Published in final edited form as:
Lab Chip. 2011 May 21; 11(10): 1801–1807. doi:10.1039/c1lc20098d.

A cell-based biosensor for real-time detection of cardiotoxicity using lensfree imaging

Sang Bok Kim[a,b,‡], **Hojae Bae**[a,b,‡], **Jae Min Cha**[a,b], **Sang Jun Moon**[a,b], **Mehmet R. Dokmeci**[c], **Donald M. Cropek**[d], and **Ali Khademhosseini**[a,b,e]

Ali Khademhosseini: alik@rics.bwh.harvard.edu

[a]Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

[b]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[c]Electrical and Computer Engineering Department, Center for High Rate Nanomanufacturing, Northeastern University, Boston, MA 02115, USA

[d]U.S. Army Corps of Engineers, Construction Engineering Research Laboratory, Champaign, IL 61822, USA

[e]Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA 02115 USA

Abstract

A portable and cost-effective real-time cardiotoxicity biosensor was developed using a CMOS imaging module extracted from a commercially available webcam. The detection system consists of a CMOS imaging module, a white LED and a pinhole. Real-time image processing was conducted by comparing reference and live frame images. To evaluate the engineered system, the effects of two different drugs, isoprenaline and doxorubicin, on the beating rate and beat-to-beat variations of ESC-derived cardiomyocytes were measured. The detection system was used to conclude that the beat-to-beat variability increased under treatment with both isoprenaline and doxorubicin. However, the beating rates increased upon the addition of isoprenaline but decreased for cultures supplemented with doxorubicin. Moreover, the response time for both the beating rates and the beat-to-beat variability of ESC-derived cardiomyocytes under treatment of isoprenaline was shorter than doxorubicin, although the amount of isoprenaline used in the measurement was three orders of magnitude lower than that of doxorubicin. Given its ability to perform real-time cell monitoring in a simple and inexpensive manner, the proposed system may be useful for a range of cell-based biosensing applications.

Correspondence to: Ali Khademhosseini, alik@rics.bwh.harvard.edu.

‡Both authors contributed equally to this paper.

†Electronic Supplementary Information (ESI) available: Supplementary movies for device operation and beating of ESC-derived cardiomyocytes under treatment of isoprenaline and doxorubicin. See DOI: 10.1039/b000000x/

Author contributions

S.B.K., H.B, J.M.C and A.K. generated idea and designed the experiment, S.B.K., H.B., and J.M.C cultured cells, performed experiments and analyzed data, S.B.K, and S.J.M. fabricated device, S.B.K., H.B., J.M.C., S.J.M., M.R.D., D.M.C., and A.K. wrote the manuscript. All authors agreed on the contents

Supplement information

1. Movie shows demonstration of a working device (S1).
2. Movie displays effects of isoprenaline on cardiomyocytes (S2 and S3).
3. Movie displays effects of doxorubicin on cardiomyocytes (S4 and S5).

Introduction

Among the causes of cardiotoxicity, drug-induced cardiotoxicity has contributed substantially to the rapidly increasing drug attrition in the drug development process.[1–6] For example, more than 30% of total withdrawn drugs during the period between 1990 to 2006 from various major markets were related to cardiac dysfunction.[7] Thus, the potential of new molecular substances to induce heart failure has become a central task in safety assessment throughout drug discovery and development as well as the usage of existing drugs. Therefore, early detection of cardiac responses against drugs is crucial for reducing the costs and time in drug development process. Furthermore, portable and real-time methods of cardiotoxicity detection are beneficial for environmental monitoring which require rapid identification of toxic materials.

Cell-based biosensors utilize live cells as sensing elements to examine biologically active reagents and have been widely applied to pharmacological screening and environmental monitoring.[8–10] Several different types of cell-based biosensors have been developed for cardiotoxicity testing such as by using digital movie analysis,[11, 12] microelectrode arrays (MEA),[13, 14] and light-addressable potentiometric sensors (LAPS).[15] The movie analysis method was adapted to analyze the changes in the beating rates of heart cells through image processing. However, a large storage capacity is required to record the variances of beating rates especially when the heart cells slowly respond to the drugs. Furthermore, by using the movie analysis method, varying heart beating rates cannot be analyzed in real-time. The MEA method measures electrical potential on the extracellular field in the multiple channels. Although MEA can detect chronotropic effects (i.e., the abnormal changes in heartbeat rate) of the drug treatment in real-time, it is limited by its high cost as well as its small measurement area which is defined by tiny electrodes. Similar to the MEA method, the LAPS system is also capable of monitoring chronotropic effects in real-time. However, it requires complicated fabrication procedures for the integrated semiconductor detector system. Moreover, the difficulty still exists in adopting the specific light source that needs to match with the energy band transition of the semiconductor detector. Like the MEA method, the active measurement area is also limited, in this case, by the positioning of the light source.

Owing to the development of various digital technologies, the compact and cheap imaging sensor modules, such as charge coupled device (CCD) and complementary field oxide semiconductor (CMOS) imaging sensors, are widely used in common electronics, such as webcams, cell phones, digital cameras, and other digital imaging devices. Lensfree imaging technique is emerging as a viable detection method due to the progress in imaging sensors. The compactness and cost-efficiency of the imaging products have been tremendously improved and have been applied to a variety of fields over the last decade such as in sub-pixel resolution microscopy,[16–18] high-throughput wide-field view detectors,[19] holographic microscopes[20, 21] and fluorescent imaging.[22]

In this study, we present a cell-based biosensor using the lensfree imaging technique with a CMOS imaging module extracted from a conventional webcam. An image processing algorithm was developed to detect the squirming objects like beating cardiomyocytes in real-time. To test cardiotoxiciy, cardiomyocytes were derived from mouse embryonic stem cells (ESCs), and cultured in a commercially available chamber slide. The variances of beating cardiomyocytes were measured under the treatments of isoprenaline and doxorubicin. To examine chronotropic effects of drugs, the change in the beating rate and beat-to-beat variations, which are well-known indicators of cardiac dysfunction,[23] were measured in real-time. With the use of microscale cell culture methods,[12, 24–32] our device may be extended to high-throughput drug screening systems.

Materials and Methods

All cell culture materials were obtained from Invitrogen (Paisley, UK), unless otherwise specified. Cell cultures and experiments were performed in a conventional incubator maintained at 37 °C and 5 % CO_2.

ESC culture and differentiation into cardiomyocytes

ESC culture—Mouse ESCs (R1 line) were cultured using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% of ESC qualified fetal calf serum, 100 units/mL of penicillin/streptomycin, 2 mM of L-glutamine and 0.1 mM β-mercaptoethanol. The medium was replaced on a daily basis. Prior to medium change 100 units/mL leukemia inhibitory factor (LIF, Chemicon, Chandlers Ford, UK) was added to maintain the ESCs undifferentiated. ESCs were passaged before reaching 70% confluence.

Embryoid bodies (EBs) and ESC-derived cardiomyocytes—ESCs were trypsinized and seeded on a non-adherent petri dish to obtain EBs using the suspension culture method. Alpha-Minimum Essential Medium (α-MEM) supplemented with 15% of heat inactivated fetal calf serum and 100 units/mL of penicillin/streptomycin was used to culture EBs. The culture medium was changed every day. After culturing for 5 days, the EBs were plated on a chamber slide (Nalge Nunc International Corp., Naperville, IL) for the differentiation into cardiomyocytes.

Immunocytochemistry—Mouse ESC derived cardiomyocytes were visualized by immunocytochemical staining. After observation of beating, cells were fixed with 4% paraformaldehyde for 20 min, washed three times with 1× PBS, followed by permeabilization with 0.1% Triton X-100, and blocked of nonspecific binding by incubation with 5% (w/v) normal horse serum in PBS. The primary antibodies, anti-sarcomeric α-actinin (Abcam, Cambridge, MA) and anti-heavy chain cardiac myosin (Abcam, Cambridge, MA) were diluted at 1:100 with 5% normal horse serum solution, and incubated overnight at 4 °C. The secondary Alexa Fluor 488 and 594 conjugated antibody was incubated for 1 hr at room temperature.

Real-time beating rate measurement of cardiomyocytes

CMOS module—A CMOS imaging module was extracted from a webcam (C160, Logitech) and was used to detect the variances of beating cardiomyocytes. The resolution of the CMOS imaging module was 640 × 480 pixels. The light source consisted of a white light emitting diode (LED) and a pinhole (300 μm in diameter). The pinhole was placed underneath the white LED to realize a point light source as seen in Fig. 1. The chamber slide containing cardiomyocytes was placed onto the CMOS imaging module. During measurements of the beating rates, the device was placed inside an incubator. To operate the device remotely, the CMOS imaging module was connected to a laptop computer.

Real-time image processing program—To operate the CMOS imaging module and measure the variances of beating cardiomyocytes, an image processing program was developed using MATLAB (MathWorks Inc., Natick, MA). In the first part of the program, the operation region of the CMOS imaging module was restricted to the beating site of cardiomyocytes which enabled us to increase the image acquisition rate. In the second part of the program, an image which was taken from the first frame (reference image) was captured and compared with the images acquired from each consecutive frame (live frame images). Before comparing the images, the reference and live frame images were transformed to binary images and stored as two different matrices. After transformation, an image difference matrix was obtained by subtracting the live frame image matrix from the

reference image matrix. In the last part of the program, the absolute value of each element in the image difference matrix were summed together, which we refer to as the image difference value. The larger the image difference value the greater is the variation between the reference and the live frame images.

Cardiotoxicity testing—Two drugs, isoprenaline and doxorubicin (Sigma-Aldrich Inc., St. Louis, MO), were used to test the device developed in the present study. Each drug was dissolved in culture medium and added into the chamber slide containing ESC-derived cardiomyocytes, and the changes in beating rates and beat-to-beat variations were measured. Before the addition of the drug, control experiments were performed using cardiomyocytes for 30 min (with no drugs) where we obtained reference values for the beating rates and beat-to-beat variations. Afterwards, we introduced drugs and measured the beating rates and the beat-to-beat variations of cardiomyocytes. Finally, we normalized the parameters obtained from the samples treated with the drugs to the values obtained from the reference group. Three independent experiments were performed for each drug to obtain statistically reliable data.

Results and discussion

ESC-derived cardiomyocytes

In our experiments cardiomyocytes were derived from mouse ESCs. Mouse ESCs were seeded and cultured in a non-adherent cell culture petri dish to obtain EBs. After 5 days, the EBs were plated on the chamber slide.

The EBs adhered to the bottom surface of the chamber slide and differentiated into cardiomyocytes. Approximately 10 days after plating, the beating colonies were observed. Fig. 2(A) shows mouse ESCs after 3 days of culture and Fig. 2(B) shows EBs after 5 days of culture in a non-adherent petri dish. The cardiomyogenic differentiation was confirmed by immunocytochemical staining of beating colonies against sarcomeric α-actinin[33] and cardiac myosin heavy chain as shown in Fig. 2(C) – (F).

Real-time image processing

To measure the variances of beating cardiomyocytes, a real-time image processing program was developed as described in the previous section. Figure 3 shows real-time image processing procedure. We first selected a portion (region of interest, ROI) manually from the full-size image and restricted the operation region of the CMOS imaging module to this ROI and then captured the reference image. The image difference between the reference and live frame images were calculated and displayed in real-time as can be seen in a sample plot is shown at the bottom of Fig. 3 (see also Supplementary Information, Movie S1). The graph of the image difference values showed a pulse pattern like electrocardiogram (ECG) signal. By calculating the average value and standard deviation of peak-to-peak intervals in image difference values, we obtained the changes in beating rates and beat-to-beat variations of cardiomyocytes. Next we validated our measurement by calculating the frame rates which was estimated by dividing the number of processed frames by the running time of the program. The estimated image processing rate was 29 frames per second. This image processing rate was large enough to detect the beating rates of ESC-derived cardiomyocytes which have approximately 1~2 beats per second.[11, 34] Although the resolution was not high to distinguish details of cells but was enough to detect cardiomyocytes beating.

Cardiotoxicity testing

To test the developed CMOS imaging module based cardiotoxicity biosensor, two different drugs (isoprenaline and doxorubicin) were used.

Device testing—To test the device, we first measured the beating rates of the ESC-derived cardiomyocytes in the normal culture media for 150 min. The beating rates were calculated by averaging a 30 s of consecutive measurement with a period of 5 min. Fig. 4 shows measurements of the beating rates and image difference value obtained from cardiomyocytes. The average beating rate was found to be approximately 100 beats per minute which is consistent with other results from literature.[11, 34] The variations in the beating rates in this control experiment were less than 3% which indicated that the device was robust and did not display any signs of cardiotoxicity.

Chronotropic effects of isoprenaline—Isoprenaline is a sympathomimetic β-adrenergic agonist drug. After its discovery, isoprenaline had been used for asthma, but, studies that followed have revealed that isoprenaline can produce tachycardia (i.e., abnormally increasing heart rate) and can potentially lead patients to cardiac dysrithmia.[35]

To evaluate chronotropic effects of the isoprenaline, different concentrations of isoprenaline (1, 5, 10 and 100 nM) were dissolved in the culture medium and injected into the chamber slide. During measurements, the device captured and processed live frame images consecutively for 20 s with a period of 1 min. Fig. 5(A) shows the change in beating rates as a function of time for various concentrations of isoprenaline. The beating rates increased immediately after injection of isoprenaline and saturated within 10 min for all concentrations of isoprenaline. As shown in Fig. 5(A), increasing the concentration of isoprenaline corresponded to an increase in beating rates. However, for 10 and 100 nM of isoprenaline, the change in beating rates was almost the same. The rise time, which is time taken to reach half maximum variation of beating rates, for 1 and 5 nM of isoprenaline were 3 and 4 min, respectively, and 5 min for 10 and 100 nM of isoprenaline. Fig. 5(B) shows the pulse patterns where the values are obtained from the image difference matrix. After injecting isoprenaline, the time intervals between spikes became shorter indicating that the beating rates increased.

Fig. 5(C) shows the increase in the beating rates as a function of concentration of isoprenaline after 12 min. For 1 and 5 nM of isoprenaline, the beating rates increased by 24 and 44%, respectively, and for 10 and 100 nM of isoprenaline, the increase in the beating rates was 70% and showed no statistical difference. These observations are in good agreement with previous studies.[11, 36]

In addition to the change in beating rates, beat-to-beat variations, which could be used as an indicator to cardiac dysrhythmia, were also measured. Fig. 5(D) shows beat-to-beat variations as a function of time. As we increased the isoprenaline concentration, we noticed that the beat-to-beat variations increased. The beat-to-beat variations against isoprenaline, similar to the beating rates, had a fast response.

Chronotropic effects of doxorubicin—Doxorubicin is an anti-cancer drug and it is commonly used against wide range of cancers, such as breast, stomach cancers and soft tissue sarcoma and others.[4] However, doxorubicin has serious adverse effects on the heart such as bradycardia (i.e., abnormally decreasing heat rate).[4]

To evaluate chronotropic effects of the doxorubicin, different concentrations of doxorubicin (10, 100, 100 and 300 μM) were dissolved in culture medium and injected into chamber slide using a pipette. During the measurement, the device captured and processed live frame images for 30 s consecutively with a period of 5 min. Fig. 6(A) shows the change in beating rates as a function of time for various concentrations of doxorubicin. Contrary to isoprenaline, the beating rates decreased gradually and the response to high concentrations of doxorubicin was immediate but slow for low concentration of doxorubicin. For 10 μM of

doxorubicin, the beating rates did not change during 100 minutes of measurement. However, concentrations higher than 100 μM of doxorubicin showed a decrease in the beating rates. For 100 μM of doxorubicin, the beating rates started to decrease 60 minutes after injection, but the beating rates decreased immediately for 200 and 300 μM of doxorubicin. For 300 μM of doxorubicin, the beating stopped 20 min after injection. As shown in Fig. 6(B), time interval between spikes became longer, that is, beating rates decreased. In addition, the amplitude of the pulse pattern was reduced compared to the control. Although the image processing program developed in the present study did not contain a beating amplitude detection algorithm, the image difference could reflect the variations of the beating amplitude qualitatively since the weak beating could results in a small difference between the reference and live frame images.

Fig. 6(C) shows the beating rates as a function of concentration of doxorubicin for 100 min after doxorubicin injection. The beating rates decreased by 26 and 54% for 100 and 200 μM of doxorubicin, respectively. The ESC-derived cardiomyocytes detached from the bottom surface of the chamber slide immediately after the injection of 350 μM of doxorubicin.

Fig. 6(D) shows beat-to-beat variations as a function of time. The response time of the beat-to-beat variations was slower than that of beating rates. These observations agree well with a previous study which used electrical measurements.[14] Based on this observation of beat-to-beat variations, the beating of the ESC-derived cardiomyocytes is more irregular after injection of doxorubicin than after injection of isoprenaline.

Conclusions

In this study, we present a portable, cost-effective cell-based biosensor system using a commercially available CMOS imaging module. This system was applied to monitor the chronotropic effects of ESC-derived cardiomyocytes under the treatment of isoprenaline and doxorubicin, and tachycardia and bradycardia were detected in real-time respectively. The detector system used in this study can be easily replaced by a different CMOS imaging module that holds the advanced image processing schemes with high frame rates, so that the area and amplitude of cellular beating can be measured. Basically, our system was capable of acquiring as much data as conventional stereo microscope with digital video capture system. Furthermore, our biosensor system can also be integrated into a high throughput screening system by the application of microscale cell culture techniques and may also be applied to *in vivo* study by combining with endoscope. Finally, induced pluripotent stem (iPS) cells isolated from a patient can be readily introduced into our system instead of ESCs, so that the cardiotoxicity testing of a drug treatment can be personalized for each patient.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This paper was supported by the National Institutes of Health (EB009196; DE019024; EB007249; HL092836), the National Science Foundation CAREER award (DMR0847287) and the Office of Naval Research Young Investigator award. S. B. Kim was partially supported by the National Research Foundation of Korea Grant funded by Korean Government [NRF-2009-352-D00032].

References

1. An J, Li P, Li J, Dietz R, Donath S. J Mol Med. 2009; 87:401–410. [PubMed: 19139834]
2. Force T, Kerkela R. Drug Discov Today. 2008; 13:778–784. [PubMed: 18617014]

3. Kerkelä R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, Walters B, Shevtsov S, Pesant S, Clubb FJ, Rosenzweig A, Salomon RN, Van Etten RA, Alroy J, Durand JB, Force T. Nat Med. 2006; 12:908–916. [PubMed: 16862153]
4. Menna P, Salvatorelli E, Minotti G. Chem Res Toxicol. 2008; 21:978–989. [PubMed: 18376852]
5. Schiller LR, Johnson DA. Am J Gastroenterol. 2008; 103:815–819. [PubMed: 18397418]
6. Sugimoto K, Okamura K, Tanaka H, Takashima S, Ochi H, Yamamoto T, Matoba R. Biochem Biophys Res Commun. 2009; 390:1214–1220. [PubMed: 19878660]
7. Shah RR. Pharmacogenomics. 2006; 7:889–908. [PubMed: 16981848]
8. Banerjee P, Bhunia AK. Trends Biotechnol. 2009; 27:179–188. [PubMed: 19187988]
9. Kang L, Chung BG, Langer R, Khademhosseini A. Drug Discov Today. 2008; 13:1–13. [PubMed: 18190858]
10. Misawa N, Mitsuno H, Kanzaki R, Takeuchi S. Proc Natl Acad Sci U S A. 2010; 107:15340–15344. [PubMed: 20798064]
11. Stummann TC, Wronski M, Sobanski T, Kumpfmueller B, Hareng L, Bremer S, Whelan MP. Assay Drug Dev Technol. 2008; 6:375–385. [PubMed: 18532900]
12. Khademhosseini A, Eng G, Yeh J, Kucharczyk PA, Langer R, Vunjak-Novakovic G, Radisic M. Biomed Microdevices. 2007; 9:149–157. [PubMed: 17146728]
13. Connolly P, Clark P, Curtis AS, Dow JA, Wilkinson CD. Biosens Bioelectron. 1990; 5:223–234. [PubMed: 2206490]
14. Xiao L, Hu Z, Zhang W, Wu C, Yu H, Wang P. Biosens Bioelectron. 2010; 26:1493–1499. [PubMed: 20732805]
15. Liu Q, Cai H, Xu Y, Xiao L, Yang M, Wang P. Biosens Bioelectron. 2007; 22:3224–3229. [PubMed: 17416514]
16. Cui X, Lee LM, Heng X, Zhong W, Sternberg PW, Psaltis D, Yang C. Proc Natl Acad Sci U S A. 2008; 105:10670–10675. [PubMed: 18663227]
17. Khademhosseinieh B, Biener G, Sencan I, Su TW, Coskun AF, Ozcan A. Appl Phys Lett. 2010; 97:221107. [PubMed: 21203381]
18. Zheng G, Lee SA, Yang S, Yang C. Lab Chip. 2010; 10:3125–3129. [PubMed: 20877904]
19. Su TW, Seo S, Erlinger A, Ozcan A. Biotechnol Bioeng. 2009; 102:856–868. [PubMed: 18853435]
20. Repetto L, Piano E, Pontiggia C. Opt Lett. 2004; 29:1132–1134. [PubMed: 15182009]
21. Seo S, Su TW, Tseng DK, Erlinger A, Ozcan A. Lab Chip. 2009; 9:777–787. [PubMed: 19255659]
22. Coskun AF, Su TW, Ozcan A. Lab Chip. 2010; 10:824–827. [PubMed: 20379564]
23. Kaushik V, Leon AR, Forrester JS Jr, Trohman RG. Crit Care Med. 2000; 28:N121–128. [PubMed: 11055680]
24. Collard D, Takeuchi S, Fujita H. Drug Discov Today. 2008; 13:989–996. [PubMed: 18835363]
25. Juncker D, Schmid H, Delamarche E. Nat Mater. 2005; 4:622–628. [PubMed: 16041377]
26. Khademhosseini A, Ferreira L, Blumling J 3rd, Yeh J, Karp JM, Fukuda J, Langer R. Biomaterials. 2006; 27:5968–5977. [PubMed: 16901537]
27. Khademhosseini A, Langer R, Borenstein J, Vacanti JP. Proc Natl Acad Sci U S A. 2006; 103:2480–2487. [PubMed: 16477028]
28. Moeller HC, Mian MK, Shrivastava S, Chung BG, Khademhosseini A. Biomaterials. 2008; 29:752–763. [PubMed: 18001830]
29. Murthy SK, Sethu P, Vunjak-Novakovic G, Toner M, Radisic M. Biomed Microdevices. 2006; 8:231–237. [PubMed: 16732418]
30. Murtuza B, Nichol JW, Khademhosseini A. Tissue Eng Part B Rev. 2009; 15:443–454. [PubMed: 19552604]
31. Nguyen MD, Giridharan G, Prabhu SD, Sethu P. Conf Proc IEEE Eng Med Biol Soc. 2009; 2009:1060–1063. [PubMed: 19965137]
32. Renault JP, Bernard A, Juncker D, Michel B, Bosshard HR, Delamarche E. Angew Chem Int Ed Engl. 2002; 41:2320–2323. [PubMed: 12203579]

33. Hwang YS, Chung BG, Ortmann D, Hattori N, Moeller HC, Khademhosseini A. Proc Natl Acad Sci U S A. 2009; 106:16978–16983. [PubMed: 19805103]

34. Boheler KR, Czyz J, Tweedie D, Yang HT, Anisimov SV, Wobus AM. Circ Res. 2002; 91:189–201. [PubMed: 12169644]

35. Waldeck B. Eur J Pharmacol. 2002; 445:1–12. [PubMed: 12065188]

36. Vandecasteele G, Eschenhagen T, Scholz H, Stein B, Verde I, Fischmeister R. Nat Med. 1999; 5:331–334. [PubMed: 10086391]

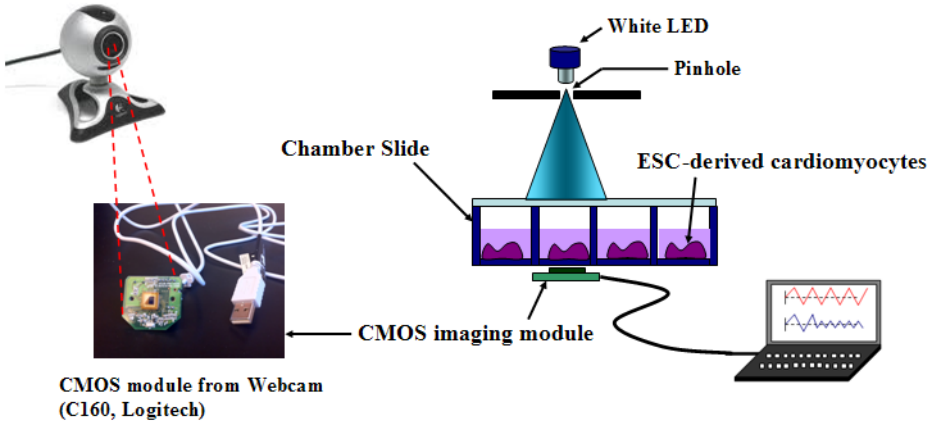

Fig. 1.

Schematic of the experimental setup. ESC-derived cardiomyocytes were plated on a chamber slide. The white LED and a pinhole combination was used as a light source for imaging the beating cardiomyocytes. The real-time beating rates of the cardiomyocytes were measured using the CMOS imaging module was controlled by an image processing program.

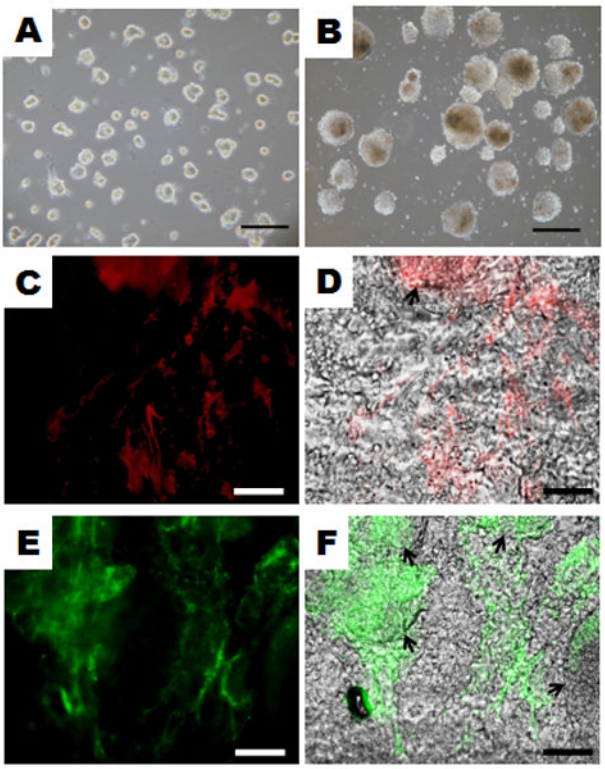

Fig. 2.

Immunocytochemical characterization of ESC cardiomyogenic differentiation. (A) Bright field image of ESCs used for cardiac differentiation. (B) Bright field image of suspension cultured EBs formed in a non-adherent petri dish. (C) Immunocytochemical staining for sarcomeric α-actinin of beating colonies cultured on a chamber slide for 10 days. (D) Immunocytochemical staining for sarcomeric α-actinin overlapped with phase contrast image. (E) Immunocytochemical staining for cardiac myosin heavy chain of beating colonies cultured on a chamber slide for 10 days. (F) Immunocytochemical staining for cardiac myosin heavy chain overlapped with phase contrast image. Arrows show beating colonies. Scale bar (A), (B) 400 μm, (C) – (F) 100 μm.

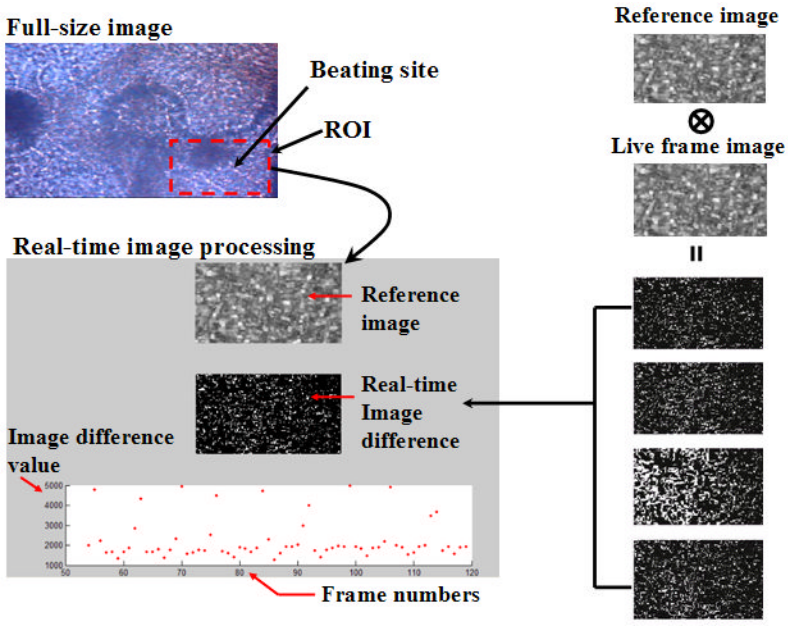

Fig. 3.

Real-time image processing procedure. ROI was selected manually from the full-size image and a reference image was captured. The image difference between reference and live frame images was calculated and displayed in real-time. The graph of the image difference value was also plotted in real-time. (see also Supplementary Information, Movie S1)

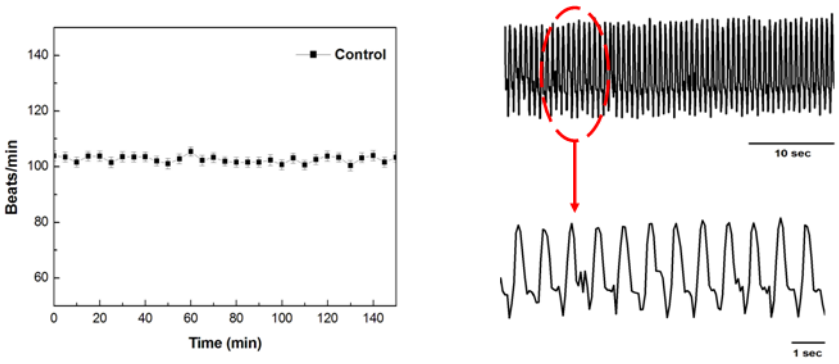

Fig. 4.

Characterization of the lensfree cardiotoxicity detector. Prior to injection of the drugs the ESC-derived cardiomyotyes were cultured in normal medium and their beating rates were observed for 150 min. The beating rates were stable and the beating signal had a regular pulse pattern.

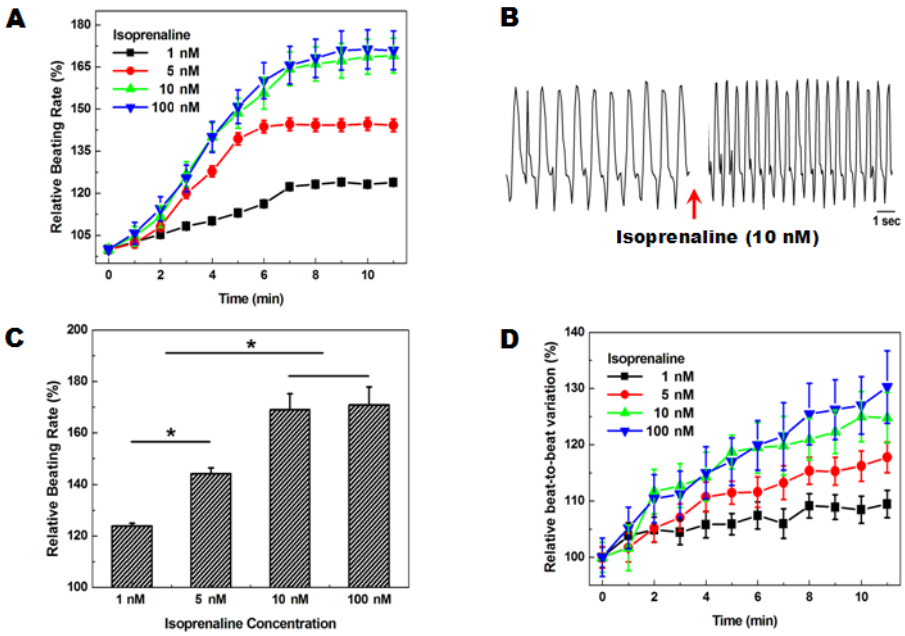

Fig. 5.

Chronotropic effects of cardiomyocytes under treatment of different concentrations of isoprenaline (1, 5, 10 and 100 nM). (A) The change in beating rate. The beating rates increased after isoprenaline injection. (B) A plot of the image difference value. A pulse pattern was observed. The decreased beating intervals were observed clearly after isoprenaline was injected. (C) The change in beating rates over time during the first 12 min after treatment of isoprenaline (D) The beat-to-beat variations. Beat-to-beat variations increased under treatment of isoprenaline. * shows a significant difference in variance ($p < 0.05$). For statistical analysis, one-way ANOVA was used. (see also Supplementary Information, Movies S2 and S3).

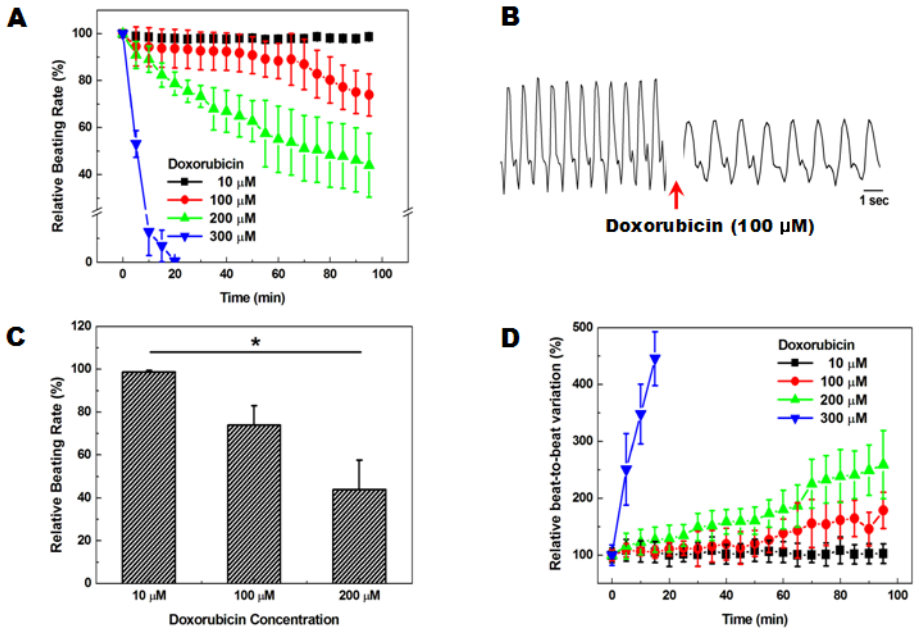

Fig. 6.

Chronotropic effects of cardiomyocytes under treatment with doxorubicin (10, 100, 200 and 300 μM). (A) The change in beating rate. The beating rates decreased after doxorubicin injection. (B) The beating signal. The increased beating intervals were clearly observed after doxorubicin injection. (C) The change in beating rates at 100 min after the treatment with doxorubicin. (D) The beat-to-beat variations. In contrary to beating rates, the beat-to-beat variations increased under treatment with doxorubicin. * shows a significant difference in variance ($p < 0.05$). For statistical analysis one-way ANOVA was used. (see also Supplementary Information, Movies S4 and S5).