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Non-Invasive Plasmonic-Based Real-Time Characterization of Cardiac Drugs on Cardiomyocytes Functional Behavior

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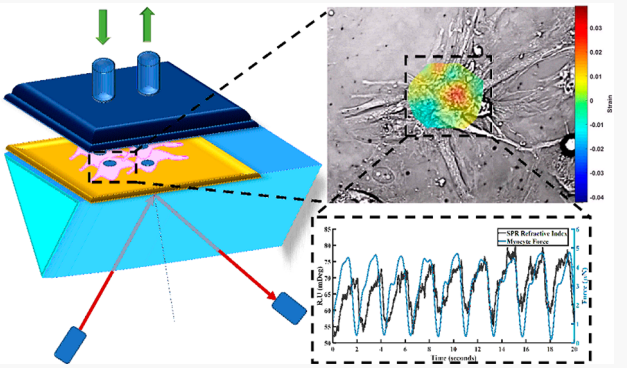
ABSTRACT: In the fabrication of cardiac tissue, an important factor is continuous measurement of its contraction features. A module that allows for a dynamic system capable of noninvasive and label-free monitoring of the contraction profile under administering chemicals and drugs is highly valuable for understanding accurate tissue mechanobiology. In this research, we have successfully demonstrated the use of surface plasmon resonance (SPR) technology for the first time to characterize the contractility of cardiac cells in response to Blebbistatin and ATP drug exposure in real tme. An optimal flow rate of 10 μL/min was selected for a continuous flow of warm media,and 10 μM drug administration effect was detected with high spatiotemporal sensitivity on contracting cardiomyocytes. Our drug screening has identified the source of the SPR periodic signal to be direct cell contraction rather than action potentials or calcium signaling. Per our results, SPR has high potential in applications in least-interference real-time and label-free tissue characterizations and cellular properties analysis from a functional and structural point of view.



It is been more than a century since not only human and animal in vivo models but also in vitro tissue cultures started being used for studying cardiovascular diseases (CVDs), drug screening, and general cardiac physiology. Yet, it takes billions of dollars for a novel drug testing protocol that might not even reach the market due to failure in nonaccurate animal trials. With emerging new technologies and design of organ on a chip devices, tissue-based platforms have become the forerunner designs for drug testing and development. However, through all the paradigms developed, there still lies a need for establishing a preferably noninterference sensing and stimulating model for recording cardiomyocytes (CM) contraction properties, in response to drug and chemical stimulations. In these *in vitro* cardiac tissue platforms, monitoring cell morphology, shape, and size provides vital indicators of excitation−contraction coupling, conduction velocity, impulse propagation, action potential depolarization velocity, total contractile force, and membrane capacitance. Several groups have used conventional methods for monitoring the CM's physiological features, through optical or electrical means (microscopy/action potential readouts using patch clamps etc.),[1−3] though either their device fabrication process is complicated and time-consuming or extensive labeling and microscopy techniques are required for analyzing a sample. Most importantly, it has also been indicated in the literature that the most favorable method, fluorescent tagging of cell proteins, which has been used for tracking cell contraction, causes perturbations in cell physiological pathways such as protein stability, protein folding kinetics and dynamics, and weak protein interactions within cells.[4−7] Hence, understanding the accurate functionality of cardiac cells and their mechanobiological pathways, using a label-free method, for establishing an exact *in vitro* model for drug testing is fundamentally crucial.

Surface plasmon resonance (SPR) is a phenomenon that occurs under specific conditions within the plasmons at a noble metal surface (usually gold or silver). When p-polarized light hits the metal surface, its photons couple to the free electrons of the metal and generate surface plasmons. At the right incident angle, total internal reflection occurs, through which an evanescent wave rises <200 nm in the medium on top of the metal surface. The wave is highly sensitive to changes in matter and hence useful for detecting sensitive changes in mass and motion.

Although SPR has been mostly used for measuring the association and dissociation kinetics of molecular bindings, in the recent years, our lab and other researchers have found potential applications of cell-integrated SPR studies for cell micromotion monitoring,[8] drug exposure and vesicle release from cells,[9] live cancer cell attachment monitoring,[10] and

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binding kinetics of membrane proteins in single cells.[11] It has even been used in a more detailed and interesting experimental design, for detecting early stage differentiation of stem cells into endothelial cells, while conventional techniques such as flow cytometry and fluorescent microscopy were unable to define the stage of differentiation.[12] SPR, as a label-free technique, exploits the use of photonics and electromagnetic particles in acquiring information from cells and possibly tissue. Therefore, the least interference will be administered to the biological features of cells and tissues, giving the most accurate response compared to conventional fluorescent tagging and electrochemical measurement of cellular mechanics. To our knowledge, no one has yet explored the potential of this label-free and noninterference optical and possibly portable technology to quantify contractile forces from spontaneously beating cardiac cells under drug exposed conditions. The design of an SPR sensing system, with a sensor plate, flow chamber, two channels, a variety of surface functionalization molecules, and control of flow, makes SPR an ideal sensing system for real time monitoring of tissue properties (cell expression, mechanical and physical characterization, etc.) with multiplex stimuli, such as rapid drug screening. An exact understanding of structural and functional changes of cardiomyocytes under different chemical stimuli, such as activating excitatory and inhibitory pathways, is needed in the field of cardiac tissue engineering and drug development. SPR technology has a very high sensitivity both spatially and temporally, is label-free and capable of recording signals from a single cell level in real time. Since its signal is sensitive to both variations in density and movement of matter, a better understanding of cell structure and function will be achieved. Our observations confirm that SPR is a versatile option for imaging, drug testing, and force quantification of cardiac cells, with minimum cell interference, taking advantage of an optophysical phenomenon.

In this study, we have demonstrated that changes in contraction of neonatal rat cardiomyocytes could be monitored using SPR technology under drug exposure. Here, we have measured the force of contraction, frequency, and regularity of neonatal rat cardiomyocytes' beating, cultured on SPR gold films. Signals acquired from SPR measured in refractive index (RI) changes have been translated to force. We further explored the effect of chemical stimulations, ATP and Blebbistatin (Blb), on the cell's behavior using the SPR technique. We believe that this label-free, accurate yet simple and highly sensitive optical technique will have the potential of being added to a microfluidic system in the future for an all-in-one organ on a chip device to observe complex cardiac tissue constructs from several physiological aspects.

EXPERIMENTAL METHODS

Gold Chip Preparation and PDMS Cell Concentrator. SPR gold chips (Biosensing Instruments, AZ) were sterilized prior to each cell culture using 15 min of dipping in 70% ethanol and 15 min exposure to UV light. The chips were then air-dried using nitrogen gas and kept at room temperature under a sterile biological flow hood. Either the cells were cultured directly on the gold chips with a medium wash prior to seeding or surface modifications for better attachment of cells, using fibronectin, collagen, and gelatin was performed on the chips. Fibronectin surface modification was carried out by coating the gold chip surfaces with a 1/100 diluted solution of fibronectin (Sigma Aldrich), incubated on a shaker for 1 h at 37 °C. The chip surfaces were then washed three times with phosphate buffer solution (PBS) and let dry for 1 h prior to cell seeding. Gelatin surface modification was done by using a sterile 2% (w/v) gelatin solution (Sigma-Aldrich). Ten microliters of gelatin solution per square centimer of culture plate was used to cover the gold chips and left to dry in a biological hood at room temperature for 2 h prior to seeding cells. For chips treated with collagen, type I collagen from a rat tail (Sigma-Aldrich) was diluted in PBS and in 0.1%(w/v) acetic acid. Chip surfaces were coated with minimum level of collagen solution and left overnight at 4 °C. The next day, chips were washed three times with PBS and allowed to dry at room temperature prior to seeing cells. To avoid the cells from being slipped off the surface to the edge of the chip and to maintain their population in the center, we developed a polydimethylsiloxane (PDMS) cell concentrator, inserted on top of the gold chips prior to cell culture (Figure 1B).

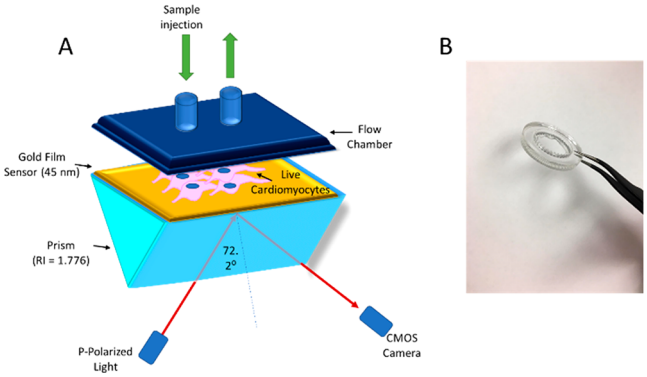

Figure 1. (A) Schematic of cell-embedded SPR sensor on a BI2000 device. P-polarized light is directed to the surface of the gold metal at an incident angle of 72.2°, and changes to the refracted angle are monitored using a CMOS camera. (B) PDMS cell concentrators are fabricated for application on gold sensors to concentrate cells in the middle of the gold film.

PDMS Cell Concentrator. We primarily observed in culturing cardiomyocytes on gold surfaces that, due to the slight elevation of gold chips from the surface of six-well plates, cells slipped off the center to the edges of the gold chip and aggregated more on the edge. This decreased the concentration and confluency of cells in the center from where the laser light is reading, leaving fewer cells behind to connect and communicate, hence a lower beating rate, which were undetectable by SPR. To have the cells attach specifically to the gold chip's center, we used a lab made approach of using a PDMS mold for isolating the cells in the middle of the chip. The mold was sterilized and, prior to sticking on gold chips within a well, was blocked with BSA to avoid nonspecific cell attachment. This allowed the cells to gather in the opening (middle) of the mold on top of the chip for better communication.

Cardiomyocyte Isolation. Neonatal rat cardiomyocytes were isolated from newborn 1−2 day old rat pups following the Thermo Fisher Scientific isolation protocol.[13] Briefly, the neonatal rats were euthanized via decapitation, and their hearts' ventricles were excised. Each ventricle was immediately put in a separate 1.5 mL vial filled with ice cold HBSS. They were then trimmed with surgical scissors into small pieces (1−3 mm^2). The cardiac tissue was digested with the addition of 0.2 mL of reconstituted Cardiomyocyte Isolation Enzyme 1

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Anal. Chem. XXXX, XXX, XXX−XXX

(papain) in HBSS and 10 μL of Cardiomyocyte Isolation Enzyme 2 (thermolysin) to each tube. Vials were incubated for 35 min at 37 °C in 5% CO_2 in a benchtop incubator. The digests were then collected. Complete DMEM (%1 penicillin/streptomycin, 10% Fetal Bovine Serum (FBS) to DMEM) was added to each vial to stop enzyme activity, and the tissue was broken up using a 1000 μL pipet and pipetting up and down for 30 times, until the tissue was a single-cell suspension. The vials were combined for determination of cell concentration and viability. Finally, 200 000 cells/cm^2 were seeded on each prepared SPR gold chip fixed in six-well plates through the PDMS chamber, and complete DMEM for primary cell isolation was added as a culture medium. After 24 h, the complete DMEM was substituted with media containing cardiomyocytes growth supplement, for cardiomyocyte purification over fibroblasts and endothelial cells. After this, the medium was changed every 3 days.

Live Cell Integration to the SPR System. Cardiomyocytes cultured gold SPR chips were mounted on the SPR prism using the provided index fluid. Instead of the flow buffer, we used 37 °C culture media with pen/strep and FBS, to maintain cardiomyocytes' activity over time. The flow rate was optimized to its final rate of 10 μL/min. Chemical injections (ATP and Blebbistatin) were carried out using the injection port, and the cardiomyocytes contractility and behavior to stimulation was monitored in real time. The concentration of the drugs was based on a former study. The highest concentration of the drug used was 10 μM. A sample rate of 100 samples/s was chosen for the SPR studies.

Real Time Image Processing. To measure the cardiomyocytes beating on gold chips and on Petri dishes, a movie of beating cardiomyocytes was captured using an Olympus BX51 fluorescent microscope, at 31 frames per second and a duration of 20 s. The video was analyzed using MATLAB (MathWorks, Natick, MA) based code built for digital image correlation (DIC) called Ncorr in conjuction with our own in-house scripts. By analyzing frame by frame differences in pixel positions, cell displacements could be calculated, which could then be converted to real world strain values based on a known scale bar and then to force using available literature information on electrode surface constitutive properties. Several CM beats were analyzed and their overlapped graphs around the specified region of interest (ROI) were established to recover contraction phase times. To eliminate intercycle noise and variability, a moving average filter was also employed, and the resultant graph was used for comparison to the experimental data.

RESULTS

Real Time Sensing of Cell Contractility under Optimized Controlled Flow Rate. In the human body, cellular metabolic exchange and respiration is a dynamic process that requires the continuous flow of blood in vessels passing through cells, for oxygen, CO_2, and metabolite exchange. In cardiac tissue, the exchange happens through active or passive diffusion from vascular (endothelial) cells' membranes into their compact adjacent cardiac cell (cardiomyocytes). Therefore, it would be a more accurate model if the cell metabolic supplies (glucose, etc.) were to be delivered in a dynamic fashion rather than a static one, as in culture dishes. It will also allow for administering chemical inductions and monitoring the mechanobiological response of cardiac cells to stimulation. As is shown in the schematic diagram for our SPR device (Figure 1), a microfluidic based module is provided which allows the flow of media on top of the cell-cultured gold chips.

The pump, which derives the fluid flow, is capable of flow rate alteration for better interaction kinetic measures, in the case of molecular−molecular interactions. We took advantage of this property and sought to explore the effect of different flow rates on signal acquisition from cells beating on the chip. We selected the flow that provides the least hindrance in the medium's refractive index changes for acquiring the highest signal-to-noise ratio. To have the cells in their most convenient environment, 37 °C medium was flowed over them continuously. We established four different flow rates of 10, 20, 30, and 40 μL/min. Figure 2 shows the resulting signals on a gold chip under the aforementioned flow rates. The signal-to-noise ratio calculated for the 10 μL/min flow was the highest with 18.9 dB compared to other rates at 2.66, 0, and 0 for 20, 30, and 40 μL/min, respectively. We observed a slight drift in baseline in SPR response under different flow rates, which will be discussed more later in the paper. For all further experiments, the flow rate was set at 10 μL/min as an optimized estimate.

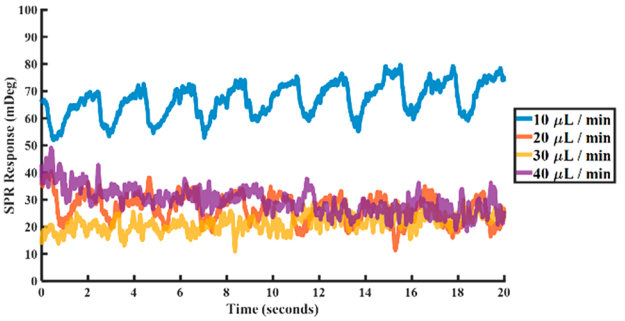

Figure 2. Optimization of flow rate effect in SPR signal acquisition on beating cardiac cells. The flow was adjusted to be 10, 20, 30, and 40 μL/min on the same sensor, and output was monitored using continuous SPR. The signal-to-noise ratio is calculated for each, and it decreases from 18.2 dB under 10 μL/min to 0 for higher rates.

SPR Force Calibration Using Digital Correlation Analysis from CMs' Videos. One of the major factors essential in the functionality of cardiac tissue and cardiomyocytes is their ability to spontaneously contract. Several standard parameters have been established for the contractility of these cells, from different sources (iPSCs or rat/mouse) and in different stages of maturity (early or late stage, neonatal or adult). The parameters we will be targeting is force of contraction and duration of relaxation/contraction periods. Contraction of a muscle happens in three known phases of latent period, contraction period and relaxation period. The latent period is defined as the time from when the stimulus (calcium in case of our spontaneous beating) is delivered to cells until the first indication of contraction. The contraction period is the time where the force of contraction goes from 0 to maximum force (F_{max}), and the relaxation period is the time where it goes from F_{max} to the resting state (0 force). The cycle is followed by another latent phase (Figure 3A).

Accurate measurement of cardiomyocyte force exertion is highly important in monitoring how healthy the cells are and how well they behave in a tissue construct. Using SPR, the recorded signal shows rhythmic changes in RI due to myocytes beating on the surface. The signal, however, is presented in

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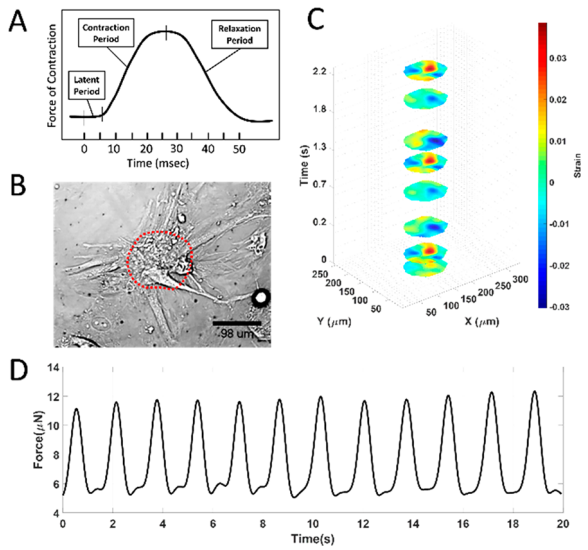

Figure 3. Digital image correlation results from Ncorr. (A) Muscle contraction phases across time (latent, contraction, and relaxation). (B) Example snapshot of cardiomyocytes from a sample chip to show ROI (red dotted curve) used for digital image correlation. (C) Strain heat map at various significant time points that correlate to periods of lowest and maximum contraction (full video can be found in the Supporting Information). (D) Force profile taken by first averaging a smaller subregion of the ROI across time and then converting from strain to force.

terms of millidegrees of SPR RI angle shifts in refracted light after the total internal reflection happens. To be able to quantify cellular forces using SPR, we proposed to calibrate the results acquired using forces calculated from the CMs videos. Hence, we recorded the spontaneous contraction of cardiomyocytes on the chip, prior to mounting the chip on SPR for signal acquisition. Each video was analyzed using a digital image correlation method (DIC) in MATLAB for computing the displacement field of a region of interest in the video throughout the frames. The open source software, Ncorr,[14] had these features built in and was thus used alongside in-house MATLAB code for video and image processing. On the basis of the displacement acquired (ΔL) and the original cell position (L), strain is calculated. Using the stiffness of the electrode's surface (gold or fibronectin treated gold), Young modulus (E), and the force exerting area (A), force can be calculated according to the following equation:

$$F = E \times A \times \Delta L/L \quad (1)$$

Results are depicted in Figure 3 and videos are presented in the Supporting Information. A section of video with high contrast between the cell and the background is selected (Figure 3B), where beating is continuous. Videos are taken at a 30 frames per second (fps) rate. The same section is analyzed over the whole time of the video for similarity and correlation analysis using a prewritten MATLAB code. The output of the analysis was an xy strain profile ($\Delta L/L$) exerted by the cell in the selected region shown in stacked heat map graphs (Figure 3C). Force is calculated from the strain profile resulting in a force profile given in Figure 3D. The F_{max} for cells is calculated as ∼11 μN. The contraction and relaxation periods are measured as 0.59 ± 0.0446 s and 0.60 ± 0.0432 s for the selected region of cells (n = 12). We observed a slight decrease in the contraction force measurements as well as a prolonged contraction−relaxation period compared with results presented in the literature, which could be due to cell exposure to room temperatures (21−25 °C) rather than being kept in a 37 °C/5% CO_2 cell incubator. Yet, the results are very much comparable with those given by SPR, as SPR experiments are all conducted at room temperature as well. All measurements will be used to calibrate the force of contraction as well as the contraction−relaxation period using SPR signals acquired from the corresponding chips.

Rapid Screening of Cardiomyocytes under Cardiac Drug Exposure. One of the major applications of developing cardiac tissues and systems for measuring their functionality is drug testing. With new drug development processes being a billion-dollar business, and the long duration of producing a drug that could reach market value, the essential need for designing an optimal drug testing system only rises. Several researchers in the field of cardiac tissue engineering have developed platforms targeted to drug testing applications within their fabricated tissues.[1,15,16] In our SPR based platform, we are offering a noninvasive, least interference strategy for measuring CMs' properties in response to drug and chemical administrations. The BI2000 SPR setup has a built-in flow chamber with two separate channels, which allows the exposure of different chemicals simultaneously to substrate material on the sensor surface. The connected pump for autoinjection allows the control of exposure conditions such as rate and time of exposure for the best output of results. Its available dynamic and static modules allow for several experimental designs to explore a variety of targets using surface plasmon resonance, investigating the pharmacodynamics (PD) and pharmacokinetics (PK) of their conjugation to cells on the sensor. A combination of its dynamic module with two separate channels for sample injection and the addition of cell culture makes this a high throughput system for the least-interfered drug screening on several tissue samples.

The flow rate is designed to be set at 10 μL/min, which is optimal in terms of both signal acquisition from cardiomyocytes as well as providing the required time for drugs to react with cells. After establishing the baseline rhythmic signal from cells, we injected a constant concentration of ATP (adenosine triphosphate, known to increase the rate of myocytes beating) and + Blebbistatin (Bb, myosin inhibitor, stops the spontaneous contraction of cardiomyocytes) in the flow-through module on top of the cell-cultured chips. The sampling rate for signals acquired is determined in the software to be 100 samples/s. A total of 80 μL of 10 μM ATP and 10 μM Bb was injected through the injection port using the provided syringe to avoid any bubbles interfering with the signals, decreasing noise.

Figure 4A and B show the prolonged exposure for the full chemical to be administered and washed away from injection of 10 μM Bb and 10 μM ATP, respectively. With the slow rate of fluid flow, it takes 246 s for the injection to move completely through the channels and be flushed toward the outlet, replaced by fresh media. Hence, it takes some time after chemical injection to see a response of signal change from cells. Samples are injected at 460 and 240 s for Bb and ATP, respectively. The expanded windows show the signal prior to, during, and after drug administrations. The drug saturation response is observed around 580−640 s for Bb (Figure 4A, middle zoom) and 260−340 s for ATP (Figure 4B middle zoom). Both are strong confirmations of the fact that results acquired are directly being read from cells' contraction due to

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Anal. Chem. XXXX, XXX, XXX−XXX

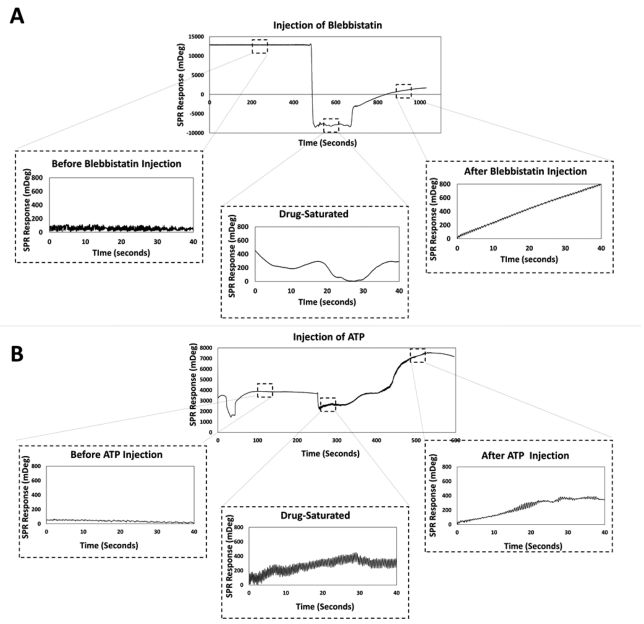

Figure 4. Drug testing experiments on rat cardiomyocytes using the dynamic flow-through module of the SPR device. The expanded windows on the left show the signal acquired before injecting the drug. The middle expansion is at the drug saturated level, and the one on the right is after the drug has been washed away. Injection of 10 μM Blebbistatin (A) and ATP (B) onto a cardiomyocyte cultured gold sensor is shown.

mass changes resulting in RI changes in the near vicinity of the gold film−dielectric interface. The sharp drop in signals is expected, as a solution containing drugs passes by the sample and causes a change in SPR signal. As is observed, after the injection is finished, the signal goes back to a different baseline than the beginning, suggesting that not all the drug is washed away, and some stays attached on their targets within cells. In Figure 4A, prior to the addition of a drug, we can see a rhythmic signal associated with the cells, while during the drug saturation phase, no activity is observed from myocytes, as expected. Finally, after the drug is removed, the myocytes resume activity and contraction. Since Blebbistatin is a myosin inhibitor, this suggests that the signals collected are indeed from cellular contractions due to calcium influx into the cells. As the signal plateaus after the drug addiction, readings should only be from the contraction force of cardiomyocytes, despite ongoing membrane potential changes or ion channel activities to produce action potentials.

Our observations from Bb show that due to the long exposure of drug and real-time monitoring of cell behavior, the immediate response of cells was not sudden silencing of their myosin, hence contraction. Rather, we observed a more spaced, less potent signal (increased latent period, decreased refractive index unit (RU) amplitude) for a course of 60 s after administration, which finally resulted in zero signal (back to baseline) with no noise, once all cells were blocked. Small disturbances within the signal could be due to contraction from cells which were not exposed to the drug (out of the microfluidic channel range), though their contraction still induced a change within the RI. However, since they are far from the center at which the surface plasmon resonance occurs more potently, their movement will not contribute much to signal change, rather random noises are produced that are received by the sensitive area. Using force calibration, a highly reproducible SPR signal with good correlation was acquired and overlapped with data from digital image correlation analysis, as seen in Figure 5, where the SPR signal is depicted

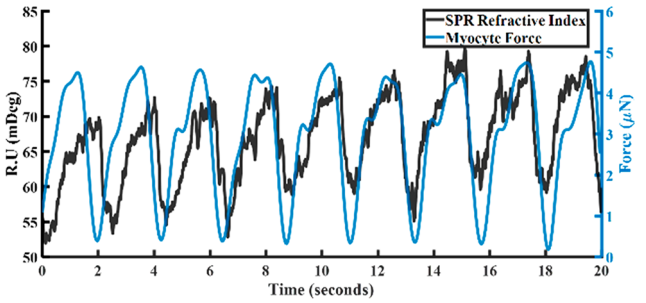

Figure 5. Calibrating the force acquired from SPR using video microscopy. Data acquired form Digital Correlation Analysis of cardiac cells on gold chips (blue) is overlapped with signals acquired from SPR of the same chip (black). This can translate the refractive index change that is received from SPR on CMs to actual force that is applied on the substrate due to their contraction.

in black and video imaging analysis in blue. Each signal is separately taken, and as observed in the aforementioned figure, the SPR signal is well validated using signals acquired from Ncorr.

DISCUSSION

Development of new drug-based treatments for cardiac disease is essential as an alternative to expensive and excruciating cardiac surgeries. Though with FDA regulations, novel drug testing costs billions of dollars due to animal and human trials. Also, the unavailability of an accurate, label-free and cost-effective in vitro human cardiac tissue model featuring the least interference with tissue results in a failure of clinical trials in phase 2 and on. A noninvasive, sensitive, and dynamic drug screening model is yet needed, which is capable of monitoring a tissue's functional and structural changes due to drug exposure. The aforementioned model in combination with human stem cell derived tissue can be an alternative to animal trials for novel drug discovery, which introduces more accurate information on drug effects in the human body, resulting in a higher chance of success in clinical trials.

Here, we demonstrate for the first time, that surface plasmon resonance technology can be used as a drug screening model to measure beating frequencies and force of a monolayer of cardiomyocytes cultured on gold chip sensors, in response to chemicals and drug exposure. We identified through testing with Blebbistatin and ATP that the signals acquired using SPR, which are corresponding to cardiomyocytes contraction directly, not only are affected by drug exposure but also possibly give pharmacokinetics data on the target drug per unit cell. Blebbistatin is a myosin inhibitor, which halts cardiomyocytes contraction, and ATP is a direct output of the glycolytic pathway that results in faster cardiac signaling, hence an increase in beating frequencies. We observed the above-mentioned physiological effect, using SPR, after injecting a dynamic flow of Blebbistatin and ATP into the system. As the evanescent wave penetrates the dielectric medium within a couple of hundred nanometers and the SPR signal being sensitive to RI changes in that medium, only spatial differences in cell membrane lipids and proteins, filaments, and organelles in cytosol near the membrane or

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Anal. Chem. XXXX, XXX, XXX−XXX

cellular movement could be responsible for variations in the SPR signal. However, there lies a concern of whether, with surface modifications, cell membranes will still be within the 200 nm window. Howe et al., using SPR Imaging (SPRI) techniques, confirmed that cells like neurons do reside within the sensitivity window of the evanescent wave, despite their poly-L-lysin surface modification.[17] However, with the modules being available for SPRI in the literature, a flow-through dynamic model cannot be achieved, hence drug screening will be inaccurate due to the accumulation of chemicals. Another group was using SPRI as a distance variation analysis method of the cardiomyocytes membrane due to contraction and relaxation modes.[18] They also confirmed that cell−substrate distance varies between 10 and 80 nm, which again lies within our sensitive SPR signal. Our results are consistent with their theory that the periodic SPR signal is coming from cells' contraction due to movement of a protein or lipid-dense section of cell membrane away from the substrate and out of the evanescent field of sensitivity. It is also well-known that the adult myocardium cell types exert a substantially higher amount of force of contraction (44 mN/mm^2) compared with iPSCs derived myocardium (4−23 mN/mm^2).[19−21] It is expected that neonatal and fetal rat cardiomyocytes' contraction forces land in a spectrum less than adult cardiomyocytes and more than iPSCs. Here, we showed that using SPR, a force of 4.44 ± 0.0531 μN can be acquired from cells. The loss in force of contraction is correlated with the absence of temperature and CO_2 control on the SPR device. A decrease in temperature can highly affect cardiomyocytes contraction, and developing a controlled environment for this technique is essential. On the basis of these data, we propose that SPR with a dynamic flow microfluidic-based module can be used as a noninvasive and label free drug screening model for monitoring structural and functional properties of excitable cells such as cardiomyocytes.

Our Blebbistatin drug experiments also confirm that the periodic SPR signals are not coming from action potential propagation and the effect of their charge on electron density cloud that is being propagated parallel to the metal surface due to resonating plasmons. Blebbistatin is a myosin inhibitor that inhibits the ATPase activity with binding halfway between the nucleotide binding pockets and the actin binding cleft of myosin. This results in relaxation of myofilaments, which stops the cardiac cells from contracting. While action potentials due to influx and outflux of calcium might still be ongoing, because of this strong block (Bb), no signal (contraction) was observed on the SPR, attesting to the assumption that signals are coming directly from cardiomyocytes contractions.

Another interesting find was the variations in signal due to different flow rates. Several researchers have used the flow rates of 10−50 μL/min for best molecular interaction response, giving the injectables enough time to react with targets on the chip for more accurate kinetic read-outs.[9,22−24] Flow rates of 10, 20, 30, and 40 μL/min are well-known rates in SPR to be used for molecular binding read-outs or for administering the least shear stress to cells in drug analysis.[15,25−27] In dynamic experiments with flow, smaller cells or apoptotic cells attached on the upper layer of tissue which is further away from the chip's surface are more susceptible to shear stress applied by continuous flow. As their attachment to their neighbor cells might not be strong enough, they could get detached from the cluster of cells, contributing to a change in RI, thus causing the SPR signal to drift. SPR is very sensitive in the sensor's close vicinity, which counts as cellular attachment points and the cell membrane, to mass and RI changes (<200 nm from sensor's surface). The overall mass change on the SPR's detection line (2 mm for BI2000) affects the RI change, linking to the drift. We determined the highest signal-to-noise ratio of SPR periodic signals to be in a flow of 10 μL/min. While the medium that is being flowed over is highly homogeneous in its RI and in the changes it makes to the baseline RI of dielectric medium, the velocity of flow can affect its laminar nature on microscopic scales. As the flow rate increases, the volume of medium moving through elevated cell areas in a contraction state becomes turbulent. The turbulence of flow will hinder the signal from a periodic one under laminar flow to a noise, which cannot be separated from contraction signals. Although the cells are yet contracting, the movement of dense membrane areas inside and out of the evanescent field will not be distinguishable as a turbulence occurring in such low volume will override the movement signal and all will be received as noise. On the basis of our observations, the flow of 10 μL/min gives a laminar behavior for the flowed media, allowing investigations on cardiomyocyte functionality.

Consolidating all into a major application, we can state that SPR is a noninvasive, label-free real-time technique that can potentially be miniaturized for operation within an incubator, and measurements can continuously be read from live tissue over the course of long time. Drug tests can be carried out using a microfluidic module using a pump for continuous fluid flow (as is physiologically expected from the human body's drug association and dissociation), hence measuring the kinetics of each drug interaction with target cells. Depending on the investigation needs, studies could be made in a static (no flow) or dynamic module. The opportunity of not having cells interfere with a fluorescent agent, mechanical pillars, or long durations of microscopy allows for studying cells and tissues in their more natural environment than what has been done so far.

In future works, using the channel heights of the microfluidic module, that range from 100 to 500 μm, multiple cell types can be incorporated within scaffolds for tissue analysis using SPR, with a thickness that can vary anywhere between 100 and 300 μm, giving a more accurate analysis of contraction properties within a 3D tissue structure. All of that being said, most of the currently commercially available SPR devices do not have a CO_2 and temperature control for longitudinal studies. Hence, a portable SPR configuration that can be kept in a biological incubator for real-time and long-term analysis of cell behavior is of high importance.

CONCLUSION

The technique developed in this study is capable of monitoring the contraction of cardiomyocytes using surface plasmon resonance simultaneous with the application of drugs and chemicals, without interfering with the tissue construct. This noninvasive label free method with its addition of a flow-through microfluidic module on top is an advantage to currently available measurement systems for cardiac cell and tissue contraction, due to its fast and noninterference response, which is being collected using photons and surface plasmons. The technology can be miniaturized using optical fibers for application within an incubator for noninterrupted recording of cell and tissue physiological responses to drug administrations.

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Anal. Chem. XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.9b04956.

Comparison of SPR signal acquired form CMs with fibroblasts, fixed cell testing, and effect of trypsin on cells on SPR (PDF)

Video of CMs beating on SPR chip (AVI)

Video of CMs beating on SPR chip (AVI)

Z-stacked images of cells beating on chip from confocal (AVI)

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Notes

The authors declare no competing financial interest.

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Anal. Chem. XXXX, XXX, XXX−XXX