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Piezoresistive sensor-integrated PDMS cantilever: A new class of device for measuring the drug-induced changes in the mechanical activity of cardiomyocytes

Dong-Su Kim [a], Yun-Jin Jeong [a], Bong-Kee Lee [b], Arunkumar Shanmugasundaram [b], Dong-Weon Lee [b,*]

[a] Graduate School of Mechanical Engineering, Chonnam National University, Gwangju-61186, Republic of Korea
[b] School of Mechanical Engineering, Chonnam National University, Gwangju-61186, Republic of Korea

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ABSTRACT

Herein, we demonstrate in detail the fabrication and evaluation procedure of a piezoresistive sensor-integrated polydimethylsiloxane (PDMS) cantilever for measuring the drug-induced changes in the contraction force of cardiomyocytes. The proposed device consists of a glass body with metal patterns, a PDMS cantilever with microgrooves (μgrooves), and integrated piezoresistive sensor. Reliability of the piezoresistive sensor and connection wires was greatly improved by using a glass substrate with metal patterns. The longitudinally patterned μgrooves formed on the PDMS cantilever was optimized to maximize cantilever deformation. The mechanical deformation of the cantilever caused by the contraction force of cardiomyocytes is directly observed by using the integrated piezoresistive sensor, whereas the existing methods rely on the optical methods to measure the cantilever displacement. The contraction force is maximized between day eight and nine after seeding the cardiomyocytes onto the PDMS cantilever. After preliminary experiments, the strain sensor integrated μpatterned PDMS cantilever was subjected to measure the change in the contraction force of cardiomyocytes under different concentrations of cardiac drugs. The experimental results showed that the strain sensor integrated PDMS cantilever can effectively verify the changes in the mechanical output of the cardiomyocytes under the drug influence.

1. Introduction

The most important function of the heart is to pump the blood, supplying oxygen/nutrients to a human body by regular contraction and relaxation cycle of the working cardiomyocytes. When there is a problem in the regulation of contractility of cardiomyocytes a person may experience an abnormal heart rhythm. This often causes a serious situation to human health [1,2]. There are several reasons behind the abnormal heart functioning, among them preclinical drug use causes the high failure rates [3]. Hence, an accurate measurement and analysis of cardiac contractility is a key component for several pharmacological studies [4–12].

The mechanical activity of cardiomyocytes under the drug influence is usually carried out by measuring the contraction force of cardiomyocytes. In these studies, the isolated cardiomyocytes are placed onto biocompatible substrates and grown in-vitro at appropriate conditions. During the culture period the cardiomyocytes rearrange their myofibrillar and start to beat spontaneously again [13–16]. Over the years several techniques have been proposed to measure the contraction force of cardiomyocytes [17–24]. Of these techniques, much attention has been paid to polymeric microposts or cantilevers that directly measure the physiological behaviours of cardiomyocytes. The micropost array (μPA)/cantilever method measures the contraction force of cardiomyocytes through the mechanical deformation of cylindrical elastomer/polymeric cantilever. Although, these technologies used extensively to measure the contraction force of cardiomyocytes [25], it has several drawbacks such as it need an external optical device to measure the displacement of the structures, inability to work within a microarray format, the periodical alignment and calibration of a laser source is really a time consuming process when we employ the array of cantilevers [26,27].

In order, to overcome the all the practical difficulties of the methods, herein we present a novel technique for measuring

* Corresponding author.
E-mail address: mems@jnu.ac.kr (D.-W. Lee).

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the contractile force of isolated cardiomyocytes. The proposed cantilever device can measures the cantilever mechanical deformation/contraction force of cardiomyocytes electrically and it does not rely on any optical methods. To measure the mechanical deformation electrically, we bring in the innovative concept of using the strain induced by the mechanical deformations of the cantilever. The bending of the cantilever caused by the contraction force of cardiomyocytes can be measured effectively and effortlessly by monitoring the change in electrical signal passing through the strain senor. The cantilever is made by a biocompatible, flexible and optically transparent-polydimethylsiloxane (PDMS) material. To maximize the contraction force of cardiomyocytes and to improve the mechanical deformation of the cantilever we introduced the three-dimensional (3D) microgroove patterns on the surface of PDMS cantilever. The strain sensor integrated onto the PDMS cantilever through the conventional photolithography and metal etching processes. Further, thermal stability and electrical reliability of the μpatterned PDMS cantilever were greatly improved through the chemical attachment of PDMS cantilever with the metal wire deposited glass electrode.

Finally, the fabricated strain sensor integrated PDMS cantilever was used to measure the drug induced changes in the mechanical activity of cardiomyocytes. For cell seeding experiment, the heart was aseptically isolated from a Sprague-Dawley rat on day 3. The separated ventricles are washed by using ADS buffer solution, then single cardiomyocytes were acquired through enzyme solution and pre-plating. The acquired cardiomyocytes are then seeded onto the strain sensor integrated μpatterned PDMS cantilever. The change in contraction force of cardiomyocytes in response to the different concentrations of two drugs (Isoproterenol and Verapamil) was measured through cantilever bending displacement. The experimental results clearly demonstrate the effect of these drugs on the mechanical activity of cardiomyocytes. Hence we sincerely anticipated that the μpatterned PDMS cantilever arrays integrated with a strain sensor opens up a great opportunity to establish the new class of device for efficient and effective measurement of the drug induced changes in the mechanical activity of cardiomyocytes under the drug influence.

2. Material and methods

2.1. Design and fabrication of PDMS cantilevers

Due to the difference in thermal expansion coefficients between the metal wires and soft PDMS substrate thermal stress often exists during the metal deposition process which leads to the cracks and wrinkles on thin metal wires. To overcome these intrinsic problems, several approaches have been investigated. For example, Chou et al. demonstrated the stable deposition and metal patterning layers on PDMS substrate. In this method the parylene C which has high young's modulus (2.76 GPa) and thermal expansion coefficient ($3.5 \times 10^{-5}\ K^{-1}$) has been deposited on PDMS substrate. Baek et al. proposes another fabrication process to increase the PDMS surface roughness through reactive ion etching (RIE) method [28,29]. However, these methods have associated with some disadvantages, such as changes in the properties of the fabricated PDMS devices and breaking of wires owing to the high tension/compression in the PDMS during the fabrication processes/long-term operation. Fig. 1(a) shows the optical images of the PDMS cantilever, fabricated by using the existing fabrication processes. The fabricated PDMS cantilever exhibits the cracks and wrinkles caused by the thermal stress. In particular, the cracks cause the wire breakdown and the wrinkles increase the surface roughness of the device (Ra = 163.11 nm), thereby reducing the reliability and stability of the device electrical resistance.

To conquer this negative aspect of the fabricated device, we proposed the novel structure to realize the crack-free metal patterns and subsequent resistance stability at the PDMS substrate. The surface roughness of the PDMS substrate is greatly improved by employing a glass substrate. As shown in Fig. 1(b) the proposed structure consists of two parts (i) strain sensor-integrated PDMS cantilever and (ii) thin metal wires deposited glass substrate. Finally, the glass substrate was chemically bonded with the strain sensor-integrated PDMS cantilever, through which the durability and resistance reliability of the device is dramatically improved.

The detailed fabrication process of strain sensor-integrated PDMS cantilever is schematically illustrated in Fig. 2. The μpatterned PDMS cantilever is prepared by using general MEMS process such as etching and lift-off. In this fabrication process first, the photoresist film patterned on Si wafer through the conventional

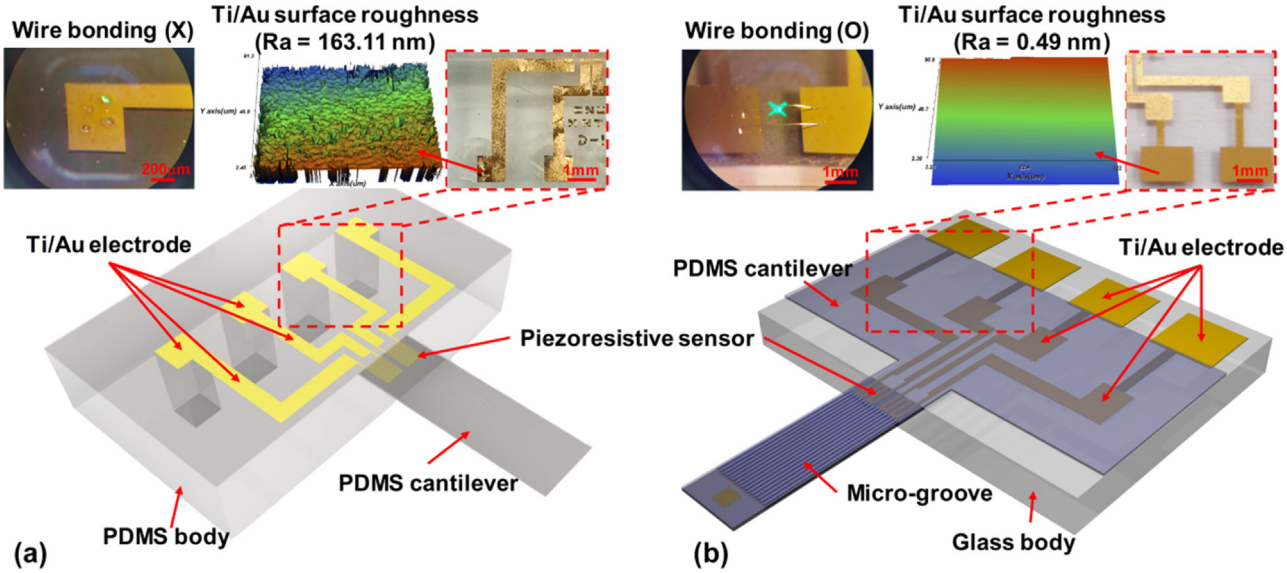

Fig. 1. Schematics of PDMS cantilevers for drug toxicity screening application. (a) Strain sensor-integrated μpatterned PDMS cantilever. (b) Advanced PDMS cantilever with improved electrical reliability.

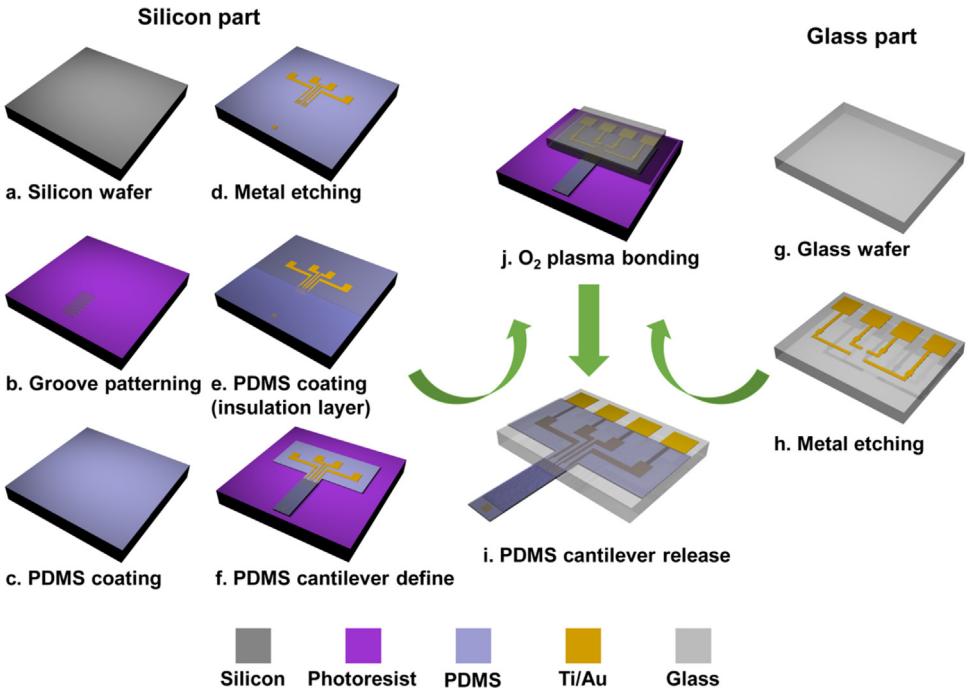

Fig. 2. Fabrication process flow of the PDMS cantilever integrated with μgrooves and strain sensor.

photolithography process. Subsequently, the μgroove structure with 1.5 μm line and space are formed on the sacrificial layer. Then 60 μm thickness PDMS is spin coated over the fabricated μgrooves molded Si substrate (Base: curing agent = 10: 1), consequently cured at 100 °C for 35 min (Fig. 2(a–c)). Next, 5 nm and 100 nm of Ti/Au layers were deposited by using the electron beam (EB) evaporator. The strain sensor is fabricated on PDMS surface through the photolithography and metal etching processes. During the cardiomyocytes culture time the PDMS cantilever is operated inside the culture medium, whereas the integrated strain sensor isolated electrically from the culture medium. To achieve this, 10 μm-thick PDMS layer is spin-coated over the strain sensor (Fig. 2(d–f)). In the fabrication process of glass part with metal wires, the photoresist was patterned on the glass wafer, subsequently, 5 nm and 100 nm of Ti/Au layers were deposited respectively by using electron beam (EB) evaporator. Then the photoresist removed by using acetone, which resulted in metal structures forming over the glass wafer (Fig. 2(g–h)). The glass wafer cut into 9 mm × 12 mm pieces using a dicing saw (AM Technology, NDS200). The fabricated strain sensor-integrated μpatterned PDMS cantilever and metal wire deposited glass substrate are chemically bonded together. In order to attain this, surface modification of the PDMS is carried out. The PDMS surface is treated by using the surface plasma treatment system (FEMTO SCIENCE, CUTE-MPR) in oxygen atmosphere at 100 W for ∼50 s. Finally, photoresist, which were used as the μgroove mold, was removed by using acetone and the PDMS cantilever was released from the substrate (Fig. 2(i–j)).

Fig. 3 shows the optical and scanning electron microscopic images of the fabricated strain sensor-integrated μpatterned PDMS cantilever. Fig. 3(a) shows the top and a cross-sectional view of the strain sensor-integrated PDMS cantilever with μgrooves and Fig. 3(b) shows the side view of the μpatterned PDMS cantilever. In this proposed work, the strain sensor-integrated PDMS cantilever designed to show the large displacement even with the small contraction force of cardiomyocytes. The length, width, and thickness of the cantilever are kept at 6,000 μm × 2,000 μm × 70 μm respectively. The measured spring constant of the strain sensor-integrated PDMS cantilever is found to be ∼0.7 mN/m. The aspect ratio of the cantilever is set to be 3:1 on the basis of information obtained from the earlier study by Park et al. [30].

2.2. Cell culture and neonatal rat ventricular myocytes (NRVM) isolation

All animal tests were performed in accordance with protocols approved by the Animal Ethics Committee of Chonnam National University. A heart was aseptically isolated from a Sprague-Dawley rat on day 3. The separated ventricles were washed by using ADS buffer solution (NaCl 120 mM, HEPES 20 mM, NaH_2PO_4 8 mM, D-glucose 6 mM, KCl 5 mM, $MgSO_4$ 0.8 mM, DI water 1L, pH 7.35) and then single cardiomyocytes were acquired through enzyme solution (Collagenase 0.5 mg/mL, Pancreatin 0.6 mg/mL, ADS buffer solution 50 mL) and pre-plating. In order to promote the attachment, spreading and proliferation of cardiomyocytes, a thin layer of fibronectin were coated over the PDMS surface. To effectively coat the fibronectin (Corning®), the fabricated PDMS cantilever was subjected to surface treatment at 80 W for 30 s. The acquired cardiomyocytes were then seeded onto the PDMS cantilever at a density of 1,000 cell/mm^2.The cell seeded PDMS cantilevers were cultured in a solution containing 67% Dulbecco's modified Eagle medium (DMEM, LONZA), 17% Heparin sodium salt from porcine intestinal mucosa (M199,Sigma-Aldrich), 10% Horseserum (HS, Sigma-Aldrich), 5% fetal bovine serum (FBS, Sigma-Aldrich), and 1% penicillin streptomycin (P/S, Sigma-Aldrich). The cardiomyocytes were cultivated at 37 °C in 5% CO_2 incubator and the culture medium was replaced every 72 h. After seeding the cardiomyocytes on the PDMS cantilever the displacement measurement analysis were conducted for 10 days.

2.3. Immunocytochemical staining

The cardiomyocytes were placed in 3.7% formalin solution for 10 min at room temperature and washed 3 times with phosphate-buffered saline (PBS Takara). Permeabilization was accomplished with 0.2% Triton-X (Sigma-Aldrich) in PBS for 15 min at room temperature. To prevent nonspecific binding, the antibodies were

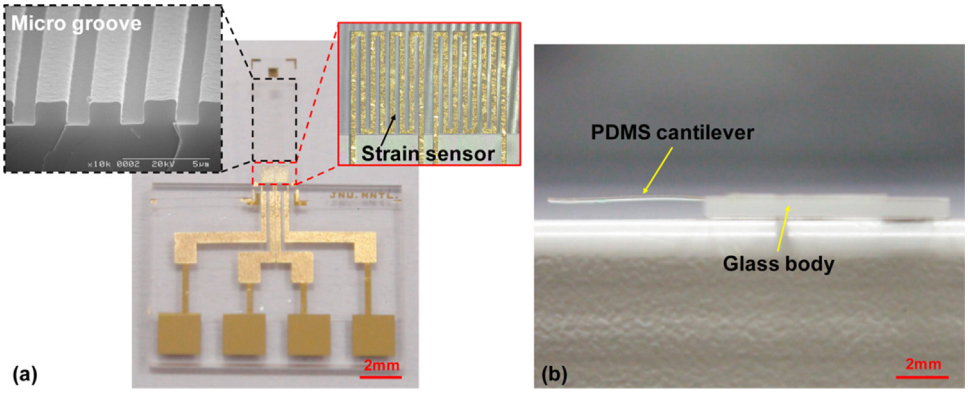

Fig. 3. Optical image of (a) the PDMS cantilever (inset: SEM image of μgrooves and optical image of the strain sensor), and (b) a side view of the μpatterned PDMS cantilever.

incubated at room temperature for 40 min by using 1% bovine serum albumin (1% BSA, Sigma-Aldrich). The primary antibody, monoclonal anti-actin (α-sarcomeric) was diluted at 1:200 with 1% BSA and incubated at room temperature for 1.5 h. The secondary antibody (Alexa-Flour 488 goat anti-mouse IgG conjugate, Sigma-Aldrich) was diluted at 1:500 in the same blocking solution and incubated for 1 h at room temperature. At last, the samples were incubated with 4′,6-Diamidino-2-phenylindole (DAPI) at 37 °C for 15 min for immunofluorescence of F-actin and nucleus [31].

2.4. Experimental setup

Schematic of the sensor setup and techniques used for sensing characteristics measurements are shown in Fig. S1(a). Displacement of the cantilever is monitored at free end of the PDMS cantilever through the CCD microscope (Dino-Lite Premier, AM4113ZTL) which is positioned in front of the cantilever. The PDMS cantilevers displacement occurring as a result of contraction and relaxation of the cardiomyocytes are measured through the change in resistance (ΔR) of the strain sensor. The change in sensor resistance is determined through the current-voltage (I *vs* V) characteristics of the strain sensor (KEITHLEY source-meter-2410). To identify the maximum sensitivity limit of the fabricated strain sensor integrated PDMS cantilever; we have measured the sensor response (ΔR) as a function of different external force (100–1000 μm) which is applied to the free end of the cantilever. As observed in Fig. S1(b) the sensor response gradually increase with increase in external force. These results clearly indicating that the fabricated strain sensor integrated PDMS cantilever exhibited wide range of detection limit and it able to show the sensor response to external force as low as 100 μm. Fig. S1(c) clearly indicates the strong linear dependence of the sensor response as a function of external applied force and slop of the plot is found to be 16.3 μΩ/μm.

3. Result and discussion

3.1. Alignment of the cardiomyocytes on μgrooved PDMS substrate

To examine the morphological alignment of the cardiomyocytes, the cells were cultured on both the unpatterned and μpatterned PDMS cantilever substrates and then the cardiomyocytes were observed by using a confocal microscope and fluorescence staining at several point of time. The optical microscope images of the cardiomyocytes cultured on with and without μgrooves PDMS cantilevers shown in Fig. S2. The cardiomyocytes cultured on the unpatterned PDMS cantilever (Fig. S2(a)) were grown isotropically. Whereas in the μpatterned PDMS cantilever, the cultured cardiomyocytes and nuclei cardiomyocytes respond to the μpattern features and become elongated in the μgrooves direction and α-sacomeric actin was also elongated more in the μgrooves direction (Fig. S2(b, c)). We also observed that the number of cardiomyocytes was found to be increased in the μpatterned PDMS cantilever although the cell spreading area was similar in both unpatterned and patterned PDMS cantilever. This consequence is now well established and many studies systematically investigated the role of micropatterning on cardiomyocytes growth and alignment on a PDMS substrate. The micropatterns created by conventional photolithography provides strong geometry constrain, permissive growth environment and a guide for cardiomyocytes growth in a pre-established directions. Additionally, the cardiomyocytes cultured on the microgrooved PDMS substrate exhibits considerably lower proliferation rates compared to those on the flat PDMS surface [32–34].

3.2. Characterization of PDMS cantilever displacement according to the culture time of the cardiomyocytes

The sensor response (ΔR) and the corresponding mechanical deformation (Fig. 4) causing from the contraction and relaxation of cardiomyocytes are measured during the culture period. The Fig. 4(a) and (b) shows the sensor response and displacement of unpatterned and μpatterned PDMS cantilever as a function of different culture period. The sensor response is calculated by measuring the change in resistance of the PDMS cantilever and it is defined as the ratio of sensor resistance caused by the contraction force and relaxation state of cardiomyocytes. The characteristic analysis of the cantilever according to cell culture period started on day 4 when the cells are synchronized and the measurements are subsequently conducted for seven days. This study clearly reveals that the cardiomyocyte contraction force is greatly influenced by the cell culture time. The mechanical deformations of the PDMS cantilever increases until day 9 and then decreases. The maximum bending and the corresponding sensor response of the μpatterned PDMS cantilever on day 9 is measured to be 6.975 ± 0.425 mΩ and its displacement is 690 ± 18 μm. Whereas in the unpatterned PDMS cantilever the maximum displacement and sensor response is found to be 2.615 ± 0.048 mΩ and 422 ± 13 μm respectively which is 167% and 40% less compare to the μpatterned PDMS cantilever. The reason for increase in contraction force of cardiomyocytes and mechanical deformation on μpatterned PDMS cantilever could be explained based on the following lines. As we know, the primary cardiomyocytes exhibits a spontaneous contractility in a range of frequencies. At the initial stages of the culture period the spontaneous contractions of cardiomyocytes exhibits

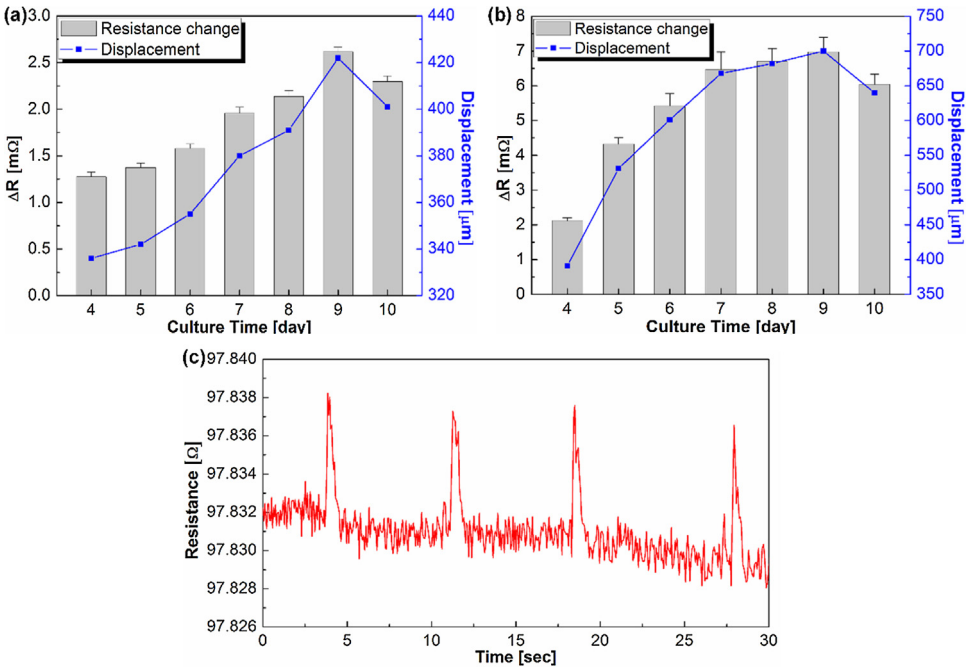

Fig. 4. Displacement of cantilever and strain sensor, ΔR, according to culture period for (a) unpatterned PDMS cantilever (the relative standard deviation in each day is 3.9, 3.4, 3.0, 3.3, 2.9, 1.9 and 2.6% respectively), (b) μpatterned PDMS cantilever (the relative standard deviation in each day is 3.5, 4.0, 6.4, 7.7, 5.6, 6.1 and 4.7% respectively) and (c) real-time measurement of beating characteristics for cardiomyocytes using the piezoresistive sensor-integrated PDMS cantilever.

only in the individual cardiomyocytes and the resultant contraction force is not sufficient to produce any deformation in the PDMS cantilever (up to day 3). The optical microscope images of the progression of cardiomyocytes shown in Fig. S3. As the culture time increases the cells started to contact with one another and the contractions force of cardiomyocytes became stronger with more synchronization (day 4). The contractile activity of cardiomyocytes improved with the increasing culture period (up to day 9), after that due to senescence of the cardiomyocytes the contractile activity decreases.

Fig. 4(c) shows the real-time measurement of the change in contraction force of cardiomyocytes. The response and recovery time of the sensor are defined as the time taken by the sensor to measure the maximum resistance caused by the contraction force and recovery back to original resistance due to the relaxation of cardiomyocytes. The response time and recovery time of the sensor is about ~1 s respectively. The beating frequency can be controlled by employing an electrical stimulator. Due to the small temperature fluctuation during the measurement, the sensor shows the slight decrease in base resistance which can be compensated by maintaining the constant temperature by using a temperature controller (Fig. S4(a, b)). The peak to peak comparison demonstrates that there is no observable change in peak shape, peaks amplitude and also the peak width is consistent with time-scales. However, as shown in Fig. 4(c) the sensor response slightly affected by the noise, but noise signal intensity is almost negligible compare to the sensor signal intensity. The measured signal to noise ratio of the μpatterned PDMS cantilever sensor was found to be ~5.44 dB.

3.3. Measurement of drug induced changes in the mechanical activity of cardiomyocytes

The strain sensor integrated PDMS cantilever was investigated to measure the drug induced changes in the mechanical activity of cardiomyocytes. Drug treatment-induced contraction force and the resultant beating frequency of the cardiomyocytes were observed on 1.5 μm grooved PDMS cantilever. The contraction force and beating frequency of the cardiomyocytes under the drug influence is determined through the mechano-physiology measurement, this technique measures the contraction force of the cardiomyocytes through the mechanical deformation of the cantilever. Two typical cardiovascular drugs, Verapamil and Isoproterenol were selected to test the performance of the fabricated sensor device. Verapamil is a calcium channel inhibitor and prevents the calcium influence into the cell, thereby decreasing the contraction force of the cardiomyocytes. Whereas Isoproterenol is a β-agonist that increases contraction force by stimulating β-adrenergic receptors. To evaluate the effect of these drugs on the contraction force of cardiomyocytes, different concentrations of verapamil (50 nM, 100 nM, 200 nM, 500 nM, and 1 μM) and Isoproterenol (1 μM, 2 μM, 4 μM, and 10 μM) based culture medium were prepared. The representative drugs were diluted with ethanol (concentration >0.1%) and the basic experimental results showed that it has no effect on the contraction force and beating frequency of cardiomyocytes.

Verapamil and Isoproterenol were introduced into the cardiomyocytes on day 7 and day 9 because the contraction force of the cardiomyocytes increase and decrease respectively at day 7 and 9. We measure the contraction force of drug treated cardiomyocytes 5 min after the treatment. Fig. 5 shows the sensor response (ΔR) and the beating frequency of the sensor in accordance with the different concentrations of Verapamil and Isoproterenol. The measurement results indicate that the mechanical deformation of cantilever and the beating frequency of cardiomyocytes decreased with increasing the concentration of Verapamil. The contraction force and heart beating of cardiomyocytes towards 50 nM, 100 nM, 200 nM, 500 nM, and 1 μM concentration of Verapamil were measured to be 6 ± 0.44, 5.5 ± 0.33, 4.4 ± 0.41, 3.1 ± 0.23, 1.2 ± 0.11 and 13, 7, 7, 4, 4 respectively. The contraction force and beating frequency of cardiomyocytes decreased approximately ~81.3% and ~75% at 1 μM Verapamil (Fig. 5(a, b)) compare to control state (without Verapamil treatment).

In the case of Isoproterenol treatment, the contraction force and beating frequency of cardiomyocytes increases with increasing Isoproterenol concentration until 2 μM and then decreases.

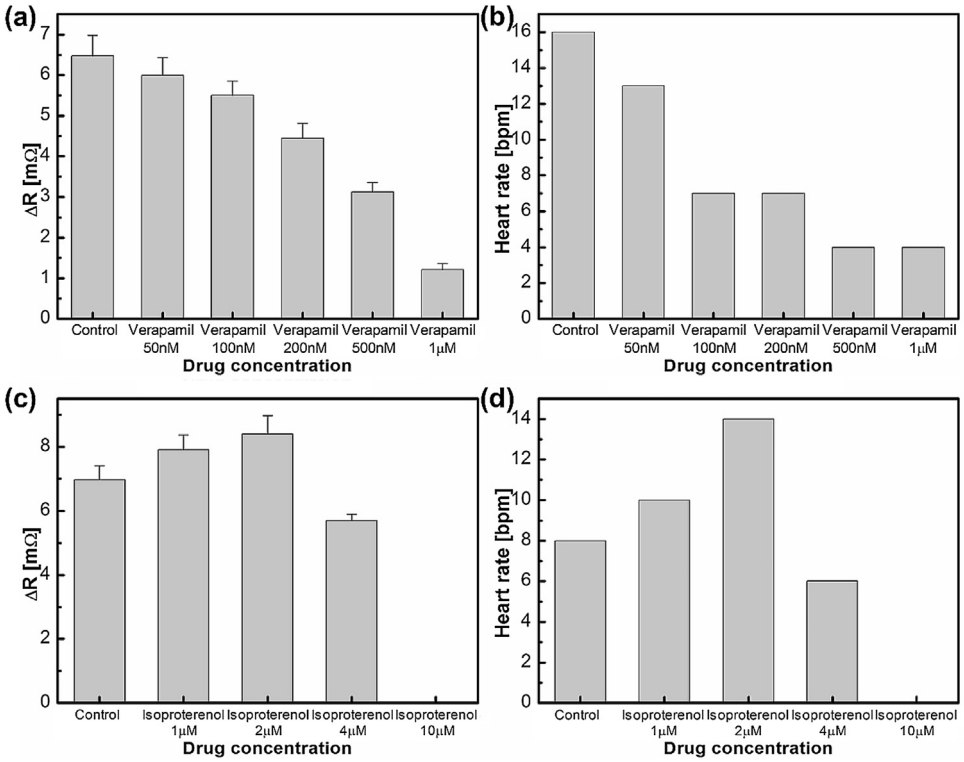

Fig. 5. Changes in contraction force and beating frequency according to drug concentration of PDMS cantilever seeding cardiomyocytes. Changes in (a) contraction force (the relative standard deviation in each drug concentration is 7.7, 7.3, 6.3, 8.2, 7.3 and 12%, respectively) and (b) beating frequency according to Verapamil concentration. Changes in (c) contraction force and (the relative standard deviation in each drug concentration is 6.1, 5.9, 6.7, 3.5% respectively) (d) beating frequency according to Isoproterenol concentration.

The measured contraction force and the corresponding beating frequency of cardiomyocytes towards 1 μM, 2 μM, 4 μM, and 10 μM concentrated Isoproterenol was 7 ± 0.4, 7.9 ± 0.51, 8.4 ± 0.54, 5.6 ± 0.11 and 8, 10, 14, 6 respectively. Conversely, the contraction force and beating frequency of cardiomyocytes decreases very rapidly when the cardiomyocytes treated with Isoproterenol beyond 2 μM concentration. Compare to control state (without Isoproterenol treatment) the contraction force and beating frequency of cardiomyocytes increased up to 20.4% and 75% at 2 μM Isoproterenol (Fig. 5(c, d)).

Further, adding the drug into the cardiomyocytes (i.e. Verapamil >1 μM; Isoproterenol >2 μM) the contraction force and beating frequency of the cardiomyocytes rapidly decreased. The preliminary experimental results clearly showed that the fabricated strain sensor integrated PDMS cantilever can verify the drug induced changes in the mechanical activity of cardiomyocytes through the beating status of cardiomyocytes.

Advantages of the proposed strain sensor integrated μpatterend PDMS cantilever: the important feature of our device compare to other contractile measurement techniques includes (i) our system can measure the contractile force on a specific microsized area in real time and produce the electrical output signal and it does not rely on any spectroscopy technique for signal read out, (ii) the thermal stability, surface roughness and electrical reliability of the device were greatly improved through the chemical attachment of PDMS cantilever with the metal wire deposited glass electrode, (iii) the manufacturing process of our device involves simple micromolding technique, therefore large number sensor arrays can be fabricated inexpensively and easily (iv) the micro cantilever structure is made by a flexible, transparent, and superior bio-compatible poly (dimethylsiloxane) (PDMS) substrate and there are no harmful elements to affect the cardiomyocytes function, hence, the cardiomyocytes structures and activation status could be efficiently observed using a confocal microscope.

Hence, we sincerely anticipate that the proposed strain sensor integrated PDMS cantilever opens up a great opportunity to establish the new class of device which can measure the drug induced changes in the mechanical activity/contraction force of cardiomyocytes.

4. Conclusions

In this study, an Au strain sensor was integrated onto a PDMS cantilever to quantitatively measure the contraction force of cardiomyocytes in real time. Cardiomyocytes were aligned by forming three-dimensional μgrooves on the upper surface of the PDMS cantilever, thereby enhancing cantilever displacement. Electrical reliability of the strain sensor was also greatly improved because the Au strain sensor-integrated PDMS cantilever was chemically bonded with a glass substrate. Further, real-time measurements were demonstrated in the media environment by integrating the Au strain sensor inside the PDMS cantilever. We verified that cardiomyocytes seeded on the PDMS cantilever were synchronized at day 4 after cell culture and that their contraction force increased up to day 9. The changes in the mechano-physiological characteristics of the cardiomyocytes with respect to drugs using Isoproterenol and Verapamil showed that the contraction force and heart beat frequency decreased as the concentration of Verapamil increased within the range 50 nM–1 μM. In the case of Isoproterenol, the contraction force and beating frequency increased as a result of the drug effect up to 2 μM concentration but decreased over 4 μM concentration as a result of the influence of the drug. We experimentally confirmed that the μpatterned PDMS cantilever with Au strain sensor has significant potential for measuring the drugs induced changes in the mechanical activity of cardiomyocytes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.08.167.

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Biographies

Dong-Su Kim received his BS degree in electronics engineering from Chosun University (Korea) in 2013 and MS in mechanical engineering from Chonnam National University (Korea) in 2016. Currently he is pursuing his doctoral degree in mechanical engineering at MEMS and Nanotechnology Laboratory, Department of Mechanical Engineering, Chonnam National University, Korea. His research activity is primarily focused on design and fabrication new class of Bio-MEMS device for drug screening applications.

Yun-Jin Jeong received his BS degree in chemical engineering followed by MS degree in mechanical engineering from Chonnam National University (Korea). He is currently working toward the Ph.D. degree in mechanical engineering at MEMS and Nanotechnology Laboratory, Department of Mechanical Engineering, Chonnam National University, Korea. His current research interests include MEMS device design and fabrication, pressure sensor, CO_2 gas sensor.

Prof. Bong-Kee Lee received his Ph.D in Mechanical Engineering from Pohang University of Science and Technology (POSTECH), Pohang, Korea. Currently he is working as a professor at School of Mechanical Engineering, Chonnam National University, Korea. His research interests lie in a development of advanced micro/nano molding technologies and their application in optical, microfluidic, and biomedical areas.

Dr. Arunkumar Shanmugasundaram completed his Ph.D from CSIR-Indian Institute of Chemical Technology, Hyderabad, India. Currently he is working as a post-doctoral researcher at MEMS and Nanotechnology laboratory, School of Mechanical Engineering, Chonnam National University, Korea. His research interests include design and development of functional nanostructured materials for applications in sensors, catalysis and energy storage devices.

Prof. Dong-Weon Lee received his Ph.D. degrees in Mechatronics engineering from Tohoku University, Sendai, Japan in 2001. He has been a Professor of Mechanical Systems Engineering at Chonnam National University (CNU), South Korea since 2004. Previously, he was with the IBM Zurich Research Laboratory in Switzerland, working mainly on microcantilever devices for chemical AFM applications. At CNU, his research interests include smart cantilever devices, miniaturized energy harvester, smart structures & materials, and nanoscale transducers. He is a member of the technical program committee of IEEE Sensors Conference, Transducers, and Microprocesses and Nanotechnology Conference.