The Effect of E-Cigarettes on the Human Heart Studied using Cardiomyocytes Generated from Induced Pluripotent Stem Cells

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***Abstract*— Electronic cigarettes (e-cigarettes) have become increasingly popular with adolescents in recent years as a result of e-cigarette companies' aggressive marketing schemes, false safety claims, and appealing flavors targeted towards teens. In the past 8 years alone, e-cigarette use amongst youth has increased by 18 times. Although many dangerous effects of smoking e-cigarettes on lungs have come to light, limited and only qualitative effort has been made to analyze the impact of smoking e-cigarettes on the human heart directly. In this study, we determined e-liquid cardiotoxicity in both healthy cells and cells with long QT syndrome by treating healthy and diseased human cardiomyocytes with e-liquids with varying nicotine concentrations. These cardiomyocytes were generated from human induced pluripotent stem cells. The cardiomyocytes were divided into 5 groups, a control group and 4 test groups, each treated with e-liquid containing varying amounts of nicotine between 0% and 70%. The cells' biological indicators such as heart rate, pulse pressure, essential protein concentration, and metabolic activity, were measured, characterized using three different functional assays: contractility, Western blot, and viability, and tracked closely over 2 weeks. The results demonstrated that acute exposure to e-liquid led to tachycardia, hypertension, decreased protein levels, and cell death. The rate of cardiotoxicity increases with higher nicotine concentrations. The basal fluid also showed non-negligible toxicity. Under identical conditions, the functionality of the diseased heart cells declined at a faster rate compared to healthy cells. Overall, this work systematically establishes the harmful physiological effects of e-cigarettes on the human heart quantitatively.**

I. INTRODUCTION

The anatomy of a typical e-cigarette is shown in Figure 1 [Brown, 2013]. E-cigarettes contain a "juice" in a cartridge, which is referred to as e-liquid. It is composed of various carrier compounds, additives, flavorings, and nicotine. The e-liquid in the cartridge is heated in the vaporizing chamber and delivered as aerosols through the mouthpiece to the user. The battery voltage is adjustable to allow users to customize vapor discharge quantity per puff [Brown, 2013]. The inhalation of this e-liquid vapor could be dangerous to pulmonary and cardiovascular health [Qasim, 2017].

Induced pluripotent stem cells (iPSCs), are pluripotent stem cells derived from somatic cells, such as blood cells or skin cells [Ye, 2013]. Since their discovery by Shinya Yamanaka in 2006 [Takahashi, 2006], numerous researchers have utilized human induced pluripotent stem cells (hiPSCs) to carry out drug discovery, health studies, and "clinical trials in a dish" [Grskovic, 2011] [Karakikes, 2015] [Matsa, 2016] [Shi, 2017] [Magdy, 2018] [Fermini, 2018] [Lee, 2019]. In particular, hiPSCs have been used to generate cardiomyocytes to conduct cardiovascular research [Chatterjee, 2010] [Matsa, 2011] [Burridge, 2016] [Narkar, 2017] [Kitani, 2019] [Wei, 2022]. Several published studies have shown that smoking e-cigarettes negatively affects pulmonary health and has carcinogenic effects on lungs [Rowell, 2015] [Hecht, 2015]. However, no direct studies have been reported to quantify the physiological effects of smoking e-cigarettes on the human heart. As such, we decided to use iPSC-cardiomyocytes to quantitatively determine e-liquid cardiomyopathy. In this project, the impact of e-liquid on the human heart was systematically studied and quantified. In addition, we compared the magnitude of the impact between healthy and diseased heart cells. We hypothesized that even the basal e-liquid without nicotine could possess compounds that induce cardiomyopathy; higher nicotine concentrations in the e-liquid cause more rapid decline in cardiomyocyte health; and under equivalent conditions, the effect would be worse for diseased cells as compared to healthy cells. Thus, we conducted a series of experiments using cardiomyocytes that we differentiated from two lines of hiPSCs – one healthy and one with long QT syndrome. To correlate the e-liquid's nicotine content to cardiotoxicity, the cardiomyocytes were divided into five groups: a control group, one group treated with pure basal fluid without nicotine, and three groups each to be treated with e-liquid containing varying amounts of nicotine of 30%, 50%, and 70%, respectively. To understand the effect of e-liquid exposure on heart rate and pulse pressure, essential protein concentration, and metabolic activity, we used three different functional assays to quantify the cardiotoxicity of the e-liquids; contractility, Western blot, and viability.

II. PRIOR RESEARCH

There have been numerous human observational studies, murine experiments, and review papers on the effect of smoking cigarettes and e-cigarettes on the heart [Qasim, 2017] [Papathanasiou, 2013] [Lee, 2018]. It has been established that nicotine has cardiotoxic effects and that smoking is a risk factor for heart attacks and myocardial infarction [Ottervanger, 1995]. Cardiovascular disease is a major cause of death among smokers [Goldenberg, 2003] [Qasim, 2017]. Papathanasiou et al. reported that nicotine causes an acute increase in heart rate and blood pressure in adult humans [Papathanasiou, 2013]. Lee et al. reported their findings on the effect of e-cigarette smoke on a mouse heart as measured by DNA damage [Lee, 2018]. In this work, to debunk the claim that e-cigarettes are noncarcinogenic, a

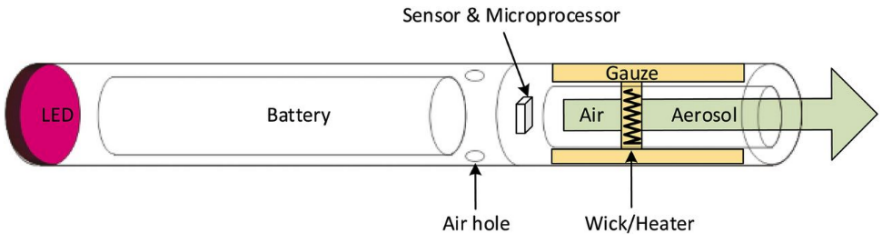

Fig. 1. Anatomy of a typical e-cigarette [Brown, 2013]. The e-liquid is vaporized in the vaporizing chamber, which consists of a nicotine cartridge, heating coil, and atomizer. This e-liquid is thought to have cardiotoxic effects on human cardiomyocytes.

group of mice were exposed to e-cigarette smoke. It was found that treatment with e-cigarette smoke caused damage to mouse DNA and slowed repair processes in the murine lung, heart, and bladder. However, it still remained unclear whether this cardiotoxic effect also extended to humans. Farsalinos et al. analyzed the National Health Interview Surveys (NHIS) of 2016 and 2017 to examine whether e-cigarette use was consistently associated with coronary heart disease and myocardial infarction [Farsalinos, 2019]. However, they were unable to draw a significant association between e-cigarette use and an elevated risk for coronary heart disease or myocardial infarction in humans. Use of hiPSCs for experimental purposes was demonstrated by Wei et al. Their study established that cannabis users had a higher risk of myocardial infarction and that genistein attenuates marijuana-induced vascular inflammation [Wei, 2022]. A review paper by Cherian et al. described e-cigarette associated lung injury. It was found that vaping products gave rise to a variety of complications, including thermal injuries, life-threatening pulmonary issues, and more [Cherian, 2020]. Although the pathological effects of e-cigarettes on human lungs are mostly established, there have been no systematic and quantitative studies to determine how e-liquid affects human heart cells physiologically thus far. Although much is known about smoking-induced cardiotoxicity, very little is known about vape-induced cardiotoxicity. This motivated our work to experimentally determine the effect of e-cigarettes on human cardiomyocytes derived from hiPSCs.

III. METHODS AND MATERIALS

A. iPSC Confluency

In this work, we passaged and cultured two lines of iPSCs until they reached 80% confluency. One line contains healthy cells and the other line contains cells exhibiting long QT syndrome. First, for each line, we precoated two 6-well cell plates with 1.5 mL of Matrigel® per well. This forms a coating at the bottom of each well to help the newly-passaged iPSCs to attach to the plates. Then, we passaged existing cells of each line at a 1 to 12 ratio, redistributing them into 12 wells. To do this, we first digested one well of iPSCs with Gentle Cell Dissociation Reagent (GCDR). After 5 minutes, we aspirated the fluid and added 1 mL of Insulin RPMI with B27 supplement; we then fully separated the layer of cells from the bottom of the plate by pipetting up and down. Once the Matrigel®-coated plates incubated at 37°C for at least 30 minutes, we added 1.5 mL of StemMACS™ iPS brew XF to each well of the two 6-well plates. Finally, we added 80 L of the dissolved cell solution to each well. After proper settling, the plates were incubated at 37°C with 5% CO2. The iPS brew medium was replaced daily until the cells reached 80% confluency, which usually takes 3 days to occur.

B. Cardiomyocyte Differentiation

The process of differentiation starts after the iPSCs reach roughly 80% confluency and generally takes between 8 to 12 days. On Day 0, we replaced the iPS brew medium with 1.2 mL of 6 M CHIR99021 (CHIR). The addition of CHIR activates the Wnt signaling pathway to induce mesoderm differentiation. We diluted 0.9 mL of 10 M CHIR stock solution with 0.3 mL of -Insulin RPMI to achieve a 6 M CHIR solution. Based on the results, concentration of CHIR may require adjustment up to 8 M or 10 M to ensure proper induction of mesoderm differentiation. Mesoderm differentiation continued on Day 1. Day 2 was the recovery phase; we neutralized the CHIR with an equal volume (1.2 mL) of -Insulin RPMI. Cardiac mesoderm specification began on Day 3 and extended to Day 4. On Day 3, we replaced the medium with 2 mL of 10 M IWR stock solution diluted to 5 M using -Insulin RPMI. The IWR inhibits the Wnt signaling pathway once fully differentiated. We then neutralized the medium with 2 mL of -Insulin RPMI on Day 5 to allow for recovery into Day 6. Day 7 marks the completion of differentiation; beating cardiomyocytes may be observed at any time from days 6-11. For the completion of the differentiation process, we replaced the -Insulin RPMI with 2 mL of +Insulin RPMI to provide nutrients to the cells. Fig. 2 shows the progression of differentiation for this experiment.

C. E-liquid Treatment

After generating the beating cardiomyocytes from iPSCs, we exposed both the healthy and diseased cells to the e-liquid. We obtained 4 different e-liquids, the basal fluid with no nicotine content, and Marcado-flavored e-liquids with 30%, 50%, and 70% nicotine concentration by volume from Red Oak. First, we replated each cell line of the cardiomyocytes onto 96-well plates, ensuring a cell count of at least 3,000 per well and 80 wells per line. We used

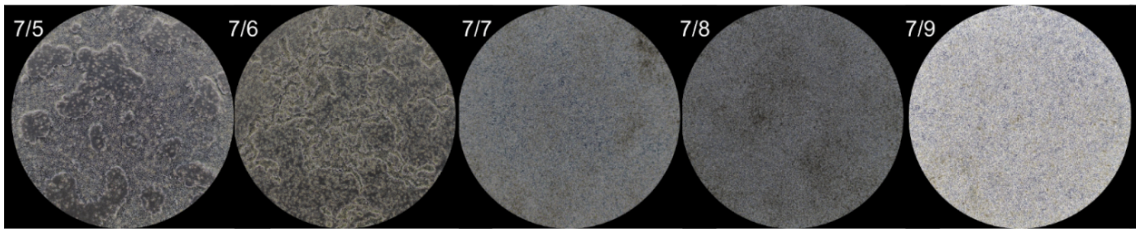

Fig. 2. Optical microscope images depicting the progression of cell growth over 5 days after reaching full confluency. Visible morphological changes can be observed throughout the process as the cells differentiate. Beating cardiomyocytes were observed on Day 6.

16 wells as control and 16 wells each for every concentration of nicotine. To emulate the e-cigarette vaporization process and to account for potential degradation in the e-liquid components, we first boiled the e-liquid in a CFX Duet BioRad polymerase chain reaction (PCR) machine. We waited for the vapor to cool and condense before adding 10 L of condensed e-liquid at each intended concentration to the wells. This research only tested the effects of acute (rather than chronic) exposure to e-liquid, so in subsequent weeks we replaced the medium with -Insulin RPMI every two days.

D. Functional Assays

We utilized three different functional assays to quantify the impact of e-liquid exposure on the cardiomyocyte health: contractility, Western blot analysis, and cell viability. We used the contractility assay to determine the general physiological effects of e-liquid on the heart by monitoring cardiomyocytes' heart rate and pulse pressure every 2 days. To analyze the e-liquids' impact at the cellular level, we tracked protein levels in the cardiomyocytes via Western blot. Finally, to correlate the rate of cell death with e-liquid treatment, we observed cell viability using the Countess Cell Counting Chamber. Each data point in the results were collected and averaged over 16 wells.

1) Contractility: We used the Sony SI8000 Cell Motion Imaging System to determine the heart rate and pulse pressure of the cells. These markers are useful in determining cell health as well as extending cellular symptoms to real-life conditions, such as bradycardia vs. tachycardia or hypotension vs. hypertension.

2) Western Blot Analysis: We lysed the cells using ThermoFisher radioimmunoprecipitation assay (RIPA) buffer and centrifuged the lysates at 13,000 rotations per minute (rpm) for 15 minutes at 4 °C. The lysates were then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using polyacrylamide gels. The Western blot was done as previously described [Pillai-Kastoori, 2020] [Smith, 2009]. We performed immunoblotting using specific antibodies in order to evaluate the expression of different proteins.

3) Cell Viability: To test the cell viability, we first stained the cells with Trypan blue dye diluted at a 1:10 ratio with phosphate buffered saline (PBS) and placed the stained cells into the Countess Cell Counting Chamber slides. Then, we utilized the ThermoFisher Countess Cell Counting Chamber to characterize the percentage of cells that are viable over time.

IV. DISCUSSIONS

After closely tracking the performances of the healthy and diseased cell lines over a course of 2 weeks, we analyzed the results and determined the e-liquids' cardiotoxic effects on the cardiomyocytes when they were acutely exposed. In the following sections, we discuss the experimental results from three different functional assays that we carried out: contractility, Western blot, and viability.

A. Impact on Contractility

The e-liquid's impact on cardiomyocytes' pulse rate, measured in beats per minute (bpm), and pulse pressure, measured in millimeters of mercury (mmHg), was studied through the contractility assay. The untreated cells served as a baseline for control; their pulse rate stayed relatively constant over the course of 2 weeks. However, the cells treated with e-liquid all experienced tachycardia, beating at an increasing rate over the 2 week duration. Higher concentrations of nicotine led to a more pronounced effect. Even exposure to the pure basal fluid without nicotine resulted in a higher pulse rate. Having a chronically increased pulse rate is dangerous [Ganz, 1995] [Umana, 2003]. Tachycardia can result in a number of complications, including hypoxia, angina, syncope, and more [Mayo Clinic, 2022]. It has been established in the past that smoking traditional nicotine products also results in a substantial increase in resting heart rate via observational [Papathanasiou, 2013] and biological studies [Csordas, 2013]. With this knowledge, it can be inferred that exposure to next-generation nicotine products would result in a similar rise in resting heart rate as well. However, a point of interest is the increase in heart rate with exposure to the basal e-liquid without nicotine, which can be observed in Fig. 3. This shows that the basal fluid contained in e-cigarettes has cardiotoxic effects even without nicotine. The comparison of heart rate over time between the healthy cardiomyocytes and cardiomyocytes with long QT syndrome can be seen in Fig. 3(A) and Fig. 3(B). Cardiomyocytes with long QT syndrome have roughly equal starting heart rate (68 bpm) as the healthy cardiomyocytes (71 bpm). However, after equivalent e-liquid exposure, diseased cardiomyocytes experienced a much more significant and rapid rise in heart rate. For example, with the basal fluid alone, the healthy cardiomyocytes' pulse rate increased steadily from 71 bpm

to 81 bpm for the first 8 days and remained stable for the next 7 days; an overall 13% increase. In contrast, the diseased cells' pulse rate climbed by 21% over two weeks; graphing trends suggest that the increase still continues even after two weeks. At 70% nicotine concentration, healthy cells' pulse rate increased by 39% to 100 bpm whereas diseased cells' pulse rate increased by 58% to 107 bpm in two weeks. Overall, at the end of the 15 days testing period, with 30%, 50%, and 70% nicotine e-liquid exposure, the pulse rate increased by 36%, 37%, and 39% for the healthy cardiomyocytes, and 52%, 56%, and 58% for the diseased cardiomyocytes, respectively. The rate of rise for the pulse rate is greater in the diseased heart cells than the healthy cells. We can then infer that individuals afflicted with long QT syndrome who smoke e-cigarettes are at much greater risk for tachycardia than their healthy counterparts. This is reaffirmed by the notion that smoking may be correlated with heightened mortality in patients with congenital heart diseases [Engelfriet, 2007]. We found that acute exposure to e-liquid also caused cardiomyocytes to beat at a higher pulse pressure, according to the measurements conducted using the Sony SI8000 Cell Motion Imaging System. Pulse pressure can also be calculated by the difference between systolic and diastolic blood pressure. Thus, a higher pulse pressure is recognized as a risk factor for cardiovascular disease [Dart, 2001] [de Simone, 2005]. Generally, a pulse pressure exceeding 40 mmHg is considered unhealthy [Mayo Clinic 2022]. As shown in Fig. 4, pulse pressure for the healthy cells after being treated with e-liquid exceeded well over 40 mmHg, indicating that smoking e-cigarettes may lead to hypertension. Once again, this finding is consistent with the already established connection between smoking cigarettes and malignant hypertension [Primatesta, 2001] [Virdis, 2010]. Complications of uncontrolled hypertension include aneurysm, myocardial infarction, stroke, and more [Sanyal, 2008] [Mayo Clinic, 2022]. The comparison of pulse pressure over time between the healthy cardiomyocytes and cardiomyocytes with long QT syndrome can be seen in Fig. 4(A) and Fig. 4(B). Similar to what was observed with heart rate, diseased cardiomyocytes were affected more drastically than healthy ones. The diseased cardiomyocytes with long QT syndrome have a higher starting pulse pressure (44 mmHg) relative to the healthy cardiomyocytes (39 mmHg). For example, with the basal fluid alone, the healthy cardiomyocytes' pulse pressure increased to 42 mmHg over 15 days, which is a 6% increase. In contrast, the diseased cell pulse pressure climbed to 50 mmHg over two weeks resulting in a 13% increase. The trends in the graphs also suggest that the basal fluid-treated diseased cells' pressure still continues to increase steadily after two weeks. At 70% nicotine concentration, healthy cells' pulse pressure increased to 55 mmHg whereas diseased cells' pulse pressure increased to 59 mmHg in two weeks. Overall, at the end of the 15 days testing period, with 30%, 50%, and 70% nicotine e-liquid exposure, the pulse rate increased to 50 mmHg, 54 mmHg, and 55 mmHg for the healthy cardiomyocytes, and 55 mmHg, 58 mmHg, and 59 mmHg for the diseased cardiomyocytes, respectively. With this equivalent e-liquid exposure, both the healthy and the diseased cardiomyocytes experienced a similar magnitude of pulse pressure rise. However, the baseline pressure for the diseased cells was higher to begin with, which may result in life-threatening levels of pulse pressure. This result also correlates well with the fact that smoking is associated with higher mortality rates in people with congenital cardiovascular conditions [Engelfriet 2007] [Gianicolo, 2010].

B. Impact on Protein Concentration

Essential proteins, also known as the housekeeping proteins, are critical molecules for maintaining overall cell health. Through this work, we also found a direct correlation between acute exposure to e-liquid and degradation of essential proteins. The comparison between the healthy cardiomyocytes and cardiomyocytes with long QT syndrome can be seen in Fig. 5(A) and Fig. 5(B). The protein concentration in the cardiomyocytes started at 77 ng/mL and 73 ng/mL for the healthy and diseased cells, respectively. These are in line with the 75-80 ng/mL range that is considered normal [ThermoFisher, 2021]. The protein levels dropped to as low as 45 ng/mL in healthy cells and 37 ng/mL in diseased cells at the end of the trial. This aligns with the fact that cigarette smoke promotes ubiquitination and significant destabilization of the proteins [Kim, 2011] [Han, 2017]. Once again, higher levels of nicotine resulted in a more drastic degradation and the basal fluid was also detrimental to protein levels. Overall, at the end of the 15 days testing period, with 30%, 50%, and 70% nicotine e-liquid exposure, the protein concentration decreased by 47%, 59%, and 71% for the healthy cardiomyocytes, and 30%, 68%, and 91% for the diseased cardiomyocytes, respectively.

C. Impact on Cardiomyocyte Viability

For the viability assay, we tracked the percentage of live cells over time. We utilized the ThermoFisher Cell Countess Chamber to check the amount of live vs. dead cells over the course of the experiment. We observed that the cell viability for both healthy and diseased cells declined over time following exposure to the e-liquid. The cardiomyocytes not only degraded in quality, but also began to die in large amounts after a few days. The comparison of viability between the healthy cardiomyocytes and cardiomyocytes with long QT syndrome can be seen in Fig. 6(A) and Fig. 6(B). The control sample stayed at 100% viability, meaning that only a negligible amount of cells died naturally over the course of the testing period. Following e-liquid treatment, the cells began to die at increasingly rapid rates. At the end of the testing period, with basal fluid, 30%, 50%, and 70% nicotine e-liquid exposure, the viability decreased by 22%, 33%, 41%, and 47% for the healthy cardiomyocytes, and 28%, 46%, 50%, and 54% for the diseased cardiomyocytes, respectively. The rate of mortality is 15% to 39% greater in disease cells compared to healthy cells under similar conditions.

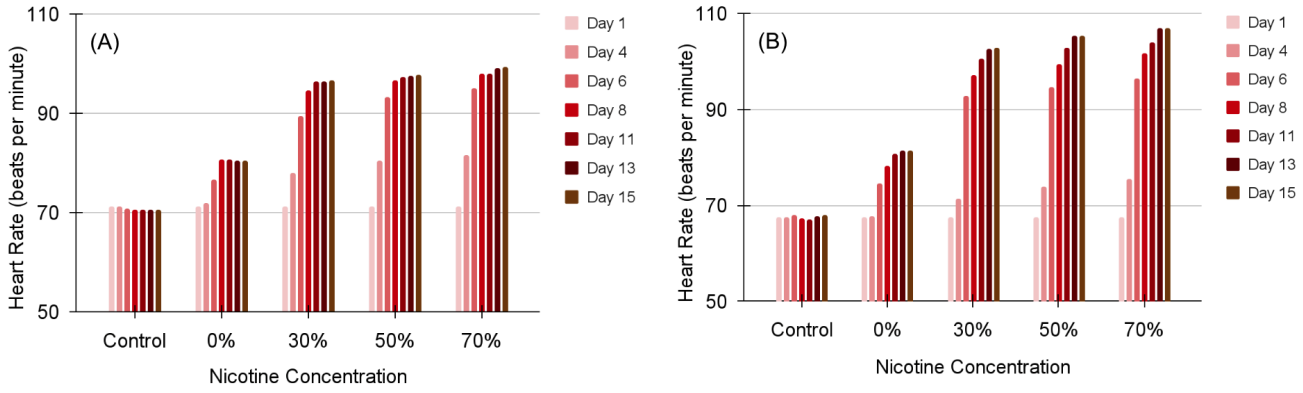

Fig. 3. Heart rate trend over time for (A) healthy cardiomyocytes (B) diseased cells with long QT syndrome. The data includes the control cells, cells treated with e-liquid basal fluid, and cells treated with 30%, 50%, and 70% nicotine concentration.

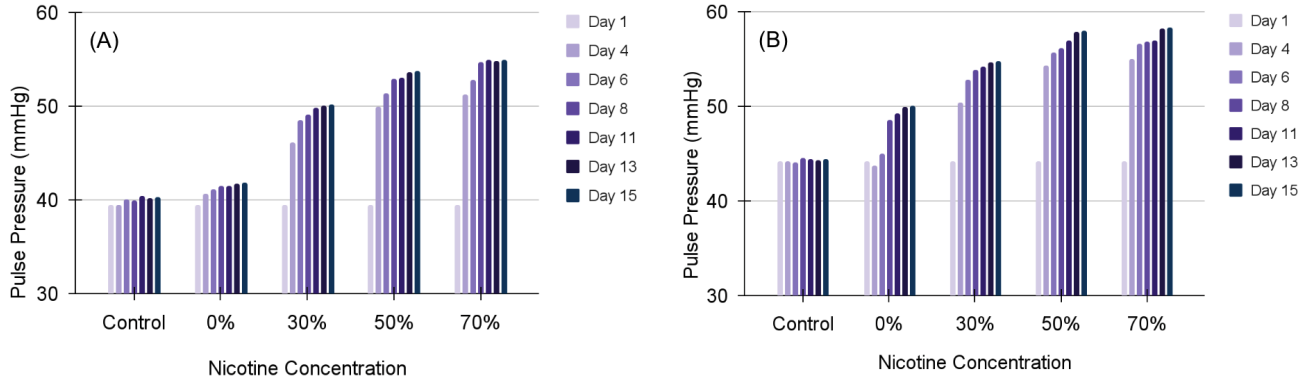

Fig. 4. Pulse pressure trend over time for (A) healthy cardiomyocytes (B) diseased cells with long QT syndrome. The data includes the control cells, cells treated with e-liquid basal fluid, and cells treated with 30%, 50%, and 70% nicotine concentration.

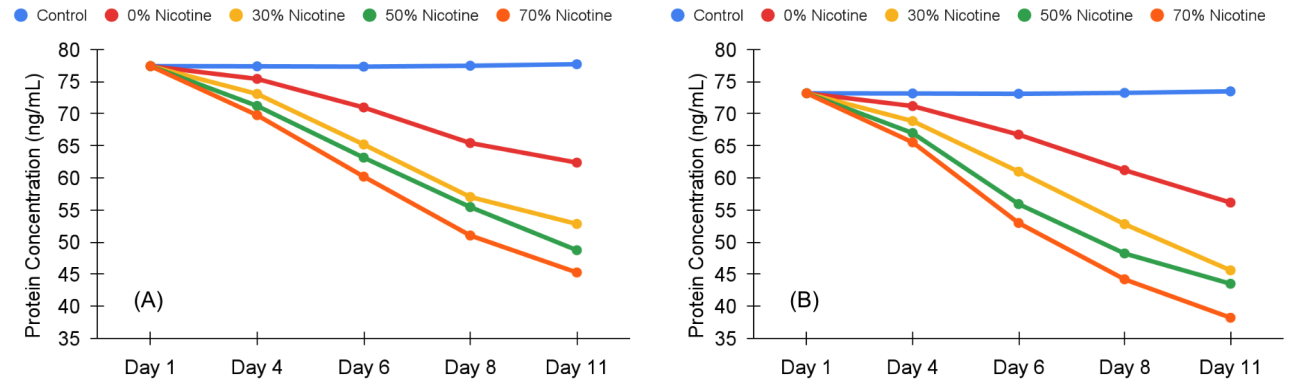

Fig. 5. Protein concentration trend over time for (A) healthy cardiomyocytes (B) diseased cells with long QT syndrome. The data includes the control cells, cells treated with e-liquid basal fluid, and cells treated with 30%, 50%, and 70% nicotine concentration.

V. CONCLUSIONS

In summary, we have conducted a systematic study using cardiomyocytes derived from human induced pluripotent stem cells to analyze the effects of smoking e-cigarettes on the healthy and diseased human heart. We differentiated one healthy line of cells and one diseased line of cells with long QT syndrome into beating cardiomyocytes. We treated both cardiomyocyte lines with basal fluid and e-liquid with 30Our studies demonstrated healthy and stable performances on key indicators from the controlled cell lines while acute exposure to e-liquid caused the conditions of the cardiomyocytes to deteriorate. Specifically, we observed an

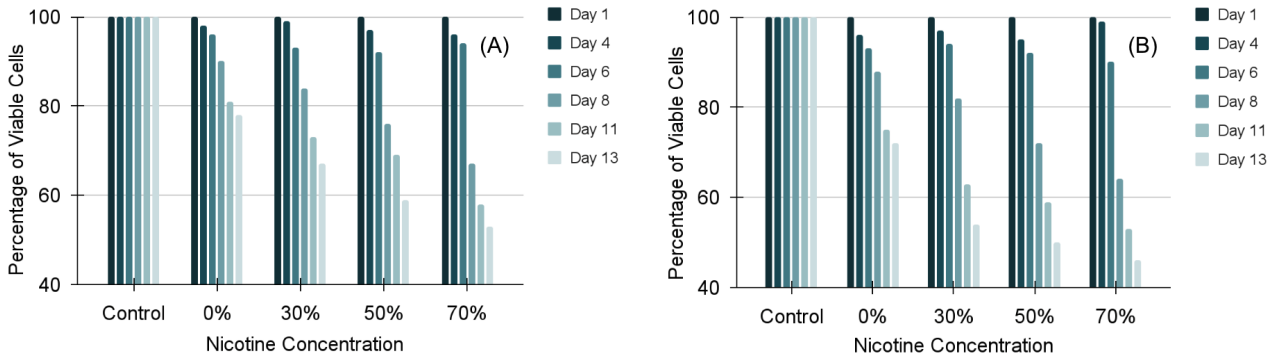

Fig. 6. Viability trend over time for (A) healthy cardiomyocytes (B) diseased cells with long QT syndrome. The data includes the control cells, cells treated with e-liquid basal fluid, and cells treated with 30%, 50%, and 70% nicotine concentration.

increase in the resting heart rate, a heightened pulse pressure, a decrease in the concentration of essential housekeeping proteins, as well as increased cell mortality. These are all risk factors for myocardial infarction, which can be lethal. The degradation of cardiomyocyte health is present when treated with pure basal fluid of the e-cigarettes and increases with higher nicotine concentrations. While nicotine is the only cardiotoxic compound in regular cigarettes, e-cigarettes incorporate not only higher levels of nicotine but also a basal fluid. The increased nicotine concentration proportionally worsens the cardiomyopathic effects. Since e-cigarettes contain more nicotine in a single cartridge than 20 traditional cigarettes and there is no way for the users to track their own consumption, it is easier for the user to over-consume nicotine than in traditional tobacco products. The fact that the basal fluid exposure alone caused significant negative impacts to the cardiomyocytes indicates that the impact of e-cigarette is even worse than that of the traditional tobacco products. Our work establishes and solidifies the cardiotoxic effects of smoking e-cigarettes. Some potential ways to expand this study would be to pinpoint the exact mechanism in which e-liquid impacts cardiomyocytes by performing additional functional assays, such as a Western blot. Furthermore, it would be useful to determine the full scope of impact of e-cigarettes on the human body by carrying out similar experiments on parts of the brain, digestive tract, musculoskeletal system, and more.

References

[1] Qasim H, Karim Z, Rivera J, Khasawneh F, Aishbool F. Impact of electronic cigarettes on the cardiovascular system. J Am Heart Assoc. 2017; 6:e006353. DOI: https://doi.org/10.1161/JAHA.117.006353

[2] Hudson L. Vaping statistics 2022. SingleCare 2022, accessed December 24, 2022, <https://www.singlecare.com/blog/news/vaping-statistics/>.

[3] Burns DM, Lee L, Shen LZ, Gilpin E, Tolley HD, Vaugn J, Shanks TG. Cigarette smoking behavior in the United States. U.S. Department of Agriculture, 1996. Pages 13-42.

[4] Mattingly DT, Hirschtick JL, Meza R, Fleischer NL. Trends in prevalence and sociodemographic and geographic patterns of current menthol cigarette use among U.S. adults. Preventive Medicine Reports 2020. Volume 20, 101227. DOI: https://doi.org/10.1016/j.pmedr.2020.101227

[5] Bold KW, Kong G, Camenga DR, Simon P, Cavallo DA, Morean ME, Krishnan-Sarin S. Trajectories of e-cigarette and conventional cigarette use among youth. Pediatrics (2018) 141 (1); e20171832. DOI: https://doi.org/10.1542/peds.2017-1832

[6] Sun T, Lim CCW, Stjepanovič D, Leung J, Connor JP, Gartner C, Hall WD, Chan GCK. Has increased youth e-cigarette use in the USA, between 2014 and 2020, changed conventional smoking behaviors, future intentions to smoke and perceived smoking harms? Addictive Behaviors 2021. DOI: https://doi.org/10.1016/j.addbeh.2021.107073

[7] Ramamurthi D, Chau C, Jackler R. Exploitation of the COVID-19 pandemic by e-cigarette marketers. Tobacco Control 2021; 30:e56-e59. DOI: http://dx.doi.org/10.1136/tobaccocontrol-2020-055855

[8] Collins, L. Glasser AM, Abudayyeh H, Pearson JL, Villanti AC. E-cigarette marketing and communication: How e-cigarette companies market e-cigarettes and the public engages with e-cigarette information. Nicotine Tobacco Research 2018, Volume 21, Issue 1, Pages 14-24. DOI: https://doi.org/10.1093/ntr/ntx284

[9] Du P, Fan T, Yingst J, Veldheer S, Hrabovsky S, Chen C, Foulds J. Changes in e-cigarette use behaviors and dependence in long-term e-cigarette users. American Journal of Preventive Medicine September 2019. Volume 57, Issue 3, Pages 374-383. DOI: https://doi.org/10.1016/j.amepre.2019.04.021

[10] Brown CJ, Cheng JM. Electronic cigarettes: product characterisation and design considerations. Tob Control 2014;23:ii4-ii10. DOI: 10.1136/tobaccocontrol-2013-051476

[11] Ye L, Swingen C, Zhang J. Induced pluripotent stem cells and their potential for basic and clinical sciences. Current Cardiology Reviews. February 2013; 9(1): 63-72. DOI: 10.2174/157340313805076278

[12] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell Press 2006. Vol 126 Issue 4 pg. 663-676. DOI: https://doi.org/10.1016/j.cell.2006.07.024

[13] Grskovic M, Javaherian A, Strulovici B, Daley GQ. Induced pluripotent stem cells– opportunities for disease modeling and drug discovery. Nature Reviews. Drug Discovery 2013. 10 (12): 915-29. DOI: 10.1038/nrd3577

[14] Karakikes I, Ameen M, Termglinchan V, Wu JC. Human induced pluripotent stem cell-derived cardiomyocytes. Circulation Research 2015. Vol 117, Issue 1, Pages 80-88. DOI: https://doi.org/10.1161/CIRCRESAHA.117.305365

[15] Matsa E, Burridge PW, Yu KH, Ahrens JH, Tennglinchan V, Wu H, Liu C, Shukla P, Sayed N, Churko JM, Shao N, Woo NA, Chao AS, Gold JD, Karakikes I, Snyder MP, Wu JC. Transcriptome profiling of patient-specific human iPSC- Cardiomyocytes products individual drug safety and efficacy responses in vitro. Cell Stem Cell 2016; 19(3): 311-325. DOI: 10.1016/j.stem.2016.07.006

[16] Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell technology: a decade of progress. Nat Rev Drug Discov 16. 2017, 115–130 https://doi.org/10.1038/nrd.2016.245

[17] Magdy T, Schuldt AJT, Wu JC, Bernstein D, Burridge PW. Human induced pluripotent stem cell (hiPSC)-derived cells to assess drug cardiotoxicity: Opportunities and problems. Annu Rev Pharmacol

Toxicol. 2018, 58: 83-103. DOI: 10.1146/annurev-pharmtox-010617-053110

[18] Fermini B, Coyne ST, Coyne KP. Clinical trials in a dish: A perspective on the coming revolution in drug development. SLAS Discovery 2018. Volume 23, Issue 8, p. 765-776. DOI: https://doi.org/10.1177/2472555218775028

[19] Lee J, Termglinchan V, Diecke S, Itzhaki I, Lam CK, Garg P, Lau E, Greenhaw M, Seeger T, Wu H, Zhang JZ, Chen X, Gil IP, Ameen M, Sallam K, Rhee J, Churko JM, Chaudhary R, Chour T, Wang PJ, Snyder MP, Chang HY, Karakikes I, Wu JC. Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy. Nature 2019. 572, 335-340. DOI: https://doi.org/10.1038/s41586-019-1406-x

[20] Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. Cardiology 2010; 115:155-162. DOI: 10.1159/000265166

[21] Matsa E, Rajamohan D, Dick E, Young L, Mellor I, Staniforth A, Denning C. Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. European Heart Journal, Volume 32, Issue 8, 2011, Pages 952-962. DOI: https://doi.org/10.1093/eurheartj/ehr073

[22] Burridge PW, Li YF, Matsa E, Wu H, Ong S, Sharma A, Holström A, Chang AC, Coronado MJ, Ebert AD, Knowles JW, Telli ML, Witteles RM, Blau HM, Bernstein D, Altman RB, Wu JC. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. Nat Med. 2016; 22(5): 547-556. DOI: 10.1038/nm.4087

[23] Narkar A, Willard JM, Blinova K. Chronic cardiotoxicity assays using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)

[24] Kitani T, Ong S, Lam C, Rhee J, Zhang JZ, Oikonomopoulos A, Ma N, Tian L, Lee J, Telli M, Witteles R, Sharma A, Sayed N, Wu JC. Human induced pluripotent stem cell model of Trastuzumab-induced cardiac dysfunction in breast cancer patients. Circulation. 2019; 139(21): 2451-2465. DOI: 10.1161/CIRCULATIONAHA.118.037357

[25] Wei T, Chandy M, Nishiga M, Zhang A, Kumar KK, Thomas D, Manhas A, Rhee S, Justesen JM, Chen IY, Wo H, Khanamiri S, Yang JY, Seidi FJ, Burns NZ, Liu C, Sayed N, Shie J, Yeh C, Yang K, Lau E, Lynch KL, Rivas M, Kobilka B, Wu JC. Cannabinoid receptor 1 antagonist genistein attenuates marijuana-induced vascular inflammation. Cell 2022; 185(10): 1676-1693.e23. DOI: 10.1016/j.cell.2022.04.005

[26] Rowell TR, Tarran R. Will chronic e-cigarette use cause lung disease? American Journal of Physiology 2015; Volume 309, Issue 12. Pages L1398-L1409. DOI: https://doi.org/10.1152/ajplung.00272.2015

[27] Hecht SS, Carmella SG, Kotandeniya D, Pillsbury ME, Chen M, Ransom BWS, Vogel RI, Thompson E, Murphy SE, Hatsukami DK. Evaluation of toxicant and carcinogen metabolites in the urine of e-cigarette users versus cigarette smokers. Nicotine Tobacco Research 2015, Vol 17, Issue 6, Pages 704-709, DOI: https://doi.org/10.1093/ntr/ntu218

[28] Ottervanger JP, Festen JM, de Vries AG, Stricker BHC. Acute myocardial infarction while using the nicotine patch. CHEST 1995. Volume 107, Issue 6, Pages 1765-1766. DOI: https://doi.org/10.1378/chest.107.6.1765

[29] Goldenberg I, Jonas M, Tenenbaum A. Current smoking, smoking cessation, and the risk of sudden cardiac death in patients with coronary artery disease. Arch Intern Med 2003. 2301-2305. DOI: 10.1001/archinte.163.19.2301

[30] Papathanasiou G, Georgakopoulos D, Papageorgiou E, Zerva E, Michalis L, Kalfakakou V, Evangelou A. Effects of smoking on heart rate at rest and during exercise, and on heart rate recovery, in young adults. Hellenic J Cardiol 2013. 54(3):168-77. https://pubmed.ncbi.nlm.nih.gov/23685653/

[31] Lee HW, Park SH, Weng MW, Wang HT, Huang WC, Lepor H, Wu XR, Chen LC, Tang MS. E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. PNAS 2018. 115 (7) E1560-E1569. DOI: https://doi.org/10.1073/pnas.1718185115

[32] Farsalinos KE, Polosa R, Cibella F, Niaura R. Is e-cigarette use associated with CHD and MI? Insights from the 2016 and 2017 National Health Interview Surveys. SAGE Journals 2019. DOI: https://doi.org/10.1177/2040622319877741

[33] Cherian SV, Kumar A, Estrada-Y-Martin RM. E-cigarette or vaping product-associated lung injury: A review. The American Journal of Medicine 2020. Vol 133 Issue 6 657-663. DOI: https://doi.org/10.1016/j.amjmed.2020.02.004

[34] Pillai-Kastoori L, Schutz-Geschwender AR, Harford JA. A systematic approach to quantitative Western blot analysis. Anal Biochem 2020. 593:113608. DOI: 10.1016/j.ab.2020.113608

[35] Smith CJ, Osborn AM. Advantages and limitations of quantitative PCR (q-PCR)-based approaches in microbial ecology. FEMS Microbiology Ecology, Volume 67, Issue 1 2009, Pages 6-20. DOI: https://doi.org/10.1111/j.1574-6941.2008.00629.x

[36] Ganz, LI, Friedman PL. Supraventricular tachycardia. N Engl J Med 1995; 332:162-173. DOI: 10.1056/NEJM199501193320307

[37] Umana E, Solares CA, Alpert MA. Tachycardia-induced cardiomyopathy. Am Journ of Med 2003, Pages 51-55. DOI: https://doi.org/10.1016/S0002-9343(02)01472-9

[38] Mayo Clinic. Tachycardia. Mayo Clinic 2022, accessed December 25, 2022, <https://www.mayoclinic.org/diseases-conditions/tachycardia/symptoms-causes/syc-20355127>

[39] Csordas A, Bernhard D. The biology behind the atherothrombotic effects of cigarette smoke. Nat Rev Cardiol 2013. 10, 219-230. DOI: https://doi.org/10.1038/nrcardio.2013.8

[40] Engelfriet PM, Drenthen W, Pieper PG, Tijssen JGP, Yap SC, Boersma E, Mulder BJM. Smoking and its effects on mortality in adults with congenital heart disease. International Journal of Cardiology, Volume 127, Issue 1 2008. Pages 93-97, ISSN 0167-5273, DOI: https://doi.org/10.1016/j.ijcard.2007.05.008

[41] Dart AM, Kingwell BA. Pulse pressure- a review of mechanisms and clinical relevance. J Am Coll Cardiol. 2001, 37(4) 975-984. DOI: doi.org/10.1016/S0735-1097(01)01108-1

[42] de Simone G, Roman MJ, Alderman MH, Galderisi M, de Divitiis Oreste, Devereux RB. Is high pulse pressure a marker of preclinical cardiovascular disease? Am Journ Heart Assoc 2005. 45:575-579. DOI: https://doi.org/10.1161/01.HYP.0000158268.95012.08

[43] Mayo Clinic 2022. Pulse pressure: An indicator of heart health?, accessed December 25, 2022,<https://www.mayoclinic.org/diseases-conditions/high-blood-pressure/expert-answers/pulse-pressure/faq-20058189>

[44] Primatesta P, Falaschetti E, Gupta S, Marmot MG, Poulter NR. Association between smoking and blood pressure. J Am Heart Assoc. 2001; 37:187-193. DOI: https:/doi.org/10.1161/01.HYP.37.2.187

[45] Virdis A, Giannarelli C, Fritsch MN, Taddei S, Ghiadoni L. Cigarette smoking and hypertension. Current Pharmaceutical Design 2010. Volume 16 Number 23, p. 2517-2525

[46] Sanyal AJ, Bosch J, Blei A, Arroyo V. Portal hypertension and its complications. Gastroenterology. Vol 134 1715-1728. DOI: https://doi.org/10.1053/j.gastro.2008.03.007

[47] Mayo Clinic. High blood pressure (hypertension). Mayo Clinic 2022, accessed December 25,2022,<https://www.mayoclinic.org/diseases-conditions/high-blood-pressure/symptoms-causes/syc-20373410>

[48] Gianicolo AL, E., Monica C, Lamia AA, Ilenia F, Andreassi G, M. Smoking and congenital heart disease: The epidemiological and biological link. Current Pharmaceutical Design 2010 16(23), 2572-2577.

[49] ThermoFisher, Quantitative western blot analysis 2021, accessed December 31, 2022. <https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/quantitative-western-blot-analysis.html>

[50] Kim SY, Lee JH, Huh JW, Ro JY, Oh YM, Lee SD, An S, Lee YS. Cigarette smoke induces Akt protein degradation by the ubiquitin-proteasome system. Journ of Bio Chem 2011. Volume 286 Issue 37. DOI: https://doi.org/10.1074/jbc.M111.267633

[51] Han S, Jerome JA, Gregory AD, Mallampalli RK. Cigarette smoke destabilizes NLRP3 protein by promoting its ubiquitination. Respir Res. 2017. 18, 2. DOI: https://doi.org/10.1186/s12931-016-0485-6