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Realistic computational modeling for hybrid biopolymer microcantilevers

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Abstract— **Three dimensional cultures in a microfabricated environment provide in vivo-like conditions to cells, and have used in a variety of applications in basic and clinical studies. Also, the analysis of the contractility of cardiomyocytes is important for understanding the mechanism of heart failure as well as the molecular alterations in diseased heart cells. This paper presents a realistic computational model, which considers the three dimensional fluid-structural interactions (FSI), to quantify the contractile force of cardiomyocytes on hybrid biopolymer microcantilevers. Prior to this study, only static modeling of the microscale cellular force has been reported. This study modeled the dynamics of cardiomyocytes on microcantilevers in a medium using the FSI. This realistic model was compared with static FEM analysis and the experimental results. Using harmonic response analysis in FSI modeling, the motion of a hybrid biopolymer microcantilever in the medium was identified as a second-order system and the influence of the dynamics of cardiomyocytes could be evaluated quantitatively.**

I. Introduction

Three-dimensional cell cultures have a wide variety of applications in basic cell and tissue studies [1], in vivo tissue repair and clinical practice. Some heart diseases, even in the early stages, can injure the ability of a heart cell to contract and relax [2], which may result from the significant difference in contractile force between normal heart cells and those involved in heart failure. Therefore, information on the contractile force of heart cells will be very helpful in understanding the precise mechanism of heart failure as well as the molecular alterations involved in diseased heart cells [3]. Previously, many researchers developed micro technology-based methods to measure the contractile force of cardiomyocytes. [4-6]. However, these studies have some limitations such as the use of injurious cell manipulation, and different effects on cell adhesion and cell locomotion compared with that on flat surfaces [7]. A measurement method needed for quantitative analysis of the contractile force of cardiomyocytes. In particular, for heart function investigations and clinical applications, more realistic numerical models that consider fluid-structural interactions (FSI) have been studied intensively [8]. Although the actuation of cardiomyocytes is dynamic and occurs in a medium, there are few reports that have considered these circumstances when measuring the contractility of cardiomyocytes on the microscale. Moreover, since fluid damping is dominant in the actuation of microdevices because of the scaling-law, its influence on the measurements of the contractile force of cardiomyoyctes need to be taken into account.

Previously, 'hybrid biopolymer microcantilevers' made from flexible polydimethylsiloxane (PDMS), on which living cardiomyocytes had been cultured, were designed and fabricated [7]. The presented device was used to measure the contractile force of the self-organized cardiomyocytes on a specific microsized area in real time. It was found that the variation in stress from the monolayered cardiomyocytes ranged from 2 to 5 nN/μm2, which is similar to that from a single cardiomyocyte [6]. The motions of the microcantilever showed good agreement with finite element modeling (FEM) of a hybrid system. The present study examined the contractile force on hybrid biopolymer microcantilevers by comparing the different analytical solutions, i.e. the FEM and realistic FSI, as well as by validating these analyses through experiments. This study used a commercial software-based dynamic three dimensional FSI model to quantify the contractile force of the cardiomyocytes on microcantilevers. The influence of the dynamic motion of a microcantilever in a medium was considered using harmonic response analysis and was modeled as a second order system using the system identification method in a frequency domain. Based on the modeling results, the influence of the dynamic motion of cardiomyocytes can be evaluated quantitatively. The proposed realistic computational model and experimental validation is expected to improve the understanding of the mechanisms of heart function and promote the further design of optimal microscaled hybrid biopolymer actuators and microdevices in a medium.

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II. MATERIALS AND METHODS

A. Cell culture

A heart was aseptically isolated from a neonatal Sprague-Dawley rat at day 1 and briefly washed with Hank's balanced salt solution (HBSS, Gibco Invitrogen Co., Grand Island, NY, USA). After removing the ventricles, the remaining tissues were minced and incubated in a 0.3 mg/ml collagenase solution containing 0.6 mg/ml pancretin (Sigma Chemical Co., St. Louis, MO, USA). The isolated cardiomyocytes were seeded directly onto the hybrid biopolymer cantilever at a cell density of 5×10^3 cells/mm^2 and cultured in Dulbecco's modified Eagles' medium (DMEM, Gibco Invitrogen) containing 10% fetal bovine serum (Sigma), 50 µg/ml streptomycin and 50 µg/ml penicillin (Gibco Invitrogen) at 37 °C in 5% CO_2 in air. The medium was changed at 48h intervals in order to maintain a continuous beating.

B. Environmental scanning electron microscopy

Environmental scanning electron microscopy (ESEM) was used to examine the cells because there is no requirement of a high vacuum in the sample environment, as in conventional SEM. The cells were fixed in 4% (w/v) paraformaldehyde (Sigma) for 30 min and then examined. Conventional procedures involving dehydration, fixation, critical point, and gold sputtering were not used. High-resolution still images of the microcantilever were recorded using an FEI XL 30 ESEM.

C. Experimental setup for measuring motion of a microcantilever

The motion of the hybrid biopolymer microcantilevers was measured using two microscopes, as shown in Fig. 1.

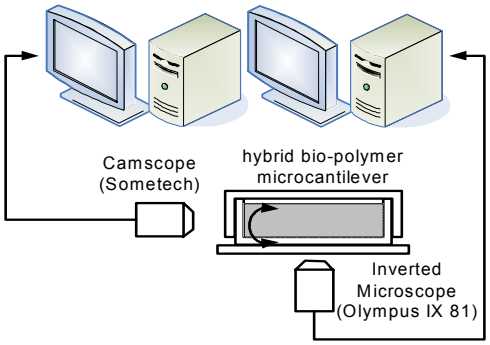


Fig. 1. A schematic diagram for the measurement system used to monitor the motion of hybrid biopolymer microcantilevers.

The microcantilevers, on which cardiomyocytes were cultured, were placed on a microstage. The lateral motion was then monitored using an inverted microscope (Olympus IX 81, Olympus) and the images were captured using a CCD camera connected to a PC at 30 frames/sec with an image pixel size of 1280 × 1024. Vertical motion was observed using a movable camscope (ICS 305B, Sometech) with a maximum magnification of 350× and a long working distance. The images were also captured on a PC with an image pixel size of 640 × 480. The position of the camscope was controlled using the microstage, and was focused on the side of the microcantilever.

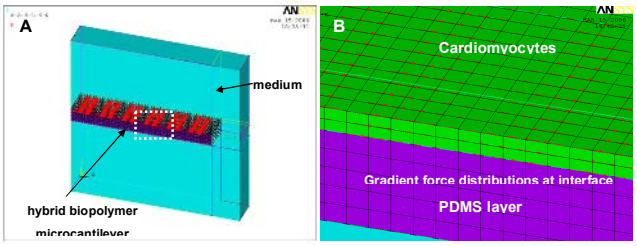
D. Fabrication of hybrid biopolymer microcantilever

The procedures used for fabricating the hybrid biopolymer microcantilevers are described elsewhere. Briefly, a master was made on a silicon wafer using a thick negative photoresist (PR) (KMPR-1050, MicroChemTM). Using two-step photolithography, a three-dimensional master for the microcantilever was produced. A PDMS precursor (Sylgard 184 Silicone Elastomer Kit, Dow Corning, Midland, MI) and a curing agent were mixed in the ratio of 10 to 1. Before the PDMS mixture was poured onto the fabricated master, the master was silanized with (tridecafluoro-1,1,2,2, -tetrahydrooctyl)-1-trichlorosilane (Sigma Chemical Co., St. Louis, MO, USA) to allow the easy removal of the PDMS after curing. The hybrid biopolymer microcantilever was made using a sandwich molding process. The PDMS mixture was poured onto the master and a transparency film was then placed on the PDMS mixture. After an extra wafer had been placed on the top of the transparency film to generate an even PDMS surface, the stack consisting of the fabricated master, PDMS, transparency film with an extra wafer, and a rubber sheet was placed between the two aluminum plates and then clamped. The clamped stack was cured for 2 h at 100°C in an oven. When the curing was complete, a thin PDMS replica was peeled from the master. Since a fresh PDMS surface is hydrophobic, which would prevent the adhesion of proteins and cells, the surface was changed to hydrophilic by exposure it to O_2 plasma in a reactive ion etching (RIE). The plasma-treated surface was then coated with mixture containing 0.001% fibronectin (Sigma Chemical Co., St. Louis, MO, USA) and 0.02% gelatin (Becton Dickinson, MA, USA). The coating solution was spread over the surface, and incubated for a day before seeding the cells. Immediately before plating the cells, the coated-PDMS microcantilever was placed on a fresh PDMS sheet in order to prevent cell adhesion between the microcantilever and the substrate.

E. Finite element model

Computational modeling was carried out using commercial ANSYS multiphysics software (ANSYS, Inc., USA). For the three-dimensional FSI, the solid element (solid45) and three-dimensional fluid element (fluid142) were used to model the hybrid biopolymer microcantilever and the medium, respectively. Fig. 2 shows a finite element model of the hybrid biopolymer microcantilever in the medium to simulate the FSI. The hybrid biopolymer microcantilever was considered a bilayer microcantilever, in which the PDMS substrate is coated with cardiomyocytes. After meshing the microcantilever, the length of the microcantilever was divided into N regions. The gradient force distributions (varying sizes of arrows in Fig. 2B) were applied to each region in the intersectional area of the cardiomyocytes and PDMS, which represents the continuously varied cellular forces in a single cardiomyocyte. The forces on each node

were determined by multiplying each element area by the stress. The maximum force was considered in each region by multiplying the element area by 2–5 nN/μm^2, as previously reported [6]. The gradient of force was assumed to be a linear relation. Young's modulus and Poisson's ratio of the PDMS were assumed to be 750 kPa, and 0.49, respectively. Young's modulus of the cardiomyocytes was assumed to be 188 kPa,



and the Poisson's ratio was assumed to be 0.49.

Fig. 2 Finite element model of the hybrid biopolymer microcantilever in the medium to simulate FSI. Arrows in right figure indicate the forces on nodes at interface between PDMS and cardiomyocytes. Continuously varying sizes of arrows represent gradient force distributions.

In this study, the medium was modeled as an incompressible fluid with a density 1000 kg/m^3 and viscosity 0.004 kgm^{-1}s^{-1}. The flow is governed by the continuity and Navier-Stokes equation. The Arbitrary Lagrangian-Eulerian formulation was used in order to conserve the mesh quality in the fluid, as well as to allow the fluid mesh to follow the FSI boundary. The fluid mesh connected with a solid domain was modeled with same dimensional mesh in a solid field. The sequential coupled technology was used to reflect the interactions between the fluid and the solid domains. The conservative formulation for the load transfer was selected in order to allow the forces to transfer from the fluid to the solid, and the displacements and velocities to transfer from the solid to the fluid across the FSI interface. In FEM, harmonic analysis was used to determine the response of a structure from loads that vary sinusoidally (harmonically) with time. Here, it was assumed that the contractile force of the cardiomyocytes was a sinusoidal input, and full harmonic analysis was performed with a 2 or 5 nN/μm^2 amplitude and a frequency sweep from 0 to 3000Hz.

The general description of the analytical solution, Stoney's equation and static FEM modeling of the contractile force of cardiomyocytes on the microcantilever to compare the FSI results are reported elsewhere [7].

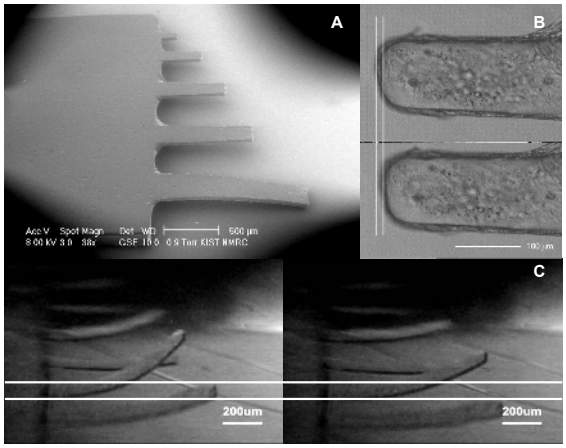
III. Results and Discussions

The hybrid biopolymer microcantilever array consisted of five different sizes of microcantilevers, 50, 100, 150, 200, and 300 μm wide, and lengths five times longer than its width. All the cantilevers had a 20 μm thickness.

Fig.3A shows an ESEM image of the array of fabricated microcantilevers prior to cell seeding. Using this array, the cardiomyocytes were seeded using the procedure described elsewhere. After cell seeding, the contraction of the cells reached the maximum level after 72 to 96 h of culture. The functional aspects of the cardiomyocytes were quantified by measuring the contractile forces from the motion of the microcantilever at 96 hours. Both the lateral and vertical displacements of the microcantilever were measured by obtaining images at the edge of the microcantilever, as shown in Fig.3B and C, respectively. The lateral and vertical displacements of the different microcantilevers were 1.79 ± 1.39, 4.01 ± 2.14 and 4.68 ± 1.93 μm, and 4.16 ± 1.96, 19.77 ± 7.50 and 34.08 ± 13.63 μm in the 50 × 250, 100 × 500, and 150 × 750 μm cantilevers (width × length), respectively. Therefore, both lateral and vertical displacements increased with increasing microcantilever size.

Fig. 3 Image and actuation of hybrid biopolymer microcantilever. (A) ESEM image of the array of fabricated microcantilevers before cell seeding. (B) Still for later motion of hybrid biopolymer microcantilever. (C) Still for vertical motion of hybrid biopolymer microcantilever.

The contractile forces were assessed using the analytical solution based on Stoney's equation and static FEM modeling, which do not consider the fluid dynamics in the



medium. Of course, the results in the previous study showed reasonable values for the contractile force of the cardiomyocytes. However, this study tried to evaluate the influence of the dynamics of cardiomyocytes in the medium quantitatively.

Simulations were performed for the 50 × 250, 100 × 500, and 150 × 750 μm cantilevers, with the frequency ranging from 0 to 3000Hz. For vertical motion, Fig. 4 shows the results from the FSI, static FEM modeling, and experimental results. The experimental data is bracketed by three simulation results for two focal pressure levels, 2 and 5 nN/μm^2. Therefore, we could conclude that the two simulation results represented the contractile force of cardiomyocytes well and they showed the similar values.

The examined their natural frequencies of three types cantilevers were about 1600, 400, and 180 Hz. These frequencies are much higher than the bandwidth of actuation frequency of cardiomyocytes, which is normally 0.1-10 Hz.

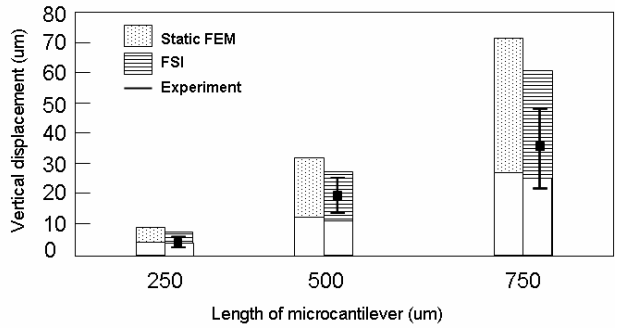


Fig. 4 Comparisons of analytical solution (Stoney's equation), static FEM, FSI, and experimental results. The stresses from the analytical solutions, static FEM, FSI varied from 2 to 5 nN/μm^2. Three simulation results bracketed the experimental data

As shown in slant line area on Fig. 5A, the actuation area is far from the natural frequency. Therefore, the theoretical differences between dynamic and static state in measurement of contractile force of cardiomyocytes are very small. However, as the length of microcantilever becomes longer, the natural frequency gets smaller.

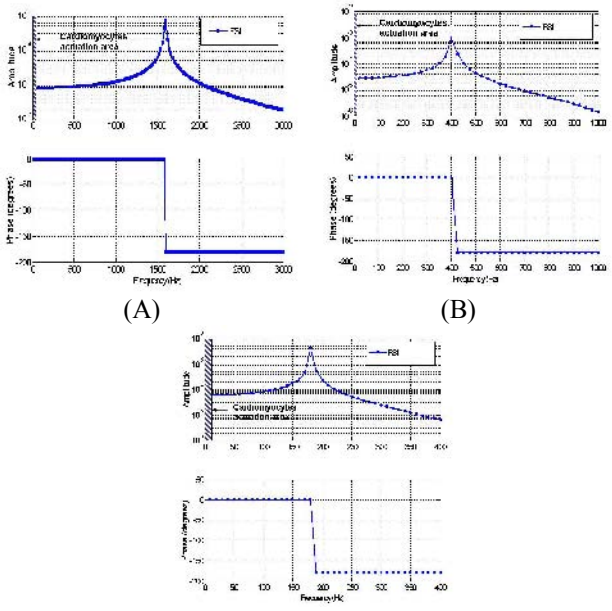


Fig. 5 Bode plots for harmonic response using FSI. (A) 50 × 250 μm, (A) 100 × 500 μm (C) 150 × 750 μm

In Fig. 5B-C, the differences between natural frequency and the actuation area of cardiomyocytes become smaller as the length is longer. Since the natural frequency is $\omega_n = \sqrt{\frac{k}{m}}$ where m is equivalent mass and k is elastic coefficient of the microcantilver, the elastic coefficient has the proportional relation, that is $k \propto l^{-3}$ (l is length of microcantilever) and the natural frequency is proportional to the length with the relation $\omega_n \propto l^{-3/2}$. If the length of microcantilever is over 1.5 mm, then according to the above proportional relation, the natural frequency will be lower than 100Hz roughly. In that case, the influence of fluid dynamics becomes significant, and we can not ignore the effect of it. PDMS has low young's modulus, and by using it we could fabricate very sensitive microcantilever sensors. We can increase the sensitivity by increasing the length of microcantilever. However, we should consider fluid dynamics in the microcantilevers with over 1.5mm length for accuracy of measurement due to this low young's modulus.

IV. Conclusions

This study proposed a realistic computational model that considers the three dimensional fluid-structural interactions (FSI) in evaluating the influence of the fluid dynamics while measuring the contractile force of cardiomyocytes. A comparison of the experimental results with the proposed model, the static FEM without considering the fluid, showed that the new proposed model represented the real system well, and the differences between FSI results and the static FEM were small. A system identification method was introduced to quantify the frequency responses. The natural frequencies of three kinds of microcantilevers (50 × 250, 100 × 500, and 150 × 750 μm) were about 1600, 400, and 180 Hz, respectively. However, if the length of the microcantilever is >1.5 mm, the fluid dynamics will need to be considered when measuring the contractile force of cardiomyocytes due to the low young's modulus of the PDMS. This study first considered the influence of fluid dynamics when measuring the contractile forces. We hope our results could help design optimal microscaled hybrid biopolymer actuators and microdevices in a medium.

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