A microfabricated piezoelectric transducer platform for mechanical characterization of cellular events

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Abstract

During the last decade, it was discovered that the mechanical properties and interactions of cells and their surrounding extra-cellular matrix play important roles in cellular activities. Substantial efforts have been made to develop various methodologies and tools to study cell mechanics. In this paper, we report an ongoing study on integrating the concept of a smart structure with a microfabricated thin film piezoelectric transducer for characterizing the various changes in mechanical properties associated with cellular events. A microbridge sensor integrated with a thin film piezoelectric transducer was created from silicon dioxide and zinc oxide sandwiched between two gold electrodes. The cells to be tested were cultured on the microbridge surface. The surface tractions exerted by the cells on the microbridge directly modulated the selected resonant behaviors, which were detected with the custom designed effective surface electrode. Our theory and simulation results showed, for the first time, that the application and changes in these surface tractions resulted in resonant and anti-resonant frequency shifts in the impedance response of the piezoelectric transducer. Both spatial and temporal information of dynamic cellular activities could be inferred from the changes in the impedance spectra. The design, theory, finite-element simulation, microfabrication techniques, and preliminary test results are discussed.

1. Introduction

The cytoskeleton provides the mechanical properties of a cell and supports the cell body with intricate connections to the extra-cellular matrix (ECM) through focal adhesion complexes on the cell membrane. Recent research in cell biology has established that the mechanical properties of living cells play important roles in cell functions such as growth, proliferation, migration, differentiation and embryogenesis as well as in health or diseases such as cancer metastasis, cardiovascular diseases, arthritis and immune dysfunction. The strong correlation between the mechanical properties of a cell and its physiological activities could be exploited to investigate cell behaviors and responses to stimuli by monitoring its mechanical properties.

The reported methodologies of investigating the mechanical properties of a cell can be classified into two categories. The first is the application of quantified static or quasi-static forces onto a localized region on the cell membrane surface while observing or measuring the resulting mechanical responses and related mechanotransduction. The tools used include atomic force microscopy (AFM), 3D magnetic twisting cytometry, optical tweezers, magnetic pulling cytometry (magnetic tweezers), and micropipette suction [1]. These methods provide a way to study the mechanotransductions of cells, but may also be invasive, which often confounds the findings. The second category is to use an intelligent substrate that can provide a means to monitor cellular activities through surface tractions exerted by the cell. Early work reported that cell tractions were evidenced by wrinkled thin polydimethylsiloxane (PDMS) substrate with an elasticity of several MPa, on which the cell had adhered [2]. This method indicated that cells adhered and pulled on the substrate in various magnitudes, directions, and distributions throughout mitosis and cytokinesis processes [3]. Subsequently,
traction force microscopy [5], where traction fields exerted by cells were investigated. This concept led to the development of quantitative maps of tractions during various cellular activities. Movements of the beads in the area where the cells are cultured, hundred nm in diameter near the surface [4]. By measuring the exerted by a cell with embedded fluorescent beads of several kPa range, was used to monitor in-plane stresses polyacrylamide-based flexible gel, with an elasticity in the several kPa range, was used to monitor in-plane stresses exerted by a cell with embedded fluorescent beads of several hundred nm in diameter near the surface [4]. By measuring the movements of the beads in the area where the cells are cultured, quantitative maps of tractions during various cellular activities were investigated. This concept led to the development of traction force microscopy [5], where traction fields exerted by cells are calculated and displaced as maps.

These methods are based on varying the elasticity and thickness of substrates for culturing cells [6]. In order to quantify the stress exerted by cells onto the substrate, microfabricated polycrystalline silicon cantilever beams were embedded in an elastic substrate [7]. The distribution of the resultant force at various points of a migrating fibroblast was found by measuring the static deflection of the microcantilever beam. However, the beams were too large to measure beyond one or two points on a migrating cell. More recently, an array of PDMS posts with various stiffnesses was developed to monitor cell tractions by recording the degree of bending of the posts when a cell was cultured on the post tips [8]. Based on these studies, the observed tractions exerted by various cell types range from 1 to 100 nN.

In this paper, we report the design, theory, computer modeling, fabrication techniques, and preliminary test results of a microfabricated platform that leverages smart structures in the study of cell mechanics. The cell-and-piezoelectric-transducer interaction theory developed in this paper shows, for the first time, that surface tractions result in modulated resonant behaviors and can be detected by the changes in impedance responses of the piezoelectric transducer. Adopting the concept of an effective surface electrode with known structural information that serves as the baseline signal [9, 10], the microbridge platform is capable of detecting changes in the surface tractions exerted by cells adhered to the surface. Both the spatial and temporal information of cellular events could be inferred from the changes in the impedance spectra of the piezoelectric transducer. The ultimate goal is to study cellular activities electronically in a massively parallel array, which would not be possible with the use of conventional optical microscopes. The current approach offers some key advantages over the use of quartz crystal microbalances [11, 12], which require a large number of cells and do not have sufficient sensitivity to investigate cellular activities. Since the operation frequency is in the MHz range and each detection can be completed in less than 1 min, this device provides non-invasive real time monitoring of cellular activities.

2. Design concept

It has been established in the field of flexible structure control that by designing the geometry and location of the electrodes as well as the shapes of the piezoelectric actuators and sensors, unique structural resonant behaviors can be excited and structural information can then be detected [9, 10]. This principle led to the development of modal sensors and actuators [10]. With this concept, we designed two different rectangular electrodes that provided optimal actuation at the first and second bending modes of doubly clamped thin film transducer bridges [13, 14]. This design also provided known structural information on the bending angles at the edges of the electrodes, allowing a better predictive model on the impedance response of the piezoelectric transducer.

Figure 1 shows the basic design concept of the transducer, where a ZnO piezoelectric thin film is supported by a layer of thermal oxide, forming a bi-layer membrane. Evaporated gold films partially sandwich the ZnO layer to form two rectangular effective surface electrodes spanning from the center to the edge of the transducer. This design is targeted to excite resonance at the second bending mode over the other modes. An impedance analyzer is used to drive the piezoelectric transducer and to infer the structural information during operation. The impedance spectrum of the microbridge in the absence of cells serves as the baseline signal, which can be analyzed by considering the bending angle at the edge of the rectangular electrode [13, 14]. Any deviations in the impedance response from the baseline are attributed to the external forces applied to the thin film transducer. In particular, the rectangular electrode has been designed to span the anti-nodal point of the bending angle distribution to provide the known maximum sensitivity for picking up mechanical information of cell tractions. This design also provides unambiguous information that distinguishes the direction of cell tractions. With the known reference signal at the anti-nodal point, the location of cell tractions can be identified.

The surface of the transducer is functionalized to promote cell adhesion on the anchor zone to maximize sensitivity. The ZnO surface not covered by the gold electrode is protected with evaporated SiO2 and can be coated with various extra-cellular matrix (ECM) proteins. At the same time, the gold-covered region is coated with a polyethylene glycol-thiol (PEG-thiol) self-assembled monolayer (SAM) to inhibit cell adhesion [15]. Since both ZnO and SiO2 are thin enough to be transparent, an inverted fluorescence microscope could then be used to optically examine the cells. Prior research in cell mechanics indicated that tractions exerted by cells are through various

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focal adhesion complexes, and each of them is small compared to the sensor structure. Therefore, the cell’s influence on the sensor’s resonant behavior can be treated as concentrated moments through these contact sites [1]. It will be shown that these concentrated moments can be monitored through the variations of the impedance responses. Furthermore, the distribution of the focal adhesion complexes and the internal cytoskeleton network are dynamically modified along with every cellular activity. Therefore, the spatial locations and magnitudes of tractions should provide spatial and temporal information of the adherent cells. In addition, by operating the thin film piezoelectric transducer at a bending mode, the mechanical quality factor ($Q$) of the structure will dramatically enhance the sensitivity compared to a static measurement [7, 8]. It is important to note that the deformation of the piezoelectric transducer due to cell traction will be in the sub-nanometer range, avoiding the issue of confounding the test results with altered substrate geometry. Furthermore, the resonance can be designed to be above the MHz range, virtually eliminating the possibility of cell adaptation to the sensor structure. Therefore, the cell’s influence on the sensor’s resonant behavior can be treated as concentrated moments, which are contributed from the concentrated moments that formed between the cell and the substrate. Furthermore, $R_m$ and $I_m$ are moment arms of the piezoelectric force and cell tractions, $L(x)$ represents the spatial dependency of the fluidic drag force, and $\mu$ and $\delta$ are the viscosity of liquid and the penetration depth [18]. The second term on the right-hand side of equation (4) represents the driving force from the piezoelectric actuator. The first and the second term in equation (5) represent the fluidic drag forces from the fluid and forces exerted by the adhered cell. Note that the forcing term of the cell forces is expressed as a summation of total $n$ concentrated moments, which are contributed from $n$ focal adhesion complexes at location $x_m$ that formed between the cell and the substrate. Furthermore, $R_m$ and $I_m$ represent its real and imaginary parts. The imaginary part contributes to the damping term of the structure, and thus the imaginary forces exerted by the cell could be determined through the measured variation of the damping ratio or quality factor.

Substituting the displacement field equation (3) into equation (4), and taking volume integration with respect to the $k$th eigen-function, the governing equation of the one-dimensional plate is
\[\frac{d^2}{dt^2}A_k(t) + \frac{\mu L}{\delta(\rho - \rho_f)} \frac{dA_k(t)}{dt} + \frac{D_{11}k^2}{(\rho - \rho_f)h}A_k(t) = P_i + C_i,\]
where

\[
P_I = \frac{-B V s_\rho}{2(\rho - \rho_s)h} \int_0^l \frac{d^2 e_{31}(x)}{dx^2} u_I(x) \, dx, \quad (7a)
\]

\[
C_i = \frac{l_c}{(\rho - \rho_s)h} \sum_{m=1}^{n} \left( \bar{R}_m + j \bar{I}_m \right) \frac{d}{dx} \delta(x - x_m) u_I(x) \, dx
\]

and \( B \) and \( l \) are the width and the length of the transducer. Equation (7a) implies that the driving force from the piezoelectric actuator is a function of the location and geometry of the electrodes \( e_{31}(x) \). Thus, we can introduce the concept of the effective surface electrode into sensor design. To achieve maximum actuation at the first and the second bending modes of this double clamped one-dimensional bridge, we have chosen rectangular electrodes [13, 14]. Introducing a rectangular function into surface electrode \( e_{31}(x) \) with one end located at the boundary is equivalent to actuating and sensing the bending angle at the edge of the electrode. Based on this argument, we have chosen the effective electrode of the transducer to operate at the second mode spanning from the boundary \( (x = 0) \) to the center of the transducer \( (x = l/2) \), and two effective surface electrodes to operate at the first mode spanning from \( x = 0 \) to 0.224 158\( l \) and from \( x = 0.775 842\ l \) to \( l \) (figure 2). These are the theoretical nodal points of bending angles in the second and first bending modes, and we can derive the respective piezoelectric driving forces from equation (7a) as

\[
P_2 = \frac{-B V s_\rho e_{31}}{2(\rho - \rho_s)h} u_2'(l/2) \quad (8a)
\]

and

\[
P_2 = \frac{-B V s_\rho e_{31}}{2(\rho - \rho_s)h} u_2'(0.224 158\ l), \quad (8b)
\]

respectively. Note also in equation (7b) that the concentrated moments that are contributed by the cell are weighted by the bending angle at the location of each focal adhesion complex \( x_m \). Since the focal adhesion complexes of adherent cells are distributed mainly on the perimeter of the cell body and point towards the center, we can lump them as surface tractions on these areas. This equation then contains both the spatial information and magnitudes of forces exerted by an adhered cell.

Based on equation (6), we can derive the generalized solution of a one-dimensional plate in terms of a displacement field with external forces contributed from the surrounding fluid and an adhered cell as:

\[
W(x, t) = \sum_{i=1}^{\infty} \frac{P_i + C_i}{(\omega_i^2 - \omega^2) + 2\zeta \omega \omega} u_i(x), \quad (9)
\]

where \( \zeta \) is the resultant damping ratio originating from the viscosity of the surrounding fluid and the adhered cell. Substituting this generalized solution into the second constitutive equation (1b) and taking the surface integration with respect to the electrode of the actuator, we can derive the impedance response of the \( i \)th resonant mode as [19]:

\[
Z_i = \frac{-1}{j \omega C_i} \left\{ \left( \frac{\omega_i^2 - \omega^2}{(\omega_i^2 - \omega^2) + 2\zeta \omega \omega} \right) + DG_i \right\}, \quad (10)
\]

where

\[
G_i = -B l_p A_2 e_{31} u_i'(l/2) \quad (10a)
\]

and

\[
G_i = -B l_p A_2 e_{31} [2u_i'(0.224 158\ l)], \quad (10b)
\]

respectively. Equation (10) indicates that the damping ratios of the resonant and anti-resonant frequency of the \( i \)th mode are functions of the fluid viscosity and the imaginary part of the cell tractions. At the same time, the real part of the cell tractions will shift the anti-resonant frequency depending on its location, direction, and magnitude. This correlation can be further analyzed by substituting equations (7b), (8), and (12) into equation (10), yielding the equation of impedance response as:

\[
Z_i = \frac{-1}{j \omega C_i} \left\{ \left( \frac{\omega_i^2 - \omega^2}{(\omega_i^2 - \omega^2) + 2\zeta \omega \omega} \right) + 2\zeta \omega \omega + \alpha_i [u_i'(x)]^2 + \beta_i [u_i'(x)][\bar{u}_i'(x_m)]^{-1} \right\}, \quad (13)
\]

where \( \bar{u}_i'(x_m) = \sum_{m=1}^{n} (R_m + jI_m)u_i'(x_m) \),

\[
u_i'(x) \quad (14)
\]

\( u_i'(x) \) equals \( u_2'(l/2) \) and \( 2u_i'(0.224 158\ l) \) for the second and first bending modes, and \( \alpha_i \) and \( \beta_i \) are constants, and the overhead bar for \( R_m \) and \( I_m \) represent the lumped traction. It is important to note that the rectangular electrode provides known structural information \( u_i'(x) \) to cell tractions (equation (14)) in an identifiable physical quantity. It offers an unambiguous multiple for identifying the direction, magnitude, and location of cell tractions. Furthermore, since the magnitude of \( u_i'(x) \) is chosen at the anti-nodal points, which are the locations with maximum bending angle, it offers the highest available sensitivity from the piezoelectric transducer.

Equation (13) shows that the anti-resonant frequency of the \( i \)th mode is determined by the piezoelectric effect of the transducer and can be derived as:

\[
\omega_i' = \sqrt{\omega_i^2 + \alpha_i [u_i'(x)]^2}. \quad (15)
\]

Furthermore, this anti-resonant frequency is modified by the real part of the cell tractions depending on its direction, magnitude and is weighted by the structural bending angle. This correlation follows a parabolic relationship at each location of applied traction force:

\[
\langle \omega_i' \rangle^2 = [\beta_i u_i'(x)]^2 \bar{R}_m + (\omega_i')^2. \quad (16)
\]
where the overhead bar represents the resultant anti-resonant frequency and the real part of the lumped traction applied at location \( x_m \). This equation can be developed to yield the equation for the anti-resonant frequency shift:

\[
(\Delta \omega'_i + \omega'_i)^2 = \left[ \beta \mu'_i(x) \omega'_i(x_m) \right] \bar{R}_m + (\omega'_i)^2,
\]

which still follows a parabolic relationship with its focus located on the right side of its locus. In addition, both equations (10) and (13) show that the imaginary part of the surface tractions alter the damping ratio of the resonant valley (\( \xi \)) and anti-resonant peak (\( \xi' \)) differently. This is because the information of the surface tractions came from the denominator. An increasing imaginary part in the denominator will actually decrease the damping ratio at the numerator. This is the key to identifying the direction of these imaginary surface tractions.

Based on the derived cell-and-piezoelectric-transduction interaction theory, we can summarize the relationships between the impedance spectra of the transducer and its external forces with the conceptual illustration in figure 3. The piezoelectric driving forces \( P \) and \( G \) determine the anti-resonant frequency \( f_a \), which is shifted by the real part of the cell tractions \( C \) and altered by the fluid- and cell-originated damping ratio \( \xi \). At the same time, although the resonant frequency is also a function of \( \xi \), it is modified by the imaginary part of the cell tractions \( C \) instead of the real part. The imaginary part of \( C \) also modifies the impedance magnitudes at resonance, \( R_r \), and at anti-resonance, \( R_a \). By monitoring the variations in these parameters in the impedance spectra at different time increments, we could track cellular activities and infer the spatial and temporal information.

4. Finite-element simulation

To verify the analytical results, finite-element analysis (FEA) was used to simulate the microbridge under the influence of external applied forces. The piezoelectric transducers simulated with ABAQUES®/CAE software were 400 \( \mu \text{m} \) long and 20 \( \mu \text{m} \) wide for the one operated in the first bending mode (transducer #1), and 200 \( \mu \text{m} \) long and 40 \( \mu \text{m} \) wide for the one in the second bending mode (transducer #2). The thicknesses of the ZnO and SiO\(_2\) were 0.5 \( \mu \text{m} \) and 1.0 \( \mu \text{m} \) respectively, and thus the ZnO layer was above the neutral axis of the sensor structure, resulting in bending vibration. Gold electrodes and titanium adhesion layers were neglected in these models for simplicity. A drive voltage of 1 mV was applied in the simulations. The quality factors of all materials were set to 10000 to allow easy identification of anti-resonant frequency shift (\( \Delta f_a \)). Figure 4 shows the simulation results of the impedance magnitude spectra for four different surface tractions, with resultant forces ranging from \(-20\), \(-1\), 1, and 20 nN, applied on a 2.5 \( \mu \text{m} \times 20 \mu \text{m} \) slender area on the edge of the electrode (\( x = 0.224 158\) of transducer #1). The results indicated that the real part of the cell tractions shifted the anti-resonant frequency (\( f_a \)).

Figures 5(a) and (b) are the simulated results by applying tractions on different locations within 2.5 \( \mu \text{m} \times 20 \mu \text{m} \) and 2.5 \( \mu \text{m} \times 40 \mu \text{m} \) slender areas across the widths of transducers #1 and #2, respectively. Positive tractions with resultant forces of 10 and 40 nN were applied on transducer #1, showing that the first half cycle of the bending angle is positive and the other half is negative. This matched the derived equation of impedance response (13), where the rectangular effective surface electrodes provide a known and positive structural information \( 2u'_{l}(0.224 158) \) to the signal. Then, the anti-resonant frequency shift is determined by the location and magnitude of the applied traction at location \( x_m \) weighted by local bending angle. This correlation is consistent with the simulation result of transducer #2, where \( u'_{l}(l/2) \) is negative and the distribution of the bending angle had been flipped upside-down. These two simulation results demonstrate that this transducer can be used to identify the direction of the applied tractions. Furthermore, the weighting effect of the bending angle on the applied surface tractions discussed in equation (13) is verified for both transducers. Since the influence of the tractions holds a parabolic relationship to the
anti-resonant frequency, its distribution will be close to the actual distribution of the bending angle at a small traction magnitude. The gray lines shown in figures 5(a) and (b) are the theoretical solutions of the bending angles with the amplitude matching reasonably well with the simulated results.

Figure 6 shows the simulation results with varying surface tractions applied at $x = 3l/8$ and at $x = l/2$ on transducers #1 and #2, respectively. It shows the parabolic relationship between the applied surface tractions and the anti-resonant frequency shift ($\Delta f_a$) derived in equation (13), where we could identify that the focus is on the right side of these loci. Figure 7 shows the effects of the imaginary part of the surface traction from the cell, illustrating the variations of impedance responses to different magnitudes ($-10$, $-5$, $0$, $5$, and $10$ pN) of resultant imaginary forces from the top curve to the bottom at $x = 3l/8$ on transducer #1. This result verified that a positive imaginary part of the traction will decrease the damping ratio of the anti-resonance and increase that of the resonance, and vice versa.

Figure 8 shows the simulation result of a 40 $\mu$m long cell migrating from the center ($x = 100$ $\mu$m) to the boundary ($x = 200$ $\mu$m) of transducer #2. This was done by applying a 40 nN surface traction at the leading edge of the cell and 10 nN at the trailing edge, which are average values of a typical live cell.

5. Fabrication of ZnO transducer

Figure 9 shows the baseline fabrication sequence integrating the ZnO thin film to form the transducer core. The sensor structure is first patterned by ion milling a layer of 1 $\mu$m thermal oxide (SiO$_2$) on top of a silicon wafer through a titanium (Ti) mask (figure 9(a)). A 100 nm thick gold
Figure 8. FEA simulation results of the anti-resonant frequency shift as a function of a cell migrating from the center to the boundary of the transducer #2.

(Au) bottom electrode with 20 nm thick Ti adhesion layer is then E-beam evaporated and patterned with a lift-off technique (figure 9(b)). A 500 nm thick ZnO thin film is then sputtered deposited and patterned with 20% aqueous ammonium chloride (NH4Cl) solution doped with copper ions (figure 9(c)). Another layer of 150 nm thick Au with 20 nm thick Ti is then E-beam evaporated and patterned with a lift-off technique to form the top electrode (figure 9(d)). The exposed ZnO area is protected with 150 nm SiO2 deposited by E-beam evaporation (figure 9(e)). Besides protecting the ZnO from the surrounding fluid, this SiO2 layer also provides a hydrophilic surface for culturing cells. The final step is to release the transducer structure using DRIE and XeF2 (figure 9(f)). Figure 10(a) is the SEM micrograph of the finished transducer array. Figure 10(b) is a photo micrograph of cultured HeLa cells on top of the array. Note that the cells selectively adhered onto the SiO2 coated ZnO surface. The picture also shows that the transducers are optically accessible. The transducers labeled ‘4L,’ ‘5L’, etc are designed to operate at the second bending mode.

The impedance response of the microfabricated piezoelectric transducer was measured with an Agilent 4395A Network/Spectrum/Impedance analyzer connected to an Agilent 43961A impedance test kit [20]. A 50 Ω-terminated GGB Model 10 picoprobe with tungsten probe tips was used to probe the Au/Ti electrodes [21]. The impedance measurement was taken on a Wentworth laboratory probe station on top of a granite shock-isolation table. Figure 11 shows the measured impedance spectra for the piezoelectric transducer with the effective surface electrode designed to maximize the second bending mode. The Q values for the first, second, and third modes are measured to be 118.82, 321.68 and 37.31 in air respectively. This indicates that the electrode successfully suppressed the first and the third modes. Further improvement is possible with better electrode alignment.

Figure 9. Cross-sectional drawing of the process flow: (a) grow 1 μm thick SiO2 on silicon and pattern with ion milling through a Ti mask. (b) Form the bottom electrode by E-beam evaporation of 100 nm of Au over 20 nm of Ti as the adhesion layer and pattern with a lift-off technique. (c) Sputter deposit 500 nm of ZnO and pattern with 20% aqueous solution of NH4Cl doped with Cu. (d) Form the top electrode by E-beam evaporation of 150 nm of Au over 20 nm of Ti as the adhesion layer, patterned with a lift-off technique. (e) Form the protection coating by depositing 150 nm of SiO2 with E-beam evaporation. (f) Release the transducer by backside etching of the underlying silicon with DRIE and XeF2.

6. Discussion

6.1. Material choices

Substantial efforts and consideration have been devoted to material choices for creating the proposed piezoelectric transducer to study cell mechanics. The major factors considered include the interrelations among chemical resistance and biocompatibility of the materials, structural dimensions and the targeted cell sizes, flexibility and sensitivity of the transducer, the operating frequency and the level of influence from surrounding fluid viscosity, among others. The chosen thermally-grown and E-beam evaporated SiO2 as well as the Au/Ti electrodes have been proven to be biocompatible. However, the RF-sputtered ZnO thin film has a very low chemical resistance to an aqueous environment. Its piezoelectric quality tends to degrade over time while altering the pH level of the surrounding culture media [22]. Therefore, a layer of E-beam evaporated SiO2 was needed to protect the ZnO surface. As a result, we have not observed any ZnO degradation for periods over several months. The SiO2 also provided a biocompatible surface for culturing cells. Other thin film piezoelectric candidate materials, such as aluminum nitride (AlN) [23] and lead zirconate–titanate [PZT, or Pb(Zr1−xTi_x)O3] [24, 25], could also be used. Similar to ZnO, AlN is chemically unstable in aqueous solution [26]. A protective coating over its surface is therefore needed. PZT is attractive due to its higher piezoelectric coefficients resulting in higher sensitivity. However, it is not a biocompatible material [27] and isolation measures need to be implemented.

6.2. Transducer design

The dimension of the piezoelectric transducer was designed based on several important aspects. First, the length and
Figure 10. (a) An SEM image of a fabricated piezoelectric transducer array, in which the scale bar is 200 μm. Note that the broken microbridge curled upward, indicating a substantial amount of residual stress. (b) A photo micrograph of cultured HeLa cells on these transducers.

The width of the microbridge must be designed with the size of the targeted cells in mind, as well as whether single cell or multiple cells are to be studied. This will determine the flexural rigidity, density and geometry of the transducer. Multiple microbridges with different electrode sizes and geometries can be incorporated into an array to allow multiple experiments with the same platform. Figure 10(a) shows a version of the microbridge array, in which each microbridge is covered with electrodes of different lengths. Second, the degree of influence from the viscosity in the surrounding media on the quality factor $Q$ is proportional to the operating frequency and width [18]. Microbridges operated at higher operating frequency will have a lower $Q$ and thus decreased sensitivity. The residual stress in different layers within the piezoelectric transducer will also shift the resonant frequency upward. The residual stress in the SiO$_2$ layer could be substantial enough that the resonant frequency could be shifted by a large amount. The broken microbridge shown in figure 10(a) curled upward, indicating a substantial amount of residual stress within the transducer multilayers. A potential solution would be to use symmetrical layering to balance the residual stress [9]. However, since our primary objective is to detect the dynamic changes in the mechanical properties of cells rather than to measure their static mechanical properties in absolute terms, the residual stress and other non-ideal sensor characteristics will likely cancel out to the first degree. In addition, the sensitivity of the piezoelectric transducer is dependent on the quality of the piezoelectric coefficient, which is directly related to how uniformly and abundantly the crystalline orientation aligns within the ZnO film [28]. A thicker piezoelectric material provides higher piezoelectric sensitivity, but the associated increase in thickness results in a thicker and more rigid bridge that is actually less mechanically sensitive [29]. In addition, a rigid bridge that exerts a higher force on the cells could provoke the cell’s adaptive reaction, confounding the measurement of the cells’ otherwise natural dynamic behaviors.

As the impedance response of the piezoelectric transducer could be influenced by the thermal noise and fluid flow in the immediate surroundings, the microenvironment in which the cells are studied needs to be controlled to achieve a usable signal-to-noise ratio. The thermal noise can be effectively suppressed since the temperature of the culture media is maintained at a constant 37 °C to keep cells viable. Fluid flow over the microbridge can be suppressed by using a perfusion-based microbioreactor to minimize the fluidic shear stress inside the chamber, particularly in the immediate vicinity of the cell under test. The design and study of the microbioreactor are outside the scope of this paper, however, and will be discussed elsewhere.

To study various cellular activities dynamically, different areas, geometries and locations of cell anchoring zone need to be designed. The slender geometry of the piezoelectric transducer is chosen to measure the traction forces in one-dimension while ignoring the tractions in the orthogonal direction. The spatial information of the cell tractions can be inferred by the weighting effect of the bending angle. To avoid uncertainty due to the cyclical characteristics of the bending angles (figure 5), the cell anchoring zone is confined within one half cycle (figure 8). To study single cell biomechanics, such as the mechanical characteristics of morphological changes during mitosis, the anchor area and the associated geometry can be designed to allow only one cell to anchor while aligning most of its cytoskeleton stress fibers along the sensitive direction [30]. Cell migration, on the other hand, can be studied by creating a linear concentration gradient of chemoattractant along the microbridge, which then promotes the migration of the targeted cell along the length of the bridge [31]. The piezoelectric transducer can also be used to study cell–cell interactions by confining and aligning a group of cells on its surface. The mechanical interactions between the cells can then be studied through the focal adhesion sites linked to the cytoskeleton network and cell–cell junctions.
Figure 11. The measured impedance spectra of the transducer with a rectangular electrode designed for operating at the second mode, where the spectra (top trace is magnitude, bottom trace, phase) show (a) the first, (b) the second, and (c) the third bending resonant modes.

7. Conclusions

This work demonstrated the feasibility of integrating effective surface electrodes with a microfabricated piezoelectric thin film transducer to form a smart structure for characterizing the mechanical properties of cellular events. A cell-and-piezoelectric-transduction interaction theory was derived to elucidate the relationship between the electromechanical responses of the transducer and the cell tractions on the transducer surface, which were further verified with FEA simulations. The results demonstrated that the applied surface traction is weighted by the distribution of the bending angle, causing a shift in the anti-resonant frequency. By varying the magnitudes and directions of these surface tractions in the FEA models, the relationship between the anti-resonant frequency shift and the applied forces was developed. The influence of the imaginary part of the tractions was found to modify the damping ratio of the overall system. The fabrication process flow was developed and the prototype device array was successfully fabricated and tested. The test results verified that by locating the rectangular effective surface electrodes at the appropriate nodal points of the bending angle distribution at the second bending mode, a $Q$ of up to 321.68 was achieved. Selecting different surface treatments for the electrodes and a SiO$_2$ coated ZnO surface promoted cell adhesion on the desired zone (SiO$_2$/ZnO/SiO$_2$ sandwich area). These results pave the way for integration into a chip-based system with massively parallel arrays for automated experimental procedures to study cellular events.

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