Direct Measurement of Contraction Force in Human Cardiac Tissue Model Using Piezoelectric Cantilever Sensor Technique

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***Abstract*—A proof-of-concept method for measuring cardiac tissue contraction force using an in-house-developed piezoelectric cantilever sensor system is demonstrated. Contracting forces of 7.2 to 16.6 µN (n=5) were measured from a human cardiac tissue construct. Beating cardiac tissue constructs were monitored in-situ under a microscope during the contraction force measurements. Development of the measurement method allows very low forces such as the ones that appear in biological small scale systems to be determined.**

***Index Terms*—**cardiomyocyte, piezoelectric sensor, cantilever, contraction force

I. Introduction

Cardiotoxicity is one of the leading causes for drug attrition during a drug development process and for post-approval drug withdrawal [1, 2]. Animal experiments are poor predictors of drug-induced toxicity in humans [3]. Human stem cell-based tissue and organ models would be more reliable and ethical than experiments on animals [4]. The development of a functional cardiac tissue model requires advanced and controlled cell culture techniques combined with highly sensitive measuring technology. The generation of the contraction force is a key element in the functioning of the heart, so drugs affecting the contraction function of cardiomyocytes pose a potentially high risk for cardiac safety [5]. Therefore, there is an urgent need for a reliable method to measure the contraction force in cardiomyocytes. At present, there are only a few reports in the literature describing varying approaches to overcome this issue [6-10].

Our highly sensitive force sensors were constructed from a metallic cantilever attached to a piezoelectric sensor, which was connected to in-house-built dedicated hardware and a user interface plate. The measurement system was a further development from a previous study [11].

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In this paper, we demonstrate a proof-of-concept technique for directly measuring the contraction force of a human cardiac tissue model in-situ under microscope. The measurement method is based on the fact that the piezoelectric sensor detects the movement of the cantilever when it is brought into contact with the cardiac tissue construct.

II. Materials and Methods

A. Cardiac tissue construct fabrication

The use of human umbilical vein endothelial cells (HUVECs) from scheduled caesarean sections and human adipose stromal cells (hASCs) obtained from surgical operations was approved by the Ethics Committee of the Pirkanmaa Hospital District (permit numbers R08028 and R03058, respectively).

The human-induced pluripotent stem cells (ATCC-BXS0116 hiPSC) were differentiated into cardiomyocytes using a PSC Cardiomyocyte Differentiation Kit (Gibco Invitrogen, A2921201). The cells were maintained at 37 °C and 5% CO_2 in a humidified incubator, and the medium was replaced every 2-3 days.

The cardiac tissue constructs were fabricated by a technique adapted from previous work [12, 13]. A punched polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, USA) sheet was reversibly bonded to a cell culture dish and the holes (d = 5 mm) were coated with fibrinogen (Sigma Aldrich, F3879). The hASCs and HUVECs were propagated separately and a co-culture was formed in the PDMS holes to produce vascular-like networks as previously described in [14]. Two days later, hiPSC-derived cardiomyocytes were seeded on top of the vascular structures. After cardiomyocyte seeding, a serum-free stimulation medium (SFSM) consisting of DMEM/F12, 2.56 mM l-glutamine, 0.1 nM 3,3′,5-Triiodo-l-thyronine sodium salt, ITS™ Premix: 1.15 µM: 6.65 µg/ml insulin, 6.65 µg/ml Transferrin, 6.65 ng/ml selenious acid, 1% Bovine serum albumin, 2.8 mM Sodium pyruvate, 200 µg/ml Ascorbic acid, 0.5 µg/ml Heparin, 2 µg/ml Hydrocortisone, 10 ng/ml VEGF, and 1 ng/ml FGF-β as described in [15] was replaced by a 1:1 Cardiomyocyte maintenance medium (Gibco Invitrogen, A209208) and SFSM. A few days later,

the cardiomyocytes regained their contracting function and the PDMS sheets were removed to allow the vascularized cardiac tissue constructs to form spontaneously. This process also detached the edges of the tissue constructs which provided attachment points to the force measurement cantilever. The beating cardiac tissue constructs remained functional over 21 days.

B. Piezoelectric cantilever sensors

A piezoelectric cantilever sensor was used to measure the force of the cardiac cell contraction. A cantilever sensor has linear elastic behavior and is characterized by a spring constant. A piezoelectric sensor element in the cantilever then converts the cantilever strain to electric energy. The operation of the piezoelectric sensor element was first simulated with a finite element method and later the simulation results were verified with actual measurements from the cantilever sensor prototype. The frequency response of the cantilever measurement system was obtained via impulse response measurements. These showed a very good fit with the theoretical computations. Labview software was used to control and capture the results of the measurements obtained with the measurement hardware. The measurement hardware itself consisted of an AD8691 (Analog Devices inc, Norwood, USA), operational amplifier circuit as a buffer amplifier with a voltage amplification of 1. This amplified signal was read with an Arduino Due analog-to-digital converter and then sent through a serial communication link to Labview software [11] for further processing and analysis.

C. Finite element simulation of the cantilever

The functionality of the piezoelectric cantilever sensor was simulated with a COMSOL (Comsol AB, Stockholm, Sweden) multiphysics finite element method (FEM) simulator. Both linear elastic and piezoelectric models were used in conjunction with each other in the simulation. In the static simulation a 200 µN force was applied to the tip while the other end was fixed. The total displacement of the tip with this 200 µN load was 192 µm. This represents a cantilever spring constant of 0.96 N/m. In addition, the electric potential field was computed with the simulator on the piezoelectric material. The material parameters were taken from the Lead Zirconate Titanate (PZT) library material. The bottom electrode was fixed to zero electric potential and the electric potential as computed on the top of the piezoelectric element. This potential strength varied between 0.14 and 0.68 V along the piezo material depending on the stress produced when the 200 µN force was applied to the cantilever tip. Furthermore, to obtain the electric potential of the top electrode an average potential over this layer was computed. This average potential was 308 mV and represented the simulated output voltage of the cantilever sensor under a 200 µN load. Thus, the simulated sensitivity was 1.55 kV/N. The deflection of the cantilever beam under the load is illustrated in Fig. 1 a) while the electric field is shown in Fig. 1 b). The simulated and measured sensitivities and spring constants are listed in Table 1.

The piezoelectric cantilever frequency response was also simulated with FEM and later an analytical transfer function model was constructed to model both the mechanical and electrical behaviour of the cantilever measurement system. The modeled and measured frequency response graphs were then compared.

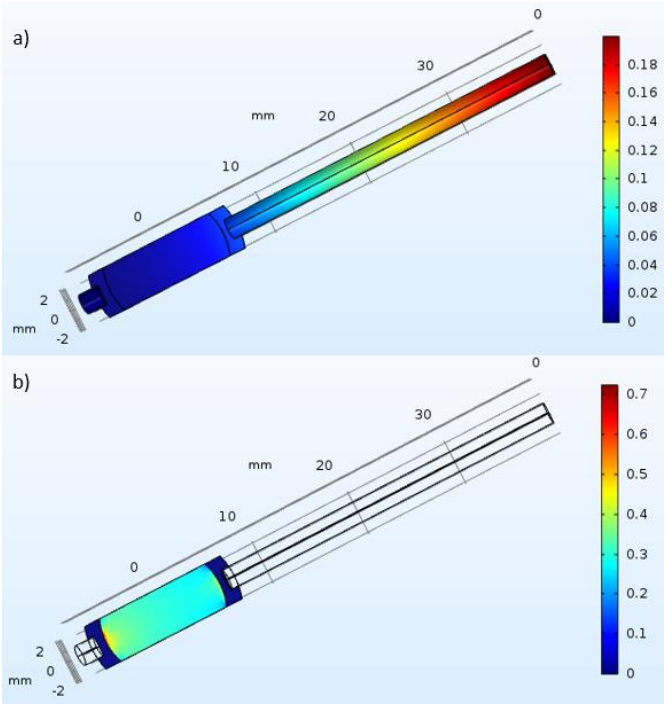

Fig. 1. A FEM computed response with a 200 µN stationary load at the cantilever tip a) total displacement in mm b) electric potential field in Volts.

D. The prototype cantilever sensor

This sensor element was constructed in-house by soldering a 33 mm long test probe pin (P100-D2-1.5mm) to a 15 mm diameter circular piezo disc with brass plate thickness of 70 µm and PZT –layer thickness of 50 µm thus a total thickness was 130 µm (Farnell P/N 2667639). The top electrode was located on top of the PZT layer. The piezo disc was further narrowed with a diamond blade cutter to a 5 mm width perpendicular to the orientation of the probe. The cantilever sensor probe prototype is shown in Fig. 2a. The other side of the narrowed piezo disc (ground electrode) was attached to a prototyping wiring board with a solder joint. This formed a fixed point for the cantilever and was also an attachment point to a 3d-printed fixture arm, which is shown in Fig. 2b. This structure was finally attached to a 3-axis linear micro manipulator (Newport Corporation, Irvine, USA) to enable accurate alignment of the probe tip during the measurements (see Fig 2c, Fig. 3). The probe was set to 30 degree angle to the cultivation dish and the tip was put into the medium as shown in fig 2c.

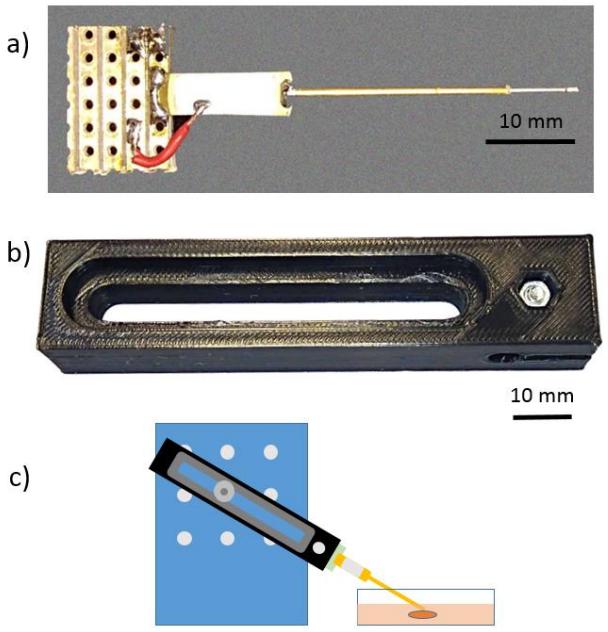

Fig. 2. a) Piezoelectric cantilever sensor probe used in the measurements. b) A 3d- printed mounting fixture for the sensor probe.c) The probe and the arm in the measurement setup.

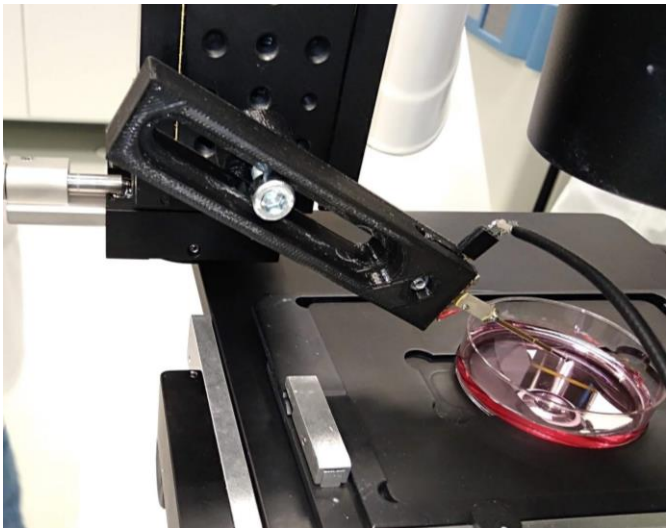

Fig. 3. Cantilever sensor probe attached into the 3-axis micromanipulator to in-situ microscope measurement setup.

E. Cantilever sensor sensitivity measurements

The voltage versus force sensitivities for the prepared cantilever sensors were measured using texture analyser Stable Micro Systems TA.XTPlus (Stable Micro Systems Ltd, Surrey, United Kingdom). In the calibration procedure a 1 Hz sinewave force excitation with 200 µm amplitude was applied to the cantilever tip and the sensor output was measured. This calibration method and data analysis are described in more detail in our previous study [11]. Here, the amplitude of the sensitivity measurement force was 157.5 µN, which yielded a 200 µm displacement and a 329 mV peak voltage reading in the sensor output. Thus, the measured sensitivity of the sensor was 1.97 kV/N and the spring constant of the cantilever 0.78 N/m. The sensor sensitivities from both the simulations and the calibration measurements are summarized in Table 1.

The differences in the simulated and measured sensitivities of the cantilever sensor may be caused by the piezoelectric material's parameters. In the specification sheet for the piezo disc there was no detailed information of the piezoelectric material, so the parameters in our simulation may not be accurate. However this was not a problem since the actual sensor operation was carefully measured.

TABLE I
SIMULATION AND CALIBRATION RESULTS FOR THE CANTILEVER SENSOR SENSITIVITY.

	Simulated	Measured
Sensitivity	1.55 kV/N	1.97 kV/N
Spring constant	0.96 N/m	0.78 N/m

The standard deviation in the force measurements during calibration was 2.11 µN. This was computed from the deviation from the sinusoidal excitation signal used in the calibration procedure. This is illustrated in Fig. 4a, in which the continuous line represents the excitation signal and the stars show the measurement observations. In the Fig 4b a calibration curve of the sensor is shown. Furthermore, the distribution of the measurement error is shown in Fig. 4c. This shows that the average error (AVG) is 0.47 µN and the standard deviation of the error (STD) is 2.11 µN.

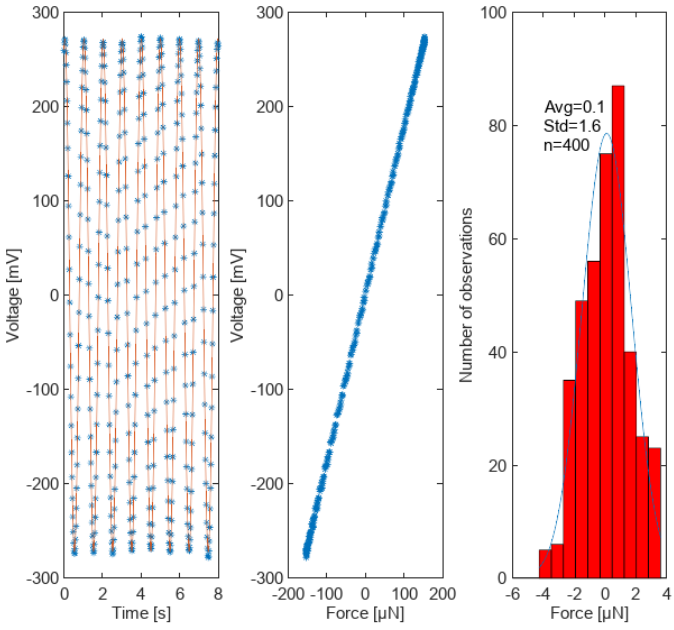

Fig. 4. a) Measurement data fitted to the sinusoidal excitation signal. B) Calibration curve c) Force measurement error distribution.

F. Impulse response measurement of the cantilever sensor

The impulse response measurement of the system was conducted to ensure that the cantilever measurement was operating in the right frequency range and that the sensitivity measurements could be interlinked in the frequency domain. The impulse response was measured by knocking the sensor element and measuring the time-domain response. This time-domain impulse response is shown in Fig. 5a. The frequency response of the cantilever sensor was then computed by performing a fourier transform on the impulse response data. The comparison of the modeled and measured frequency responses of the measurement system is shown in Fig. 5b. There, the measured and computed responses show that the usable frequency band is ~0.5 Hz to 10 Hz with a resonance peak at ~15 Hz.

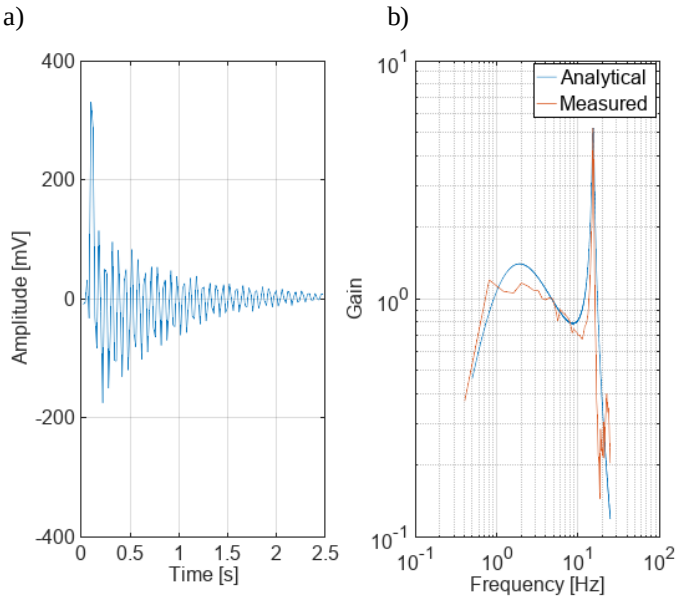

Fig. 5. a) Time domain impulse response of the cantilever measurement system. b) Computed and measured frequency response of the cantilever sensor system in frequency domain.

G. In-situ contraction force measurement setup

An optical microscope (Zeiss Primovert, Carl Zeiss AG, Oberkochen, Germany) was used for in-situ characterization of the cardiac tissue constructs during the force measurements. The use of in-situ microscopy was crucial, both for maneuvering the cantilever into its desired position in the selected cardiac tissue constructs, and also for quantifying the synchronised movement of the cantilever and the cardiac tissue construct. The in-situ microscope setup with the cantilever sensor and cell culture dish are shown in Fig. 6.

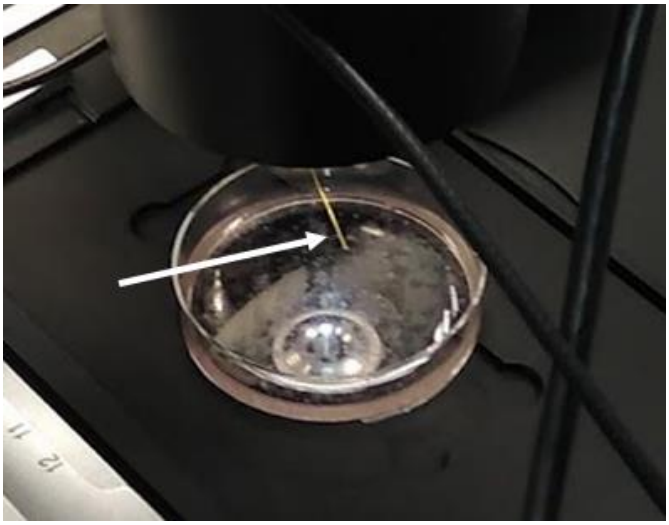

Fig. 6. In-situ measurement setup with a cell culture dish containing the cardiac tissue construct. The measurement cantilever is visible above the petri dish and marked with the white arrow.

The contraction force of the cardiac tissue constructs was measured by placing the cell culture dish under the microscope and preparing the sensor probe tip with fibrin coating to enhance its adhesion to the cardiac tissue construct. The coating procedure was to dip the tip into a 5.5 mg/ml fibrinogen solution 5 times and then let it dry for 60 seconds. Non- coated and PDMS-coated probe tips were also tried, but they did not give reliable measurement results. Altogether, 5 measurements were conducted using this probe measurement system on the cardiac tissue constructs.

III. Results and Discussion

A. Microscopic analysis of cardiac tissue constructs

Fig. 7 shows an example of a cardiac tissue construct on a cell culture dish 2 weeks after cardiomyocyte seeding. The cardiac tissue construct has not become partially detached from the cell culture dish like the cardiac tissue construct that was used in the measurements. The diameter of the beating cardiac construct was approximately 5 mm and had a circular shape and the contact area of the tip is marked with amber circle.

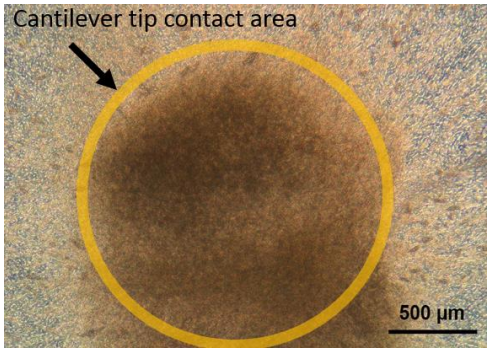

Fig. 7. An example of a cardiac tissue construct 2 weeks after cardiomyocyte seeding with cantilever tip contact area marked with amber circle.

B. In-situ imaging during force measurement

Fig. 8 shows an example of the images captured from one of the videos (in Supplementary material) filmed during the contraction force measurements. There, the cantilever tip and part of a cardiac tissue construct is shown. The contact area of the cantilever tip and the cell construct is approximately 1 mm^2. Furthermore, the supplementary video material shows the synchronous movement of the probe tip and the cell contraction.

The beating frequency computed from the measured data varied between 43 and 49 beats per minute. This corresponds to beating frequencies of between 0.71 and 0.81 Hz, and thus the measurements fall into the usable range of the cantilever frequency band.

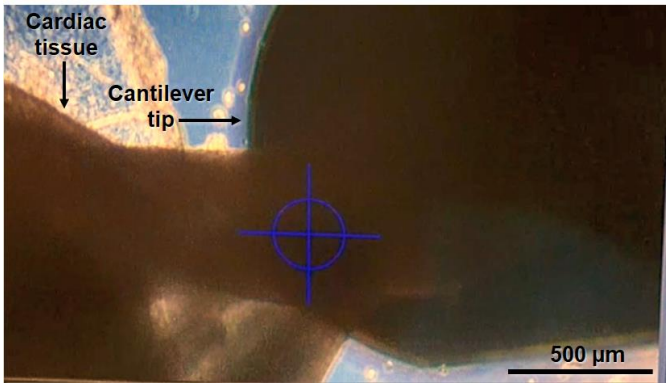

Fig. 8. Microscope image of the cardiac tissue construct and the cantilever during the contraction force measurements. This image is a still from the in-situ video, which is available in the supplementary material.

C. Contraction force measurements

Fig. 9 shows all the 5 force measurements in a time domain with a 5 second period for the measurements. There are systematic beating patterns for the cardiac tissue constructs. Fig. 10 shows individual plots of the measurements where the contracting and relaxing phases of the cardiac activity can be seen. These stable periodical signals suggest that the fibrin coating enables a good attachment between the tip of the measurement probe and the cardiac tissue construct so that the contracting force is transferred to the probe tip. The beating force from peak to peak of the cardiac tissue construct varied between 7.2 µN and 16.6 µN over the 5 measurements. These values are listed in Table 2 according to the order in which the measurements were taken. As the force measurement results can be considered to be reliable by themselves it should be noted that a biological organism itself may induce variation to the force measurement results. For example the cantilever tip location in the cell construct may be critical in the actual force measurement.

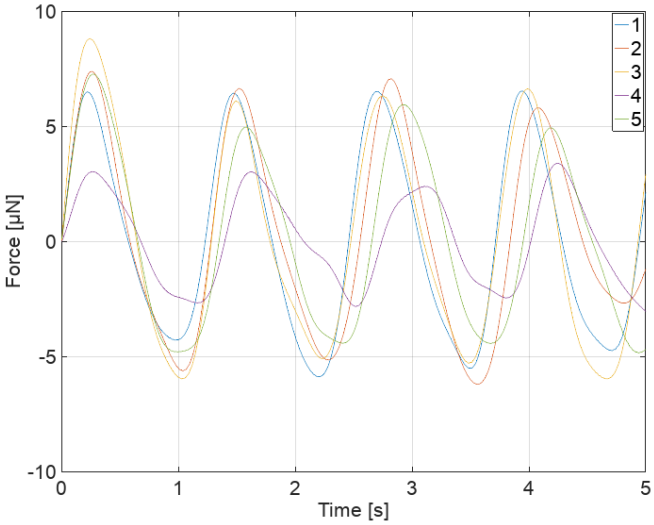

Fig. 9. Contraction force measurement in time domain (all measurements).

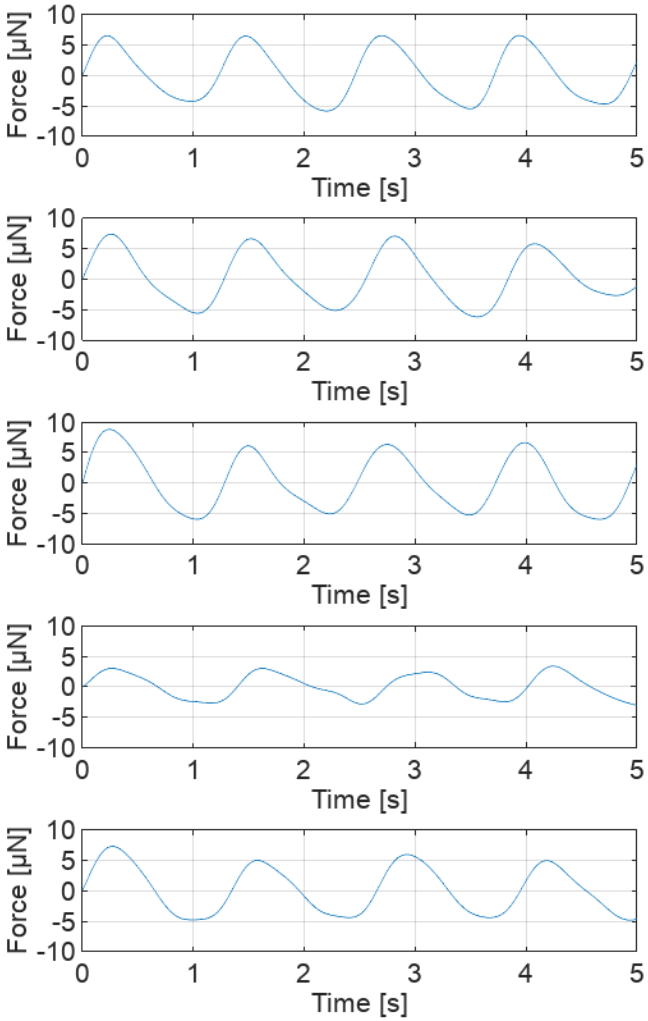

Fig. 10. Individual beating waveforms where the contracting and relaxing phases can be seen.

TABLE II
PEAK TO PEAK CONTRACTION FORCE AND RECORDED BEATS PER MINUTE FOR THE 5 MEASUREMENTS. THE STANDARD DEVIATION OF THE MEASUREMENT ERROR IS 2.11 μN.

Measurement	Peak to peak contraction force [μN]	Beats per minute	Beating frequency [Hz]
1	13.9	48.9	0.81
2	15.2	47.0	0.78
3	16.6	46.9	0.78
4	7.24	43.0	0.71
5	13.6	44.5	0.74

The obtained contraction force measurement results are in line with the previously reported data. Although much higher reported contraction forces have been reported, such as over 1 mN by Sasaki et al., it should be noted that with engineered tissue the total contraction force is a function of the size of the cell population [10]; the larger the cell population, the larger the forces. Accordingly, much lower contraction forces have been recorded right down to the single-cell scale. Such small-scale measurements, however, may require cell cultivation directly onto an Atomic Force Microscope (AFM) tip, for example, or onto a dedicated mechanical structure, as described by Linder et al. and Kim et al. [6, 9].

In this study, one consideration for the development of this approach is the very concept of force measurement itself. As the elastic force measurement principle combines a force and a displacement measurement, it is important to account for how much the displacement reflects the force measurement readings. This issue is common to any application of this measurement principle, regardless of the scale of the measurement. For example, if the cantilever is very elastic the system may not show the maximum force production, if that is point of interest. Instead, it may more or less measure only the displacement or the movement of the tissue structure. On the other hand, if the probe arrangement is very stiff it may not give any force readings at all as the tissue is too weak to cause any measurable displacement to the tip. Therefore, the role of the spring constant, or the elasticity of the sensor element, become critical. In theory, they need to be tuned to the right range or value to obtain the desired force measurement results. One possibility is to construct a variable load mechanism on the sensor element. With piezoelectric devices, it should be possible to combine a piezoelectric actuator and sensor to provide such a variable load. On the other hand the force measurement probe could be used along with existing force measurement concepts. For example the linear elastic wire video monitoring based measurement (Sidorov et al) could be replaced with the probe. Potentially simplifying the measurement arrangement. for Nevertheless this is an interesting prospect for the future development of piezoelectric cantilever biomeasurement systems.

The practical arrangements for the force measurement system also require attention. For example, how can the measurements' repeatability be ensured. There are a number of factors that can raise significant challenges for this, not just the sensor itself, but also the mechanism used for attachment to the cells and the properties of the engineered tissue. Repeatability requires standardisation of the cardiac tissue construct itself and its cultivation. Although these challenges have not been much addressed here, it is willingly acknowledged that these are all important considerations if the system is to be applied in, for example, toxicity studies or personalised medicinal applications.

IV. CONCLUSIONS

In this study we have shown that it is possible to measure cardiac tissue construct contraction forces with a direct force measurement apparatus. The measurements were done with relatively simple devices in a straightforward measurement setup. As this aim of this work was just to prove the concept, there are still many issues to be resolved before this technology reaches its final form. For example, at this stage the method has proved to be suitable only for a multiple cell construct, and is not yet sensitive enough to measure single-cell contraction forces.

However, in this proof-of-concept paper we have demonstrated that a fibrin-coated cantilever connected to a piezoelectric plate sensor can be utilised for measuring cardiac tissue contraction force. The fibrin coating proved itself able to produce reliable force readings, and the measurements of the cells' beating frequency were consistent with the beating frequency obtained from the video (see Supplementary material). The measured cardiac tissue contraction forces from peak to peak ranged from 7.2 to 16.6 μN in amplitude.

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