-14 \text{ ppm}/^\circ\text{C}$, but also slightly degraded the acoustic reflectance of the Bragg mirror.

Fig. 3 displays a scheme of the cross-section and a picture of the actual chip containing four devices.

In summary, the gravimetric biosensors consisted in Ir/tilted-AlN/Ir stacks lying over fully insulating $\text{SiO}_2/\text{TaO}_x$ acoustic mirrors grown on highly-resistive silicon wafers, which helped reducing

Fig. 3. a) Scheme of the transversal section of the SMRs. b) Plain view photo of a chip with four resonators.

the parallel parasitic capacitance of the structure. The thickness of high and low impedance layers deviated from $\lambda/4$ to reduce the TCF of the devices. The device layout included electrical extensions for taking the contact pads away from the active area in contact with electrically conductive liquids. They were designed using microwave considerations to minimize their effect on the resonator response. The contact to the bottom electrode was achieved by capacitive coupling with an extended contact coplanar with the top electrode to reduce the series resistance. The non-active metallic areas were regrown with a thick Mo layer to reduce series resistance.

3.2. Sensor functionalization

Surface functionalization of biosensors is key for the final performance of the device. Apart from obtaining a uniform distribution of the specific receptor molecules in the surface, rejection of non-specific binding of other molecules and reproducibility are the main issues for adequate operation. The ultimate aim of the functionalization process was to cover the entire active area of the resonator with receptors (aptamer or antibody) tightly bound to the surface with the right orientation, so that they could expose their binding site to the target. To achieve this goal we took advantage of the high affinity of streptavidin to biotin, and the fact that any receptor can be biotin-modified at any position within the molecule.

The Ir top electrode (active area) was the starting point of the functionalization platform, although the entire surface of the device shown in Fig. 3 (Ir active area, Mo path regrowth and AlN exposed areas) was exposed to the functionalization process that started with an O_2 plasma treatment to create OH groups. The functionalization process was successful on the Ir active area, provided it was initially covered with IrO_x or SiO_2 ad-layers. Regarding the other materials exposed to the plasma, the functionalization was poorly reproducible on AlN and ineffective in Mo. Because of its easier processing, we finally chose to cover the active areas with a SiO_2 ad-layer to form the functionalization platform.

The functionalization protocol, based on the standard APTES-GA method, had two stages; the first one was identical for every target (target-independent), while the second one involved specific receptors for each particular target. The first stage consisted in obtaining streptavidin molecules uniformly distributed over the surface of the sensor, whereas the second stage aimed at attaching biotin marked receptors for the chosen target. First, the resonator was immersed in oxygen plasma at a pressure of 13.3 Pa for 2 min to generate OH groups on the SiO_2 surface. Then, the resonator was incubated for 1 h at room temperature in a 2% solution of APTES in absolute ethanol. Afterwards, its surface was washed in absolute ethanol, dried with N_2 and cured in a furnace at 110 °C for 1 h. Following this step, the samples were immersed in a 1% glutaraldehyde in water solution to saturate the silane dangling bonds. After these four steps we obtained a surface with free aldehyde groups that could bind to the primary amine groups (NH_2^-) present in any protein, and especially in streptavidin. The next step consisted in incubating the samples for 1 h in a 10 μ g/ml streptavidin solution. After streptavidin binding, a blocking agent (typically BSA 1% in NaCl 50 mM) was used to minimize non-specific binding. At this point, the surface was prepared for the binding of the specific receptor for the targeted species.

The second stage consisted in the binding of a specific receptor containing a biotin molecule opposite to its binding site to promote its bond with the streptavidin-covered surface on account of the high affinity between biotin and streptavidin. For example, for thrombin detection, the resonator was immersed in the binding buffer (50 mM Tris-HCl pH 7.5, NaCl 100 mM, 1 mM $MgCl_2$) Tween-20 0.05%, BSA 0.1% (BBT-BSA) for TBA29 to equilibrate the surface previous to the assay. TBA29 biotin-modified aptamer 10 μ g/ml

Fig. 4. Mass sensitivity calibration of sensors of the same area with different k_s^2 expressed as the shift of the resonant frequency vs. added mass of SiO_2 .

BBT-BSA was recirculated for measuring the binding in real-time. In the case of IgG antibodies, we used phosphate buffer saline (Sodium Phosphate 10 mM pH7.4, NaCl 150 mM) Tween-20 0.05%, BSA 0.1% (PBST-BSA).

We first tested the viability of our devices as biosensors by detecting species with different molecular weight: streptavidin (52.8 kDa), TBA29 aptamer (9.75 kDa) and IgG antibody (150 kDa). Secondly, we calibrated the sensors with a particular affinity reaction. Functionalization was made with TBA29 aptamer, and the targeted molecule was thrombin with concentrations from 4 nM to 270 nM.

3.3. Sensor calibration

As mentioned in the introduction, sensor calibration must address two specific issues. The first is the mass calibration, consisting in quantifying the amount of signal (frequency shift) per mass unit. The second one is the concentration calibration, dealing with the relationship between the amount of signal with the concentration of the targeted species. This last is dependent on the specific affinity reaction occurring in the detection process. In this section, we will show both types of calibration.

To calibrate the mass sensitivity of the transducer, we analyzed the response of the resonator weighted with sputtered SiO_2 film of thicknesses ranging from 10 nm to 100 nm. This material had been extensively studied in our lab for its use in acoustic mirrors [17], being its actual mass density well known even for films as thin as 10 nm, which enabled us to calculate the mass added to the resonators as function of the thickness, provided the area of the resonator is known. The obtained value was 140 pg/nm. Fig. 4 shows the frequency shift of the shear mode as a function of the SiO_2 mass deposited on the surface of the top electrode for two types of resonators with different k_s^2 operating at 1.35 GHz. These data are compared with the theoretical response simulated with Mason's model, also included in the figure, which accurately fits the experimental data. A linear shift of the resonant frequency of 1.19 kHz/pg is observed, yielding a sensitivity of around 1800 kHz/pg·cm². Resonators with the same resonant frequency, but different k_s^2 coupling factor (1% and 2.5%) displayed roughly the same sensitivity.

These data pointed out that the sensors were suitable to characterize our functionalization protocol. After the silanisation of the surface, we could follow step by step the whole process by monitoring the changes in the resonant frequency. Since the weight of each

Fig. 5. Temporal frequency shift evolution when passing different species on functionalized sensors. TBA29 (9.75 kDa), Streptavidin (52.8 kDa), and polyclonal IgG antibody (150 kDa).

molecule was known, we could derive the number of molecules bonded to the surface in each step. To avoid errors due to changes in other parameters, like density or viscosity of the liquids, each measurement started by circulating the bare liquid used to prepare the solution containing the analyte. We assumed that the addition of the analyte in very low concentration did not alter the density or the viscosity of the sample.

Temperature changes was an additional issue to take into account, since small variations may cause significant frequency shifts, even though the TCF of our resonators had been reduced to $-14 \text{ ppm}/^\circ\text{C}$. We took a special care to ensure that all the liquids used in the assays were always kept at room temperature, which was continuously monitored and controlled within $\pm 1^\circ\text{C}$ during experiments. As mentioned above, the functionalization protocol included a common first stage that ended with the binding of the streptavidin, after which biotinylated specific receptors had to be used for each targeted species. Fig. 5 shows the evolution of the resonant frequency with time when sensors were exposed to streptavidin and to two biotinylated receptors.

The amount of detected mass in each experiment can be derived by dividing the maximum frequency shift by the mass sensitivity (1.19 kHz/pg). Once the mass determined, the density of molecules attached to the surface is obtained by dividing the total mass it by the mass of a single molecule (molecular weight over Avogadro's number) and by the sensor area ($5.5 \times 10^{-4} \text{ cm}^2$). As the change in frequency after TBA-29 (an aptamer against thrombin) detection is around 75 kHz , the total mass detected is 63 pg , which corresponds to a 6×10^{12} molecules/ cm^2 . In the case of the streptavidin, the frequency shift is 280 kHz , which corresponds to 235 pg of detected mass or a molecular area density of 4.1×10^{12} molecules/ cm^2 bound to the surface. Finally, for the IgG antibody, the change in frequency is 400 kHz , which corresponds to 335 pg bound to the surface, equivalent to an area density of 2.1×10^{12} molecules/ cm^2 . The lower density of molecules on the surface for larger molecules could be due to the size of such molecules, which may hide more than one receptor.

All these data show that, assuming that the frequency shift is only due to the addition of mass, the functionalization protocol was suitable for the different targeted species and provided a high density of binding sites for the receptors. Tests using non-biotinylated aptamers did not show any frequency shift, thus suggesting that the non-specific binding is very unlikely, probably because of the effective blocking effect of the surface with BSA.

To complete the characterization of our devices we measured the response of our sensors functionalized with TBA29 to different concentrations of thrombin. The frequency shift experienced as a

Fig. 6. Shift of resonant frequency of sensors when exposed to thrombin solutions of different concentrations. A steady state is reached for all concentrations tested (not shown in the figure), although the saturation value is only achieved for concentrations of 80 nM and above.

function of time for different concentrations of thrombin is shown in Fig. 6.

It is worth noting that the curves show a larger slope (proportional to reaction velocity) as the concentration of thrombin increases. A maximum frequency shift of 180 kHz is observed for concentration higher than 80 nM , suggesting that the surface reaches the saturation. For lower concentration the frequency shift in steady state decreases as the concentration becomes lower. As expected, the saturation of the surface is reached sooner as the concentration is increased. Actually, the rate of thrombin binding (slope of the curves) follows a monotone variation with thrombin concentration, as shown in Fig. 7. Therefore, for obtaining the actual value of the concentration, one should monitored the slope of the variation of the frequency shift with time and not only the total shift achieved.

Finally, there is an additional issue that we have disregarded in the present work were only small molecules are involved, but that could have a significant influence when dealing with the detection of large specimens. We assume that small molecules bind to the surface in such a way that they perfectly follow the movements of the sensor surface, increasing only the effective mass of the resonator. However, inertial forces and viscous forces between molecules and liquid are always present, being larger as the mass and size of the molecules increase. These forces make the acoustic integration harder, decreasing the gravimetric effect (shift of frequency by increasing mass of the resonator). This raises a relevant question concerning the use of high frequency resonators as biosensors: what is the size of the species above which these forces have to be considered? Some references in the literature dealing with the detection of whole bacteria with QCM address this issue [26,27]. A

Fig. 7. Maximum of the slope of the frequency variation with time and maximum frequency shift of the sensors after exposure to thrombin solutions of different concentrations.

whole bacterium is obviously larger than a protein, DNA chain, or antibody, but there must be an intermediate situation from which the size of the species starts to influence the gravimetric sensing. This important issue should be the focus of a deeper analysis.

4. Conclusions

Gravimetric biosensors based on AlN shear-mode SMRs are fabricated and tested. The design of the devices is optimized in order to address most of the technical problems that may arise when a sensor operates in liquid media. Firstly, AlN films are engineered to grow with their c-axis tilted, to achieve the excitation of the shear mode, required for in-liquid sensing applications. Secondly, fully insulating substrates, which include high resistivity silicon wafers and SiO₂/TaO_x-based acoustic reflectors, are used to reduce the electrical parasites arising from the extension of the electrical contacts. Additionally, these insulating acoustic reflectors are engineered to reduce the TCF of the whole structure to $-14 \text{ ppm}/^\circ\text{C}$, while keeping the transmission coefficient below -30 dB . These resonators display a mass sensitivity of $1800 \text{ kHz/pg}\cdot\text{cm}^2$, as determined by analyzing their response when thin SiO₂ films of different thicknesses are deposited on their active area. A streptavidin-biotin functionalization protocol of the sensing surface enables to use the devices as gravimetric biosensors to detect IgG and thrombin. In the latter case, thrombin concentrations as low as 4 nM are detected.

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References

- [1] G. Sauerbrey, Verwendung von schwingqarzen zur wagung dunner schichten und zur mikrowagung, *Z. Physik* 155 (1959) 206–222.
- [2] A. Shons, F. Dorman, J. Najarian, An immunospecific microbalance, *J. Biomed. Mater. Res.* 6 (1972) 565–570.
- [3] J. Weber, W.M. Albers, J. Tuppurainen, M. Linka, R. Gabla, W. Wersinga, M. Schreiter, Shear mode FBARs as highly sensitive liquid biosensors, *Sens. Actuators A: Phys.* 128 (2006) 84–88.
- [4] G. Wingqvist, J. Bjurston, L. Liljeholm, V. Yantchev, I. Katardjiev, Shear mode AlN thin film electro-acoustic resonant sensor operation in viscous media, *Sens. Actuators B: Chem.* 123 (2007) 466–473.
- [5] E. Schmidhammer, H. Heinze, M. Schmiedgen, M. Mayer, A. Link, High Volume Production of a fully matched 5050 PCS-CDMA-BAW Duplexer, *IEEE Ultrason. Symp. Proc.* (2006) 329–2332.
- [6] R. Ruby, A snapshot in time: the future in filters for cell phones, *IEEE Microwave Mag.* 16 (2015) 46–59.
- [7] M.L. Johnston, I. Kyriassis, K.L. Shepard, FBAR-CMOS oscillator array for mass sensing applications, *IEEE Sens. J.* 10 (2010) 1042–1047.
- [8] E. Iborra, M. Clement, J. Olivares, M. DeMiguel-Ramos, T. Mirea, J. Sangrador, On the effectiveness of lateral excitation of shear modes in AlN layered resonators, *Ultrasonics* 54 (2014) 1504–1508.
- [9] M. DeMiguel-Ramos, T. Mirea, M. Clement, J. Olivares, J. Sangrador, E. Iborra, Optimized tilted c-axis AlN films for improved operation of shear mode resonators, *Thin Solid Films* 590 (2015) 219–223.
- [10] X. Zhang, W. Xu, J. Chaeuna, Temperature effects on a high Q FBAR in liquid, *Sens. Actuators A: Phys.* 166 (2011) 264–268.
- [11] U. Mastromatteo, F.F. Villa, High sensitivity acoustic wave AlN/Si mass detectors arrays for artificial olfactory and biosensing applications: a review, *Sens. Actuators B: Chem.* 179 (2013) 319–327.
- [12] M. DeMiguel-Ramos, M. Barba, T. Mirea, J. Olivares, M. Clement, J. Sangrador, E. Iborra, Influence of the electrical extensions in AlN BAW resonators for in-liquid biosensors, *Proc. Eur. Freq. Time Forum (EFTF)* (2014) 301–304.
- [13] Y. Yoshino, Piezoelectric thin films and their applications for electronics, *J. Appl. Phys.* 105 (2009) 061623.
- [14] K.M. Lakin, K.T. McCarron, J.F. McDonald, Temperature compensated bulk acoustic thin film resonators, *IEEE Ultrason. Symp. Proc.* (2000) 855–858.
- [15] J. Rosenbaum, *Bulk Acoustic Wave Theory and Devices*, Artech House, Norwood, 1988.
- [16] A. Devos, E. Iborra, J. Olivares, M. Clement, A. Rastogi, N. Rimmer, Picosecond ultrasonics as a helpful technique for introducing a new electrode material in BAW technology: the iridium case, *IEEE Ultrason. Symp. Proc.* (2007) 1433–1436.
- [17] J. Olivares, E. Wegmann, J. Capilla, E. Iborra, M. Clement, L. Vergara, R. Aigner, Sputtered SiO₂ as low acoustic impedance material for bragg mirror fabrication in BAW resonators, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* 57 (2010) 23–29.
- [18] J. Capilla, J. Olivares, M. Clement, J. Sangrador, E. Iborra, A. Devos, High-acoustic-impedance tantalum oxide layers for insulating acoustic reflectors, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* 57 (2010) 23–29.
- [19] M. DeMiguel-Ramos, J. Olivares, T. Mirea, M. Clement, E. Iborra, G. Rughoobur, L. García-Gancedo, A. Flewitt, The influence of acoustic reflectors on the temperature coefficient of frequency of solidly mounted resonators, *IEEE Ultrason. Symp. Proc.* (2014) 1472–1475.
- [20] N.S.K. Gunda, M. Singh, L. Norman, K. Kaur, S.K. Mitra, Optimization and characterization of biomolecule immobilization on silicon substrates using (3-aminopropyl)triethoxysilane (APTES) and glutaraldehyde linker, *Appl. Surf. Sci.* 305 (2014) 522–530.
- [21] V. Gupta, N. Madaan, D.S. Jensen, S.C. Kunzler, M.R. Linford, Hydrogen plasma treatment of silicon dioxide for improved silane deposition, *Langmuir* 29 (2013) 3604–3609.
- [22] T. Mirea, V. Yantchev, Influence of liquid properties on the performance of S0-mode Lamb wave sensors: a theoretical analysis, *Sens. Actuators B Chem.* 208 (2015) 212–219.
- [23] S.H. Kim, J.H. Kim, J.K. Lee, S.H. Lee, K.H. Yoon, Bragg reflector thin film resonator using aluminium nitride deposited by RF sputtering, in: *Proc. IEEE Microwave Conf., Asia-Pacific*, 2000, pp. 1535–1538.
- [24] M. Clement, E. Iborra, J. Olivares, N. Rimmer, S. Giraud, S. Bila, A. Reinhardt, DCS Tx filters using AlN resonators with iridium electrodes, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* 57 (2010) 518–523.
- [25] J.W. Lobeek, R. Strijbos, A. Jansman, N.X. Li, A.B. Smolders, N. Puisford, High-Q BAW resonator on Pt/Ta₂O₅/SiO₂-based reflector stack, *Proc. IEEE Int. MTT-S Dig.* (2007) 2047–2050.
- [26] F.N. Dultsev, E.A. Kolosovsky, Forces affecting nanoparticles in fluid: and the shape of the acoustic signal accompanying particle detachment from QCM surface, *Sens. Actuators B* 202 (2014) 454–460.
- [27] M. Saitakis, E. Gizeli, Acoustic sensors as a biophysical tool for probing cell attachment and cell/surface interactions, *Cell. Mol. Life Sci.* 69 (2012) 357–371.