| 논문원고접수 |

2018년 12월 3일(월) ~ 12월 31일(월)

| 논문심사결과 통보일 |

2019년 2월 11일(월)까지 홈페이지 (http://www.micronanos.org)에 공지 및 책임저자에게 이메일로 통보

| 초록 및 논문접수처 |

http://www.micronanos.org

| 논문범위 |

1. Materials, Fabrication, and Packaging Technologies
2. Fundamentals in MEMS/NEMS
3. Micro/Nanofluidics and Lab-on-a-Chip
4. Bio/Medical Micro/Nano Devices
5. Micro/Nano Sensors and Actuators
6. RF/Optical Micro/Nano Devices
7. Micro/Nano Energy Devices
8. Flexible and Printed Devices
9. MEMS/NEMS Applications and Commercialization

주최 : (사)마이크로나노시스템학회 (E-mail : master@micronanos.org, 전화 : 02-749-6482)

제21회 KMEMS 학술대회 POSTER SESSION

Poster Session 1 (TP-1) — 4월 4일 목요일 16:50 ~ 18:00

논문 No.	발표장소	Journal Title	First Author	Corresponding Author	Presenting Author	Organization
TP-1-01	무궁화룸	Bio-oriented perm-selective structures for micro-nanofluidic applications	박성민	김성재	박성민	서울대학교
TP-1-02	무궁화룸	높은 감도와 극저농도 측정 능력을 가진 가스 센서의 제작을 위한 이황화몰리브덴의 안정적인 기능화 기술	김대기	김준협	김준협	고려대학교
TP-1-03	무궁화룸	웨어러블 응용을 위한 광 투과방식 유연 인장 센서 패키징	구지민	박인규	구지민	한국과학기술원
TP-1-04	무궁화룸	탄소나노튜브와 은나노와이어 복합체 기반 신축 직물 히터	안준성	박인규, 정준호	안준성	한국과학기술원
TP-1-05	무궁화룸	Electrochemical charge storage evaluation of nanostructured manganese sulfide thin film	Rahul B. Pujari	이동원	Rahul B. Pujari	전남대학교
TP-1-06	무궁화룸	프리즘기반의 비접촉 레이저 간섭 리소그래피를 통해 제작한 나노패턴 수치 시뮬레이션 및 AFM 측정	이성재	신보성	이성재	부산대학교
TP-1-07	무궁화룸	Hierarchical구조 기반의 신축성 투명 omniphobic PDMS 필름 제작	유채린	이동원	유채린	전남대학교
TP-1-08	무궁화룸	Dynamics of nanoelectrokinetic preconcentrated DNA leveraged by convection and diffusion	백성호	김성재	백성호	서울대학교
TP-1-09	무궁화룸	전극 위 절연층 에칭 윈도우 위치에 따른 유전영동 트랩 현상 비교	여강인	이상우	여강인	연세대학교
TP-1-10	무궁화룸	Electrode design for pH control in nano-electrokinetic device	오지환	김성재	오지환	서울대학교
TP-1-11	무궁화룸	제브라피쉬 정렬을 위한 유체 교환식 마이크로 유체 채널	이유현	김소희	이유현	대구경북과학기술원
TP-1-12	무궁화룸	확산 영동을 이용한 연속적 나노 입자 분리에서 pH의 효과 Effect of pH on Diffusiophoresis-based Continuous Nanoparticle Separation	서명진	김성재	서명진	서울대학교
TP-1-13	무궁화룸	Patternable particle microarray utilizing sequential particle delivery	이상현	김준원	이상현	포항공과대학교
TP-1-14	무궁화룸	강제적 정상 상태 달성을 통한 이온 선택적 전류에서의 음의 전도도 제거.	권순현	김성재	권순현	서울대학교
TP-1-15	무궁화룸	Spontaneous Selective Preconcentration Leveraged by Convective Flow through Paper-based Micropores over Diffusiophoresis	이도근	김성재	이도근	서울대학교
TP-1-16	무궁화룸	마이크로 표면구조 및 전기전도성에 따른 심근세포의 성숙에 관한 연구	김종윤	이동원	김종윤	전남대학교
TP-1-17	무궁화룸	세포 자극 및 실시간 관찰을 위한 스테이지-탑 바이오리액터의 제작 및 평가	정윤진	이동원	정윤진	전남대학교
TP-1-18	무궁화룸	Microfluidic channel based stretchable pressure sensor for wireless health monitoring	Munirathinam Karthikeyan	이동원	Munirathinam Karthikeyan	전남대학교
TP-1-19	무궁화룸	Characterization of the membrane capacitance and permeability changes caused by cholesterol depletion based on Dielectrophoretic System	김채원	이상우	김채원	연세대학교 의공학부
TP-1-20	무궁화룸	A PDMS 기반의 유연한 피질뇌파측정용 전극 어레이	이경연	김소희	이경연	대구경북과학기술원
TP-1-21	로즈룸	Effect of wrinkled metallic thin film in cardiomyocyte growth and maturation	노민	이동원	노민	전남대학교
TP-1-22	로즈룸	고정된 PCR Assay를 이용한 와파린 약물 관련 SNP 검출	배서진	김상효	배서진	가천대학교
TP-1-23	로즈룸	폴리머 기반의 유연한 3 차원 전극의 장기간 사용적합성 평가	장재원	김소희	장재원	대구경북과학기술원
TP-1-24	로즈룸	Novel silicon cantilever integrated with surface-patterned polymer thin layer and strain sensor for biomedical applications	Mingming Dong	이동원	Mingming Dong	Chonnam National University

Effect of wrinkled metallic thin film in cardiomyocyte growth and maturation

[1]Nomin-Erdene Oyunbaatar, [1,2]Dong-Weon Lee *
[1]School of Mechanical Engineering, Chonnam National University,
[2]ACenter for Next-Generation Sensor Research and Development, Chonnam National University
*E-mail: mems@jnu.ac.kr

주름진 금속 박막이 심근세포의 성장과 성숙도에 미치는 영향에 관한 연구

[1] 노민, [1,2] 이동원*,
[1] 전남대학교 기계공학부, [1,2] 전남대학교 차세대센서연구개발센터

Abstract

A systematic study of cardiac tissue structure is expected to provide a therapeutic approach to myocardial infarction, one of the major causes of death. Therefore, many research groups have been studying the effect of substrates such as conductivity and surface morphology. In this paper, we describe a cantilever structure based on a thin metal substrate with surface wrinkles in cardiomyocyte shapes and growth. In the case of the cantilever structure, since the contractile behaviours of the cardiomyocyte can be directly measured, it is possible to quantitatively analyze the mechanical properties of the growing cardiomyocyte. Observation of the contractile force and cell morphology revealed that cardiomyocytes showed better characteristics in terms of contractility when grown on the cantilever with the wrinkled thin metal film surface. In addition, it was experimentally confirmed that the number of Cx43 protein was greatly increased. RT-PCR experiments were also performed to quantitatively analyze gene expression.

Keywords: *Wrinkled metal thin film (주름 금속 박막), Engineered cardiac tissue (조작된 심장 조직), PDMS-cantilever (폴리 디메틸 실록산 외팔보)*

1. Introduction

Myocardial infraction is one of the leading causes of death worldwide. Numerous methods have been developed to study cardiomyocyte morphology and growth by changing the substrate material in vitro experiment [1]. The large amount of technology has been attempting to develop cardiac cell maturation for helping sequenced testing technology such as drug toxicity screening and the implementation of the cardiac patch etc. For instance, by changing the microenvironmental cues based on biocompatible materials presents highly potentiated engineered tissue like a natural myocardium. Various methods such as photolithography and micro-nano molding technologies have been attempted to produce cardiac tissues such as naturally aligned structures [2].

On the other hand, the use of electrical stimulus is one of advanced techniques to support the maturation of the cardiac cells. The electrical energy provides the cell binding energy that the cell is located near. Many different types of conductive substrates and composites have been utilized to produce electrical energy through cell culture platforms. However, information on gold metal thin films as cell culture platforms is still lacking. This work focuses on the effects of thin film metal films implemented on polymer cantilevers to study the morphological and mechanical properties of primary cardiomyocytes.

2. Material and methods

2.1 Substrate preparation

A thin metal patterned cantilever is prepared by following method. First, a four-inch single polished silicon wafer was utilized as a substrate. Then, AZ 4620 positive photoresist (PR) was coated on the silicon wafer as sacrificial film with 2000 rpm rotating speed. After proper backing the PR on a hot plate, 120 µm-thick PDMS layer was formed on the PR using a spin-coating method. Subsequently, the PDMS layer was cured at 80ºC at 1 hour. Following, 10/100 nm Ti/Au layers were deposited by electron beam (E-beam) evaporator. Various patterns of the thin metal films were carried out by a photolithography and an wet etching processes. Finally, the metal thin film with micro-sized wrinkles on the surface was formed through a tensile strain.

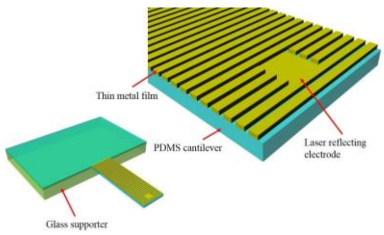


Fig. 1. Schematic of PDMS cantilever with wrinkled thin metal film on the surface

2.2. Cardiac cell isolation and expression ICC, PCR marker

This investigation was approved by the Animal Care Committee at the Chonnam National University of South Korea. Cardiomyocyte cells were isolated from 1-3-day old neonatal rat heart, which follows a standard isolation method. The culture medium was a plating medium supplemented with 10% fetal bovine serum (FBS). When the CMs is seeded on the functional surfaced cantilever, fibronectin was utilized as an ECM (extra cellular matrix).

Immunocytochemical staining was performed by using following antibodies. In the typical process, the cardiomyocytes were placed in 4% formaldehyde dissolved DPBS solution for 20 min at room temperature and washed 3 times with Dulbecco's phosphate-buffered saline (DPBS). Then, the cardiomyocytes permeabilization was completed with 0.1% Triton X-100 (Sigma-Aldrich) in DPBS for 5 min and blocked for 30 min in 3% Bovine serum albumin (BSA) (Sigma-Aldrich). The primary and secondary were diluted in 1:100 with 3% BSA solution and incubated at room temperature. After the staining experiment, the cardiomyocytes were analyzed through inverted confocal laser scanning microscopy (Leica TCS SP5 XCLSM, Germany).

In PCR experiment, cultured cells were harvested, centrifuged and resuspended. RNA isolation was performed according to the manufacturer's instructions. Isolated total RNA was purified M-MLC cDNA synthesis kit from Thermo Fisher Scientific. cDNA quality was evaluated with regular PCR reaction for β-actin gene expression.

3. Result

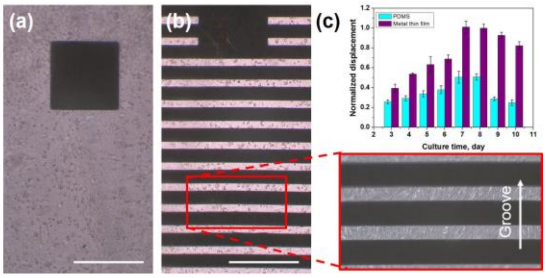


Fig. 2. Cardiomyocyte growing on the different surfaces. (a) cardiomyocytes on the flat surface cantilever (b) cardiomyocyte on the metal-patterned cantilever. (Scale bar: 500 μm).

Fig. 2 (a-b) shows the optical images of the CMs morphology on different PDMS cantilever surfaces. The bending displacements of the cantilevers with two different functional surfaces were measured using a laser vibrometer. The bending displacements and beating frequencies of two different cantilevers were compared from day 3 to day 10 after the cell seeding. The maximum bending displacement were on day 7 after cell seeding. The experiment result is shown in Fig. 2c.

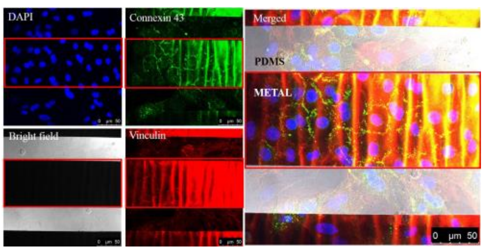


Fig. 3. Functional protein expression and cell population on different substrates with and without a wrinkled thin metal layer.

Based on enhancement of contraction force data, ICC (immunocytochemistry) staining experiment was performed to more deeply understand effect of metal thin film on the cardiomyocytes. To identify regulating protein expression, vinculin and connexin 43 (Cx43) proteins were characterized. The obtained Cx43 (green) protein expression (Fig.3) clearly reveals that the wrinkled metal thin film highly influences the cardiac cell coupling during the cell culture period. In addition, it was found that the density of the cell was much higher when the wrinkled metal thin film was employed on the PDMS cantilever.

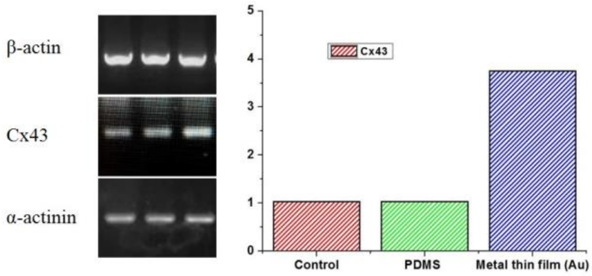


Fig. 4. PCR data for functional protein expression.

RT-PCR experiments were also performed to quantitatively analyze gene expression levels. The results of the experiment are shown in Fig. 4, and a large amount of Cx43 was observed on the metal thin film substrate. In contrast, the control and PDMS substrates did not show significant differences in Cx43 gene expression. The results clearly demonstrate the wrinkled Au metal thin film substrate, one of the competitive platforms for cell culture. In conclusion, the present study quantitatively assessed the effect of wrinkled metal thin films on the cantilever surface. We have experimentally confirmed that the wrinkled metal film shows a good cell culture platform and supports the maturation of cardiac cells in a short period of time.

Acknowledgments

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MISIT) (No. 2017R1E1A1A01074550).

References

1. M. Maddah, J.D. Heidmann, M.A. Mandegar, C.D. Walker S. Boloku, B.R. Conklin, K.E. Loewe, A Non-invasive platform for functional characterization of stem-cell-derived Cardiomyocytes with Application in Cardiotoxicity Testing, *Stem Cell Repots. 14*, 4 (Apr. 2014).
2. A.W. Feinberg, P.W. Alford, H. Jin, C.M. Ripplinger, A.A. Werdich, S.P. Sheehy, A. Grosberg, K.K. Parker, Controlling the contractile strength of engineered cardiac muscle by hierarchal tissue architecture, *Biomaterials. 33*, 23 (Aug. 2012).

The 21st Korean MEMS Conference
April 04-06, 2019, JEJU, SOUTH KOREA

The Society of Micro and Nano Systems

Absrtact

A systematic study of cardiac tissue structure is expected to provide a therapeutic approach to myocardial infarction, one of the major causes of death. Therefore, many research groups have been studying the effect of substrates such as conductivity and surface morphology. In this paper, we describe a cantilever structure based on a thin metal substrate with surface wrinkles in cardiomyocyte shapes and growth. In the case of the cantilever structure, since the contractile behaviours of the cardiomyocyte can be directly measured, it is possible to quantitatively analyze the mechanical properties of the growing cardiomyocyte. Observation of the contractile force and cell morphology revealed that cardiomyocytes showed better characteristics in terms of contractility when grown on the cantilever with the wrinkled thin metal film surface. In addition, it was experimentally confirmed that the number of Cx43 protein was greatly increased. RT-PCR experiments were also performed to quantitatively analyze gene expression.

◆**Keywords : Wrinkled metal thin film** （*주름 금속 박막*）**, Engineered cardiac tissue** （*조직된 심장 조직*）**, PDMS cantilever** （*폴리 디메틸 실록산 외팔보*）

EFFECT OF WRINKLED METALLIC THIN FILM IN CARDIOMYOCYTE GROWTH AND MATURATION

주름진 금속 박막이 심근세포의 성장과 성숙도에 미치는 영향에 관한 연구

Nomin-Erdene Oyunbaatar[1], and Dong-Weon Lee[1,2*]

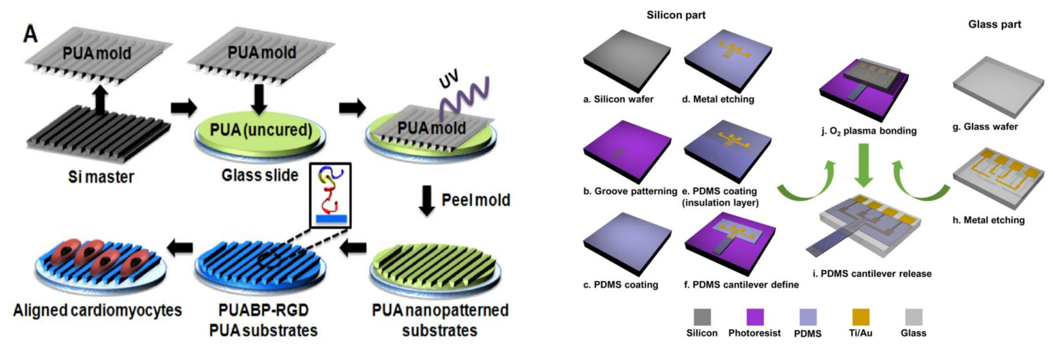
[1,2]Department of Mechanical Engineering, Chonnam National University,*
[2]Center for Next-generation Sensor Research and Development, Chonnam National University
E-mail : mems@jnu.ac.kr

전남대학교 CHONNAM NATIONAL UNIVERSITY

MEMS & Nanotechnology Laboratory

MNTL CHONNAM NATIONAL UNIVERSITY

Introduction



Ongoing bio mimicked nano- μgroove patterned cantilever

- Poor adhesion
- Complex fabrication technique (microcontact printing, photolithography, laser ablation etc.)

Motivation

- **Self-assembled multiscale topographical structure for aligning and supporting maturation of cardiomyocyte**
- **Electro-conductive metallic layer for increasing adhesion**
- **High resolution laser-vibrometer measuring system with real time**
- **High capable for detecting drug toxicity screening in early stage**

Device fabrication

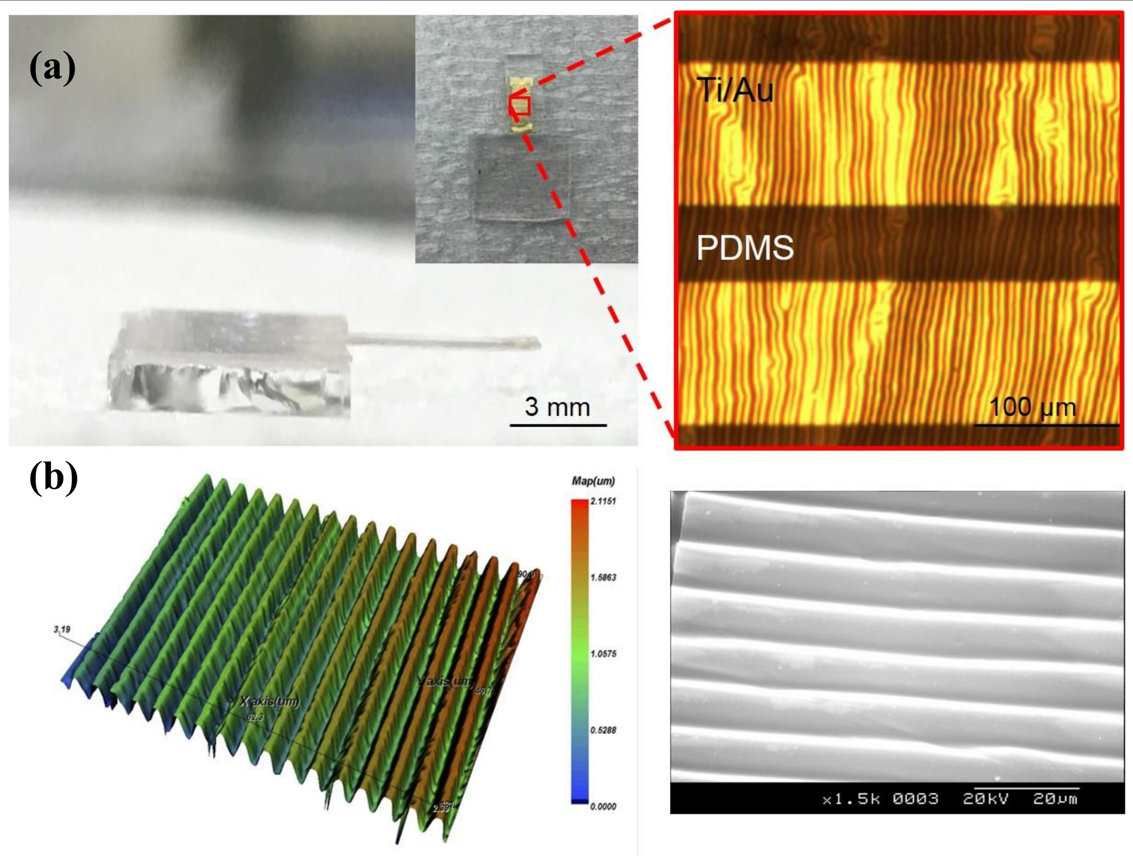


Fig. 1. (a) Optical image of fabricated polymer cantilever, Optimized surface topography (b) Optical image (c) SEM image of metal thin film wrinkle.

NRVM culture and morphology

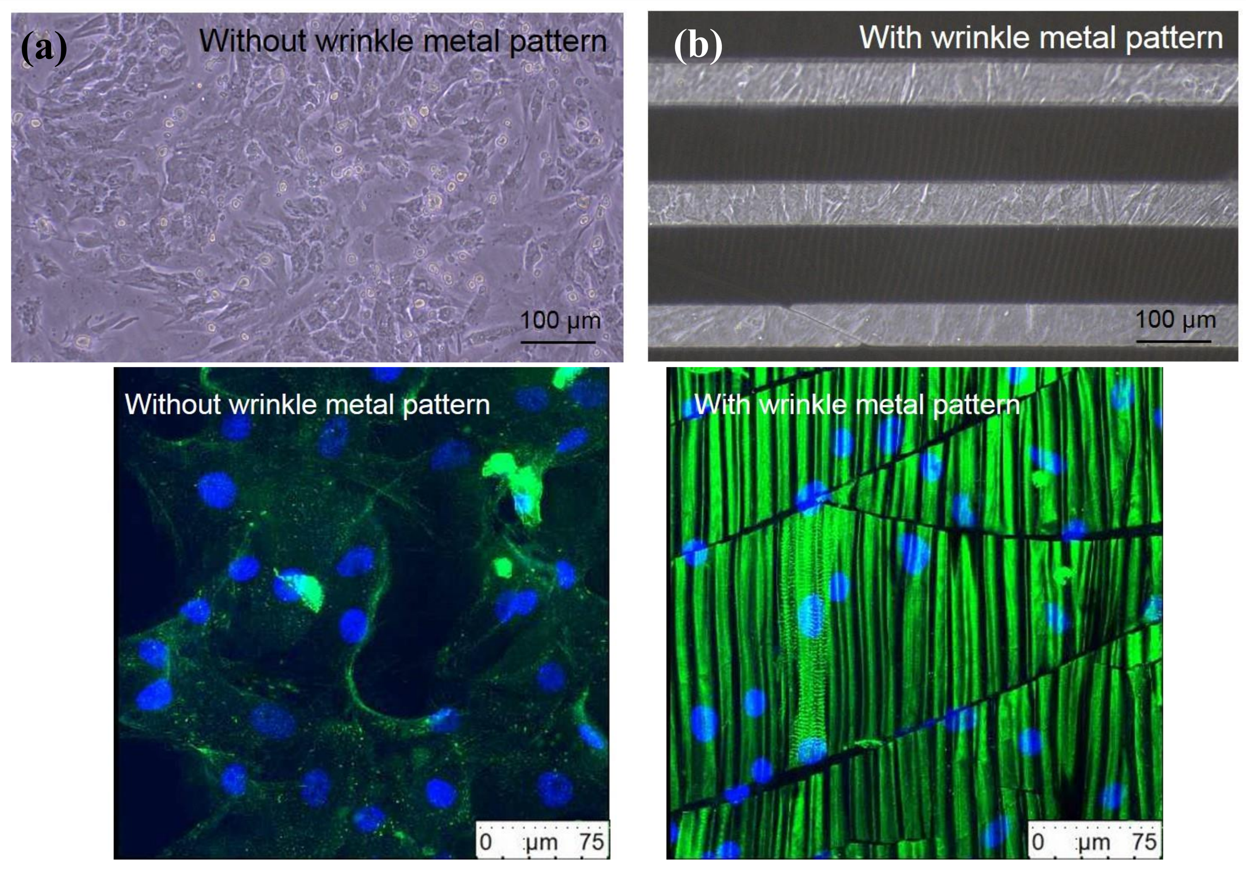


Fig. 2. Optical and ICC images of cardiac cell morphology. Cardiac cell growing on the different cantilever surface: (b) without and (c) with metal wrinkle. (Cell seeding after day on 7)

Cantilever bending displacement and RT-PCR

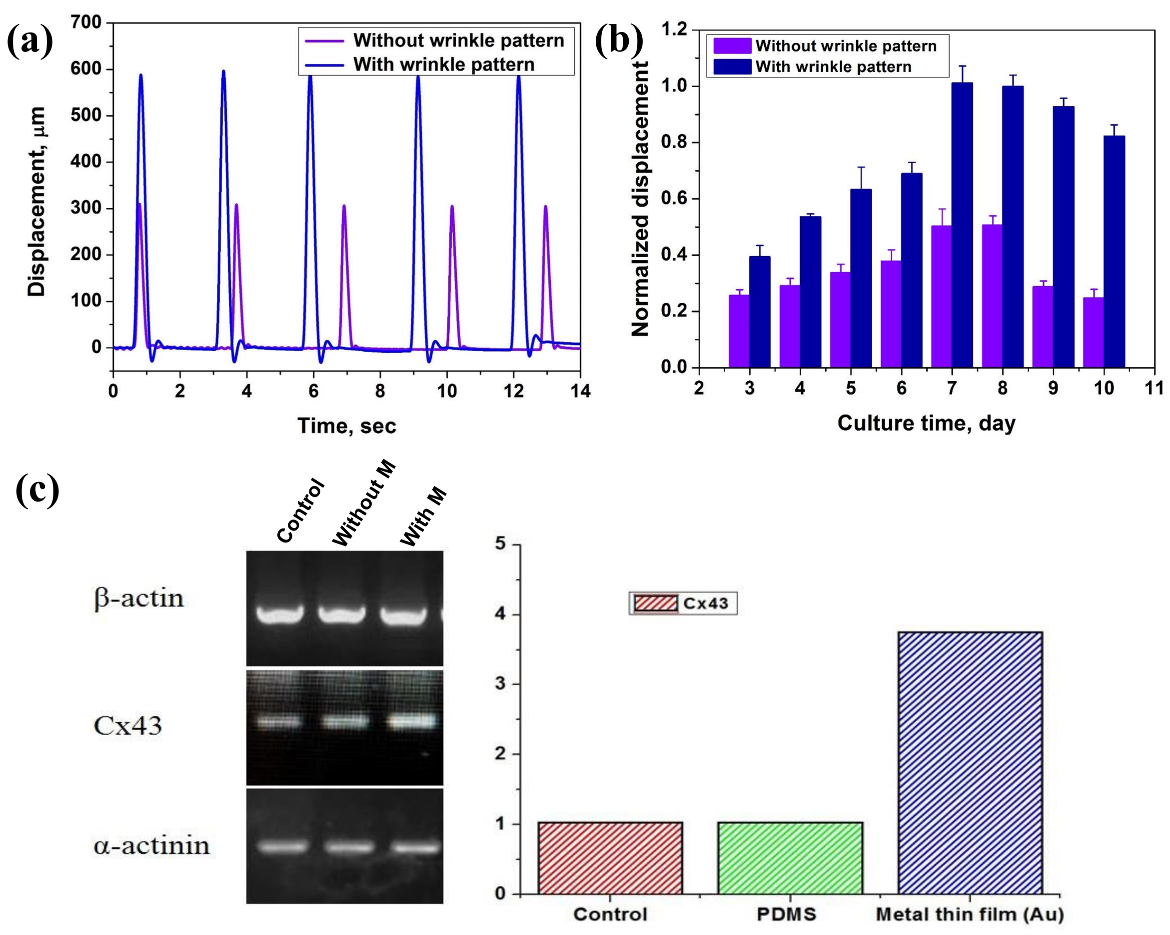


Fig. 3. (a) and (b) Cantilever bending displacement on different topographical surfaces (c) RT-PCR data on different surfaces.

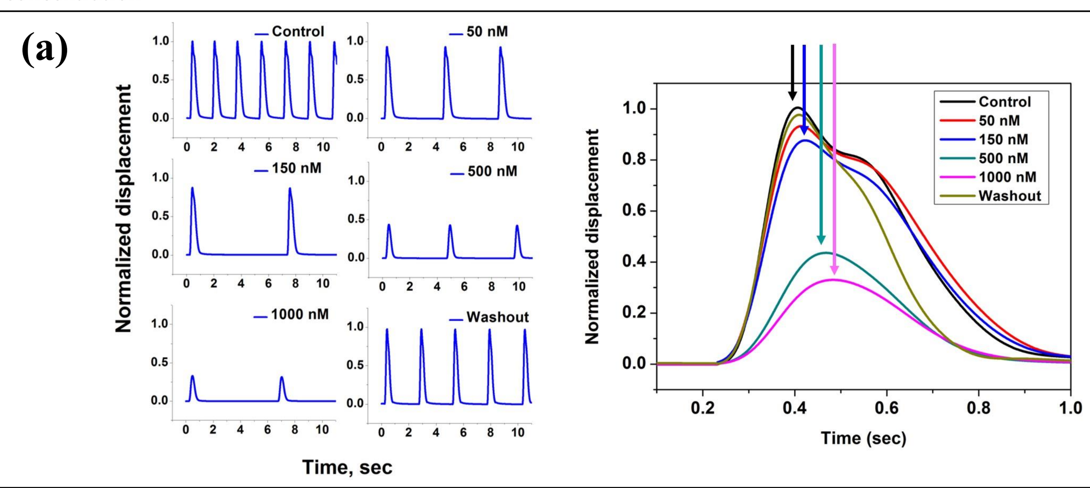
Drug-induced cardiac toxicity test

Table 1. Molar concentration of applied drugs

VERAPAMIL	50 nM	150 nM	500 nM	1000 nM	Washout
Stock Solution (0.8mM)	0.25 μL	0.75 μL	2.5 μL	5 μL	4ml medium
Ethanol concentration	0.00625%	0.01875%	0.0625%	0.125%	-

QUINIDINE	250 nM	500 nM	1000 nM	1500 nM	Washout
Stock Solution (1.6 mM)	0.625 μL	1.25 μL	2.5 μL	3.75 μL	4ml medium
Ethanol concentration	0.0156%	0.03125%	0.0625%	0.0937%	-

E-4031	12 nM	24 nM	48 nM	96 nM	120 nM	Washout
Stock Solution (48μM)	0.25 μL	0.5 μL	1 μL	2 μL	4 μL	4ml medium
Ethanol concentration	0.00625%	0.0125%	0.025%	0.05%	0.1%	-



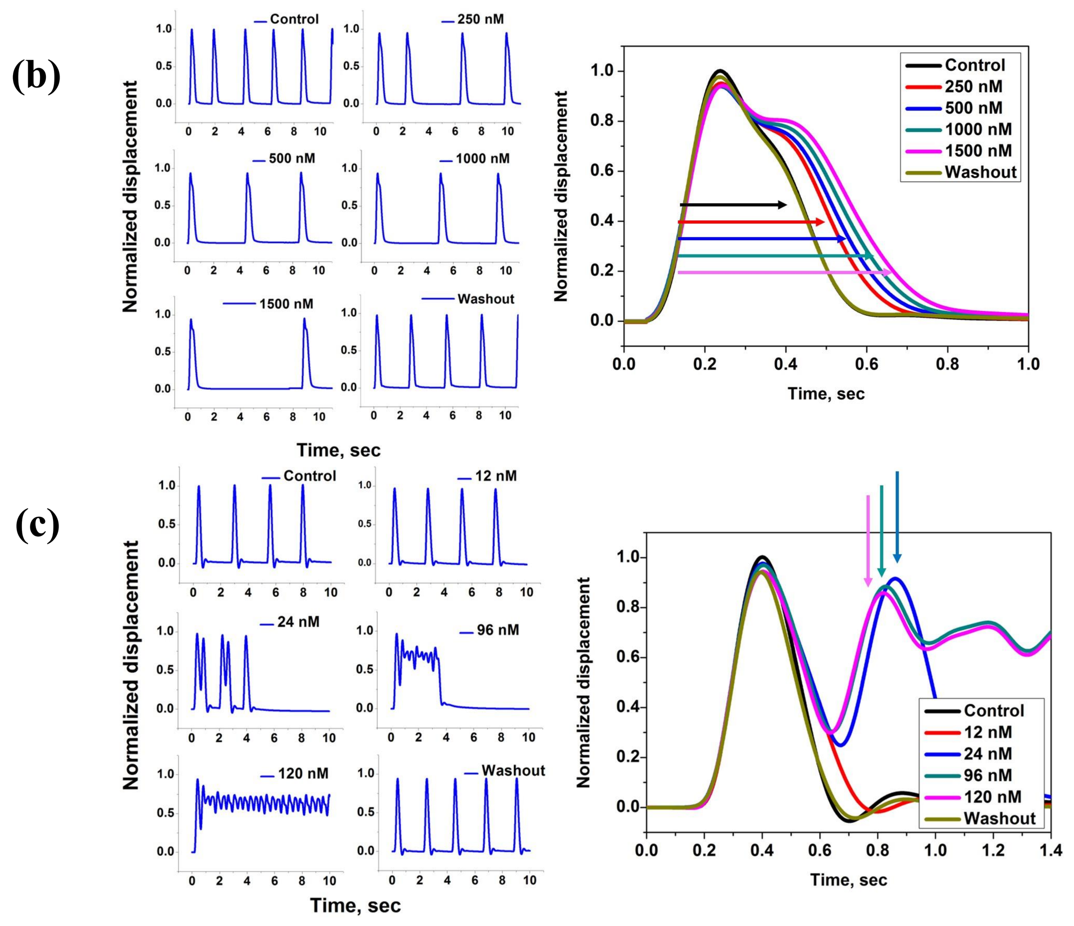


Fig. 4.Cardiotoxicity result for (a) Verapamil (calcium channel blocker), (b) Quinidine (Sodium channel blocker), (c) E4031 (potassium channel blocker) respectively.

Immunocytochemistry staining test

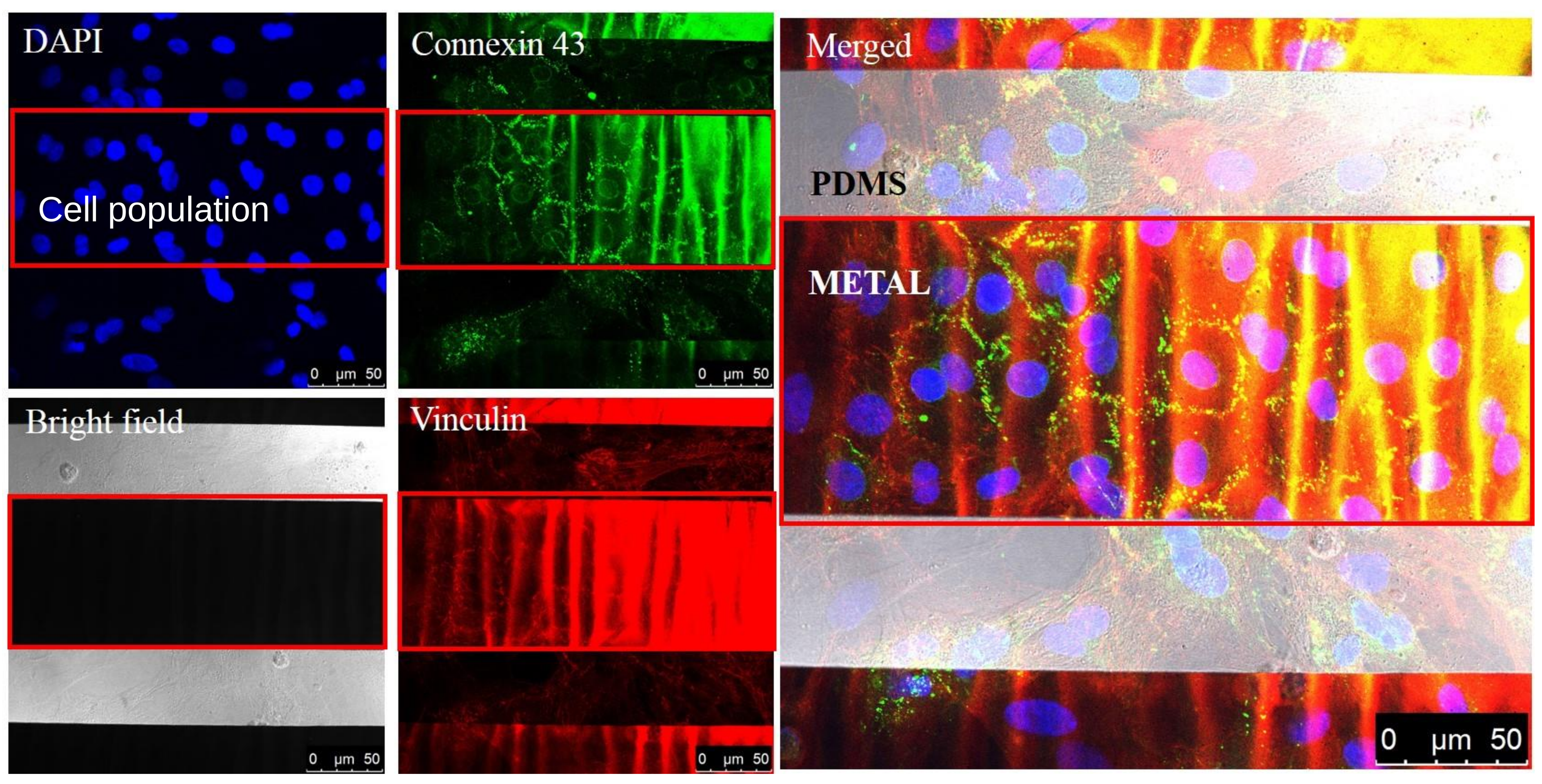


Fig. 5. Increasement of cell population and adhesion result by ICC on the wrinkled metal thin film.

Conclusion

The fabricated cantilever structure with the wrinkled metal thin film shows a quick and easy way of making from a biologist's perspective studying the mechanical properties of cardiac muscle cells. Moreover, metal substrate effectively supporting the cardiac cell maturation. Additionally, we experimentally confirmed that the wrinkled metal thin film could effectively align cardiac muscle cells and enhance their contraction forces up to 2 times as compared to the cardiac cells growing on the flat cantilever. Increase of Cx43 was also experimentally confirmed by a staining process. The proposed cantilever-based device with metallic wrinkle patterns has a great potential to further stimulate the maturation of cardiac cells from a biomimetic engineering standpoint and thereby enhances the accuracy of monitoring the drug toxicity of cardiac cells.

Reference

1. M. Maddah, J.D. Heidmann, M.A. Mandegar, C.D. Walker S. Boloku, B.R. Conklin, K.E. Loewe, A Non-invasive platform for functional characterization of stem-cell-derived Cardiomyocytes with Application in Cardiotoxicity Testing, *Stem Cell Repots. 14*, 4 (Apr. 2014).
2. A.W. Feinberg, P.W. Alford, H. Jin, C.M. Ripplinger, A.A. Werdich, S.P. Sheehy, A. Grosberg, K.K. Parker, Controlling the contractile strength of engineered cardiac muscle by hierarchal tissue architecture, *Biomaterials. 33*, 23 (Aug. 2012).

Acknowledgment

This study was supported through a National Research Foundation of Korea (NRF) Grant funded by the Korean government (MSIT) (No.2017R1E1A1A01074550).