Curriculum Vitae

Olutomiwa O. Fadiran
tfadiran@gmail.com

EDUCATION

University of Maryland, Baltimore (UMB)
Baltimore, MD
M.S. Cellular and Molecular Biomedical Sciences
12/2021
University of Maryland Baltimore County (UMBC)
Baltimore, MD
B.S. Biological Sciences
5/2018

RESEARCH EXPERIENCE

UMB
Master's Thesis
12/2020-12/2021
Mentor: Ivy Dick
Project: Generating and Improved iPSC-CM Model System for studying the effects of Cav1.2 Mutations

- Used optical mapping to demonstrate that a hormone cocktail treatment which has been shown to improve maturation of iPSC-CM, provides a partial rescue of the iPSC-CM verapamil response.

UMBC
Baltimore, MD
Laboratory Aid
1/2018- 5/2018

- Main job was preparing agar plates for a lab that performs research on *Caenorhabditis Elegans,* a model organism. I also cleaned and organized the lab, as well as washed and autoclaved their glassware.

Undergraduate Research Associate
9/2017- 5/2018

- Worked for two different labs that are studying the fruit fly *Drosophila Melanogaster*, another model organism. I created and prepared the food for these flies in small vials, bottles or beakers. Both these labs are studying different aspects of the fly genome and depend on me to make and adjust the amount of food accordingly each week.

PROFESSIONAL EXPERIENCE

BioIVT
Baltimore, MD
Associate Scientist
9/2020-Present

- I work directly within the Products group in the production of hepatocytes, media, microsomes, and S9. Extensive experience with primary cell hepatocyte isolations

and cell culture specifically. Work in accordance to standard operating procedures but am also involved in optimizing SOPs as well as developing new ones. Maintain instruments and equipment logbooks. Assist with tasks necessary for product shipment, inventory control, and record keeping in the Products department. Maintain stock levels of internally utilized products. Handling of lab animals, performing small animal surgery, to include sacrificing and organ harvesting.

Assistant Scientist
6/2018-9/2020

- Isolated hepatocytes from several different sources. Also produced media, microsomes, and S9. Worked according to standard operating procedures and departmental policies.

SKILLS

Laboratory: Aseptic Technique, Cell and tissue culture, Primary cell isolations, Optical mapping and fluorescence imaging, Light microscopy, Animal Handling, Centrifugation techniques, DNA and RNA isolations, Fluorescent and Non Fluorescent staining, PCR-gel electrophoresis, Agar and plate preparation, Western blotting, Spin-column Chromatography, Spectrophotometry, Protein purification

Computer: GraphPad Prism, Rstudio, Microsoft Office

CONFERENCE AND SPEAKING

Graduate Research Conference
3/2021

- Gave a talk on what would eventually be my thesis, discussing Long QT type 8 and how cardiomyocytes derived from iPSC's are currently used to inconsistently model this disease. Also discussed that this system can potentially be improved by treating the cells to specific hormones.

VOLUNTEER EXPERIENCE

UMB
Baltimore MD
Graduate Student Association Representative
8/2020- Present

- I represent my program and act as a liaison to the Graduate School in order to properly communicate student concerns and ideas.

Abstract:

Cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) are a useful model system for studying cardiac diseases such as long QT syndrome (LQTS), a condition in which repolarization of the heart is stalled resulting in life-threatening arrhythmias. Mutations within the calcium channel $Ca_v1.2$ are known to cause LQTS, making iPSC-CMs a useful model system for studying the mechanism of this form of LQTS. However, iPSC-CMs are often immature and don't mimic adult heart cells in several ways, as their complement of ion channels seems to differ from adult CMs. Consequently, we found iPSC-CMs and adult CMs responses to calcium channel blockers such as verapamil, to not be comparable. Using optical mapping, we demonstrate that a hormone cocktail treatment which has been shown to improve maturation of iPSC-CM, provides a partial rescue of the iPSC-CM verapamil response. We therefore identified a method for improving the usefulness of iPSC-CMs in studying LQTS.

Generating an Improved iPSC-CM Model System for Studying the Effects of $Ca_v1.2$ Mutations

by
Olutomiwa Fadiran

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Master of Science
2021

Acknowledgements

I would like to thank Dr. Ivy Dick for her guidance throughout this entire project. She has been very patient with me and I learned so much from just being in her lab surrounded by like-minded and passionate lab mates. Frankly, it's been an eye-opening experience, and through the ups and downs I wouldn't trade it for anything else. This has been the first time that I've ever written something based on my own actual research at this level, and I feel lucky to have been given that opportunity.

Also, I would like to thank Elice Garcia-Baca for her patience with me as well. I may have been a little over my head juggling work, my personal life, and school at the same time but because of her I was able to push through everything and graduate on time. For that I am forever grateful, especially since COVID could have easily made my experience here significantly less fulfilling and more difficult than it turned out to be.

Of course, I would like to thank everyone in Ivy's lab: Debbie, Josiah, Daiana, Mora, Jack, and Kevin for being so welcoming and encouraging as well.

Also, thanks Mom and Dad. Love you very much.

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List of Abbreviations

AP	Action Potential
APD80	Action Potential Duration at 80% Repolarization
CCB	Calcium Channel Blockers
CV	Conduction Velocity
CM	Cardiomyocyte
iPSC	Induced Pluripotent Stem cells
LQTS	Long QT syndrome
TD	Triiodothyronine + Dexamethasone
TS	Timothy's Syndrome

Chapter 1: What is Long QT Type 8?

Long QT syndromes (LQTS) are inherited cardiac diseases that can be generally described by the prolonged QT phase shown on an ECG, which correlates with the unnatural lengthening of the depolarization phase of the cardiac action potential (AP). Ion channels control the cardiac AP and there are numerous mutations in those channels that can cause a change in the AP which can lead to LQTS. This is why there are so many LQTS types with similar symptoms but different causes. These symptoms include cardiac arrhythmias, seizures, syncope, and sudden death. LQTS is fairly common, affecting 1 in 5,000 to 1 in 10,000 people (Schwartz et al., 2009). Many patients may not even know they have it until it causes symptoms.

A rare form of LQTS called Timothy's syndrome (TS; Long QT type 8), is caused by mutations in the $Ca_v1.2$ channels, which are L-type voltage gated calcium channel (Splawski et al., 2004). In a normal AP these calcium channels open and calcium ions flow down their gradient into the cells, which corresponds with the 'plateau' phase of the cardiac AP. Gain of function mutations in $Ca_v1.2$ channels can cause the channel to stay open for too long and prolong this plateau phase for longer than usual which can cause the typical symptoms associated with LQTS. However, because these $Ca_v1.2$ channels are expressed throughout the body, the mutations result in a multisystemic disorder. Because of this, symptoms associated with TS are rather unique compared to other LQT diseases. Including cardiac arrhythmias and seizures, those with TS may also experience syndactyly, cardiac hypertrophy, immune deficiencies, autism, and other developmental delays (Splawski et al., 2005).

Typical treatments for TS include beta blockers, ion channel inhibitors, and most commonly implantable defibrillators (Keating and Sanguinetti, 2001). Beta blockers have been commonly used in other forms of LQTS as it slows down the heart, thus decreasing the risk of arrhythmias. However, in Timothy's syndrome, beta blockers have not been shown to be effective (Keating and Sanguinetti, 2001). Common ion channel blockers that have also been tried to treat TS, were Mexiletine and Ranolazine. They both inhibit persistent or late sodium inward current, which does shorten the AP and can also reduce arrhythmias. Both these drugs have been used in TS patients and have some efficacy in reducing the QT(Gao et al., 2013; Shah et al., 2012). However, the prognosis was still not favorable, as patient still suffered from fibrillation (Gao et al., 2013; Hermida et al., 2021; Shah et al., 2012). As a result, most patients with TS rely on the use of an implantable defibrillator, and this has been shown to be effective at shocking and resetting the heart during an arrhythmic episode (Keating and Sanguinetti, 2001; Walsh et al., 2018). An implantable defibrillator is a last resort though, and not an ideal treatment method. Overall, these different treatment options can address some of the symptoms of TS to varying degrees, but none are sufficient, nor do they target the underlying cause.

Another treatment option that could possibly address this treatment deficit are calcium channel blockers (CCBs). These drugs come in 3 classes, according to their chemical structure: dihydropyridines (DHP, ex: amlodipine, bepridil), phenylalkylamines (ex: verapamil), and benzothiazepines (ex: diltiazem) (McDonagh et al., 2005). DHPs like amlodipine or nifedipine are typically used to treat hypertension as they preferentially bind to $Ca_v1.2$ channels within vascular smooth muscle tissue (McDonagh et al., 2005). This selectivity is a result of differential expression of specific $Ca_v1.2$

isoforms within peripheral vascular muscle tissue as compared to the heart (Godfraind, 1998). On the other hand, phenylalkylamines like verapamil are more often used so to treat cardiac arrhythmias. As a result, verapamil has been tried as a treatment for TS. The idea is that limiting the calcium flow could return the AP back to a normal length and alleviate symptoms, similar to the mechanism of mexiletine and ranolazine, but with the added advantage of directly targeting the calcium channel which underlies the TS phenotype (Gao et al., 2013; Jacobs et al., 2006). Unfortunately, treatment of TS patients with verapamil, have not been proven to be very effective, and additional treatments and ultimately reliance on an implantable defibrillator were required. This can possibly be explained through the data acquired by my lab mate Moradeke Bamgboye, in which she demonstrates that TS mutations within the $Ca_v1.2$ channel can reduce the efficacy of verapamil on the mutant channel as compared to WT $Ca_v1.2$. This can explain the less-than-ideal response many TS patience have with verapamil. Thus, there needs to be more research done to identify novel treatment options for these patients. It would be invaluable to have a human disease model system that can effectively respond to drugs such as verapamil. In regards to TS, having a model system that accurately mimics the normal function of $Ca_v1.2$ in determining the AP morphology, would make it easier to study treatments and screen for different drugs. Stem cells represent one promising option for a model system.

Chapter 2: Induced Pluripotent Stem Cells as a Model System

Stem cells have always been a promising, albeit controversial field of study due to involvement of embryonic stem cells raising ethical concerns. However, in 2006, Takahashi and Yamanaka, made a breakthrough when they demonstrated that it was possible to induce adult fibroblasts back into a pluripotent state by introducing four transcription factors Oct4, Sox2, Klf4 and c-Myc. These factors were chosen because they were found to function in the maintenance of the pluripotency of embryonic stem cells (Takahashi and Yamanaka, 2006). Since then, induced Pluripotent Stem cells (iPSC) have been shown to be a promising model system. These cells can be differentiated into numerous cell types including cardiomyocytes (CM). Aside from avoiding past ethical dilemmas of stem cell research, this model system has important advantages. IPSC's are not only a human model system, but they also have the added benefit of being personalized to whomever the cells are isolated from.

But as with any model system, iPSCs have their disadvantages. Although gene expression analysis has revealed that cardiomyocytes derived from iPSCs (iPSC-CMs) expressed all the major cardiac ion channel genes found in the adult cardiac tissue, they differ in expression levels. Compared to iPSC-CMs, adult CMs express higher levels of calcium channels and other proteins which are known to be important components the cells Ca^{2+} cycling machinery that aren't highly expressed in iPSC-CMs. Ion channels such as the inositol trisphosphate and ryanodine receptors, and regulatory proteins like calsequestrin 2 and calreticulin (Itzhaki et al., 2011; Jung et al., 2012; Rao et al., 2013). Calcium ions plays an essential role in nearly every basic cardiac function, from excitation-contraction coupling (EC- coupling) to modulating gene expression, and it's

therefore highly regulated. A large part of this regulation is performed by the Ca^{2+} ion channels such as those mentioned here, and many more found in CMs. The disfunction, absence, or overabundance of any of these proteins can potentially lead to many diseases in adult CMs. So, a model system must be able to express the right complement of calcium channels, and ion channels in general, as they all play a role in proper cardiac function.

In addition to altered expression profiles, iPSC-CMS also do not display comparable morphology to adult CMs. iPSC's do not exhibit the brick-like functional unit that adult cardiomyocytes show, as they are more blob like (Fig. 1b). Particularly in early-stage culture, iPSC-CMs are smaller and exhibit an immature like structure more akin to fetal CMs (Itzhaki et al., 2011; Jung et al., 2012; Rao et al., 2013). They also lack transverse- tubules, which are invaginations in the cell membrane found in muscle cells and are a necessary component of excitation-contraction coupling. Lastly, *in vivo* CMs exhibit distinct electrophysiological properties, depending on where in the heart they developed from. Based on the AP morphology, CM's can be described as atrial, ventricle, or nodal-like (Ma et al., 2011). When iPSC's start to differentiate, the cells can exhibit a heterogenous mix of properties. This can be a disadvantage when trying to work with the cells, because they won't represent a single area of the heart.

Chapter 3: Improving iPSC-CM response to Verapamil

To that end, there have been various attempts to further differentiate, or mature, iPSC-CMs that have been proven successful. Electrical stimulation and mechanical stimulation, for example, both seek to mature iPSC's by mimicking the *in vivo* environment of adult cardiomyocytes. Ruan et al demonstrated that mechanical stress conditioning and electrical stimulation promote contractility and force maturation of iPSC cardiac tissue (Ruan et al., 2016). This method appears very promising; however, it is costly and may not be realistic for every lab. Another example is the use of 3-d culturing, which is a type of technique that allows the cells to interact with their environment in all dimensions, which again mimics *in vivo* heart development. This technique has also been shown to not only improve the morphology of the CM's but also the metabolic maturation state (Correia et al., 2018), since iPSCs also differ in its preferred energy source (primarily glucose and lactate, whereas adult CMs mostly break down fatty acids). The data for 3-d cultures is encouraging, however, the difficulty in maintaining and controlling these cultures has been challenging, especially when attempting to dissociate and replate the cells (Machiraju and Greenway, 2019). Next, is the use of hormone treatment. Triiodothyronine (T3+dex or TD), is a thyroid hormone and Dex, or dexamethasone, is a synthetic corticosteroid hormone. These hormones are critical for normal heart development *in vivo*, and literature suggests that adding T3 and Dex during iPSC-CM maintenance increases cardiomyocyte size, anisotropy, and sarcomere length (Yang et al., 2014). Insulin treatment is also commonly used in tandem with TD, as it has been established that insulin significantly improves the expansion of iPSC colonies and cellular proliferation (Shahbazi et al., 2019). For this study, we chose

to use TD as a method for maturing the cells due to the promising results shown by others, higher throughput, and lower cost as compared to other methods.

We demonstrate that iPSC-CMs without TD don't respond to verapamil in the same way as an adult CM. Given what we know about verapamil's mechanism of action *in vivo*, the AP of a CM should shorten in response to increasing concentrations of the drug. But rather than shortening the AP, iPSC-CMs either do not respond at all to the addition of low-dose verapamil, or the cells lose the ability to fire an AP entirely. We indicate that by maturing the cells through the addition of TD, the verapamil response is improved. IPSC-CMs cultured with TD display a dose-dependent shortening of the AP, without the unusual loss of AP firing seen in iPSC-CMs cultured without TD. Through this we infer that maturing iPSC-CMs using TD can potentially normalize the response of the cells to CCBs.

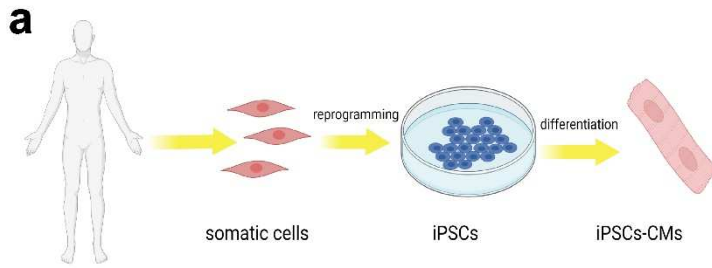
Chapter 4: Results

Using iPSC-CMs as a model system for $Ca_v1.2$ channelopathies has been challenging due to common problems associated with iPSC immaturity. Here, we address the efficacy of maturing iPSC-CMs, by culturing them with the addition of TD. IPSC-CMs were cultured until they were 90-95% confluent, then differentiated using a CHIR based protocol adapted by lab manager Debbie DiSilvestre. Insulin treatment has always been a component of the differentiation process in our lab, so all iPSC-CMs were receiving this regardless of TD status. Selected iPSC-CMs then received TD treatment after around 20-22 days (Fig. 1 a,b). The cells were grown on a coverslip as a monolayer, which was critical, as these cell-to-cell interactions mimic *in vivo* cardiomyocyte syncytia (Fig. 1b).

The iPSC-CMs were stained with Di-4 ANNEPS (4-(2-(6-(dibutylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl)-pyridinium), a fluorescent dye that is sensitive to changes in voltage. The cells were imaged using a SciMedia optical mapping system featuring a high speed MiCAM03 camera (Fig. 1c). Stained cells were excited using a high-powered green LED filtered at 531 nM (+/- 25 nm). Increases in membrane voltage of the iPSC-CMs due to the initiation of an AP corresponded to a decrease in intensity of the Di-4-ANNEPS. The resulting fluorescence signal was analyzed using the software BV workbench by SciMedia.

We used point stimulation, to pace our iPSC-CMs at a constant frequency. This involved stimulating a small population of cells at the edge of the coverslip and allowing the signal to propagate through the electrically connected cells That allowed us to properly visualize the propagation of the AP across the monolayer. The added benefit of

a



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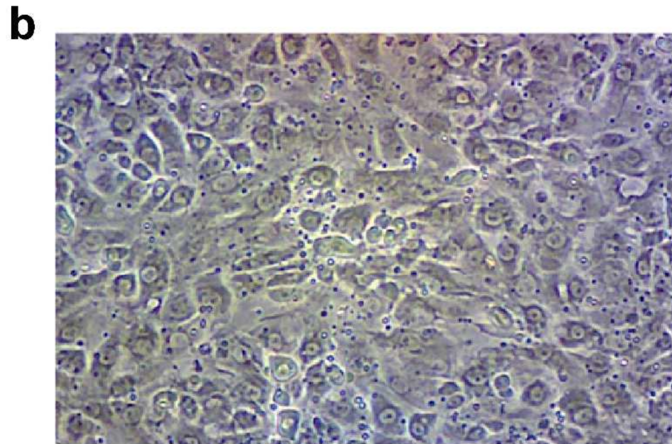

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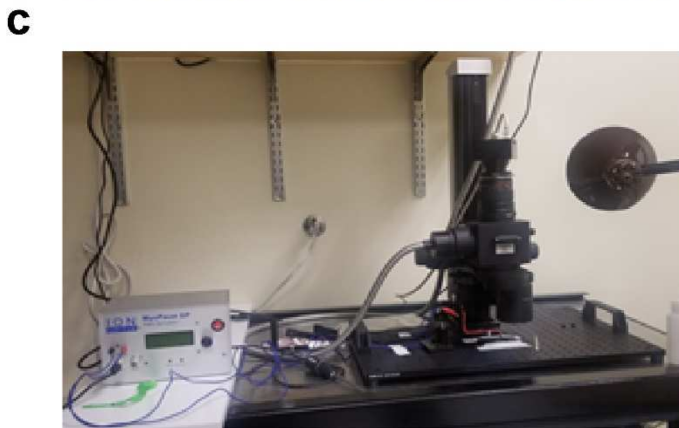

Figure 1: iPSC differentiation protocol and Optical mapping setup. a) IPSC's are grown and differentiated into cardiomyocytes (CM's) using CHIR and IWR, and treated with TD after day 20. b) Example of iPSC-CMs grown on a monolayer done in our lab. c) Custom optical mapping set up with stimulator on the left and MiCAM03 camera on right

point stimulation is that it more accurately models *in vivo* stimulation, where initiation and propagation of the AP starts at a single point in the heart, the sinoatrial (S/A) node.

The iPSC-CMs were first imaged without any stimulation to determine if they could spontaneously initiate an AP. This was a quality control measure, to ensure every coverslip contained electrically active iPSC-CMs. Then the cells were then paced using an Ion-optics electrical stimulator at 0.5hz. Because the frequency of pacing can change AP duration (APD), we chose a single pacing frequency for all experiments. An activation map was generated to illustrate the direction of propagation, by indicating the time at which each point on the coverslip initiates an AP (Fig. 2a). We show here an exemplar coverslip with smooth propagation starting at the point of stimulation on the left side (Fig. 2a). This shows that iPSC-CMs can generate an AP response that spreads evenly across the coverslip over time.

$Ca_v1.2$ channels play a major role in the cardiac AP morphology, and are largely responsible for the plateau phase. These APs can be viewed in our iPSC-CMs by plotting the change in intensity of the fluorescent signal (Fig. 2b). Early stage cultured iPSC-CMs have been shown to have differing AP morphology compared to adult CMs, indicating that the ion channel complement of these myocytes may not be fully mature. However, there is evidence that suggests maturing iPSC-CMs can improve this (Kim et al., 2010; Zhang et al., 2009). Therefore, we need to see how maturing iPSC-CMs, by adding TD, affects the shape of the AP. In particular we focused on the AP durations (APD), as this is a direct correlate to the QT interval measured by ECG in patients. We therefore took the average action potential duration at 80% repolarization (APD_{80}) of the cells across the coverslip. The APD_{80} showed no significant changes between iPSC-CMs with and

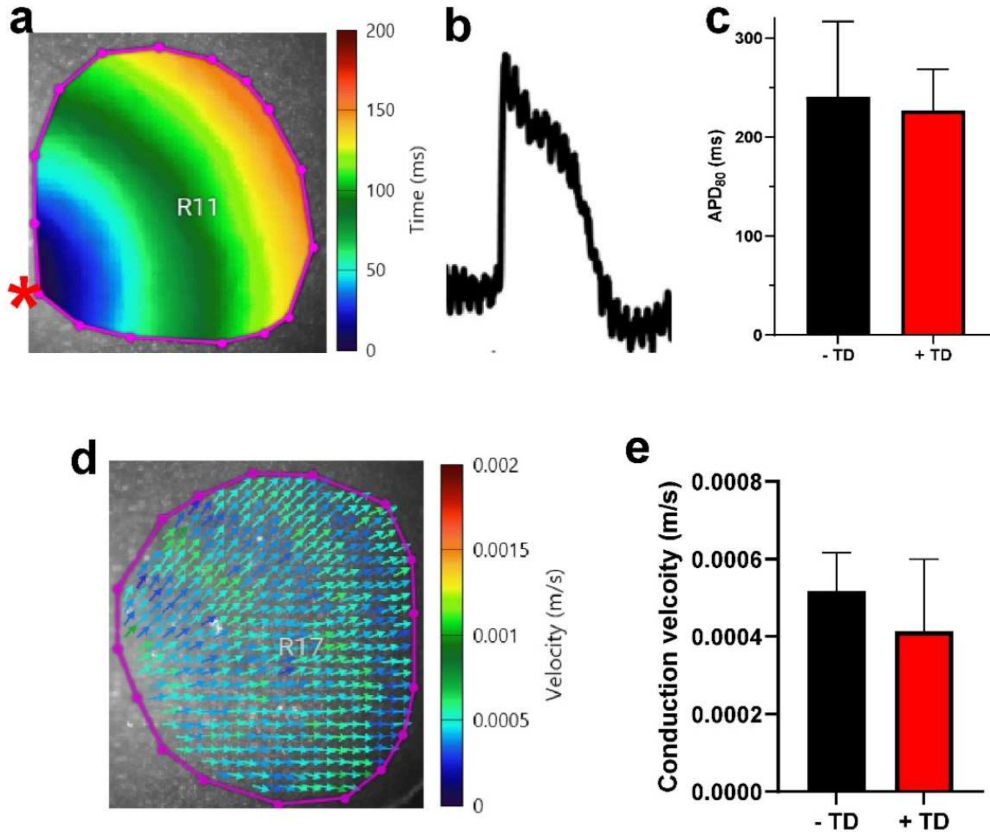

Figure 2: Activation and conduction velocity map, average APD_{80} and Conduction velocity -/+ TD. a) Activation map of iPSC-CM's with the locus of point stimulation (25V) indicated by the red asterisk. The different colors are indicative of different points in time, with blue being the earliest and yellow being latest, as shown on the scalebar on the right. b) Example of an optically recorded AP. c) Average APD_{80} of cells without and with TD (n=6). Without TD is in black and with TD is in red d) Conduction velocity map of iPSC-CM's with the locus of the point stimulation (25V) indicated by red asterisk. The different colors indicate speed of propagation, with blue being the slowest and red being the fastest as indicated by the scale bar on the right. e) Average conduction Velocity of WT cells without and with TD (n=6). Without TD is in black and with TD is in red. Using an unpaired t test indicated that the differences are insignificant for both average APD_{80} and conduction velocity ($p>0.05$)

without TD (Fig. 2c). So, TD did not appear to significantly alter the APD of iPSC-CMs in our system.

We next measured the conduction velocity (CV) maps of the iPSC-CMs. Mapping the CV onto the coverslip shows the, directionality of AP propagation (similar to an activation map), while also indicating the overall speed of the propagation throughout the monolayer (Fig. 2d). In the exemplar shown, the velocity of the propagation down the monolayer is uniform and smooth. The CV at which an AP propagates through the heart is an important electrophysiological property of adult CMs and $Ca_v1.2$ channel disfunction would affect this. Previous data indicated that iPSC-CMs displayed a slower CV than seen in adult CMs (Denning et al., 2016). We therefore tested whether maturing iPSC-CMs through the addition of TD, would alter CV. We compared the average CV of the iPSC-CMs treated with and without TD and found no significant difference between the two (Fig. 2e). Therefore, in our experiments treating the iPSC-CMs with TD did not affect the CV.

Next, we aimed to assess the potential differences in iPSC-CM CCB response, with or without the addition of TD. We chose to evaluate the CCB verapamil, due to its history in the treatment of TS. We therefore applied variable concentrations of verapamil and stimulated the cells in order to visualize any difference in the APD (Fig. 3a). From what is known about verapamil in adult CMs, the APD should gradually shorten as doses of verapamil increase. However, we actually found that a significant number of coverslips containing iPSC-CMs without TD no longer displayed a signal after the addition of 0.3μM of verapamil (Fig 3c). Moreover, the coverslips that did display a signal, showed no statistical difference between the APD_{80} before and after a 0.3μM dose

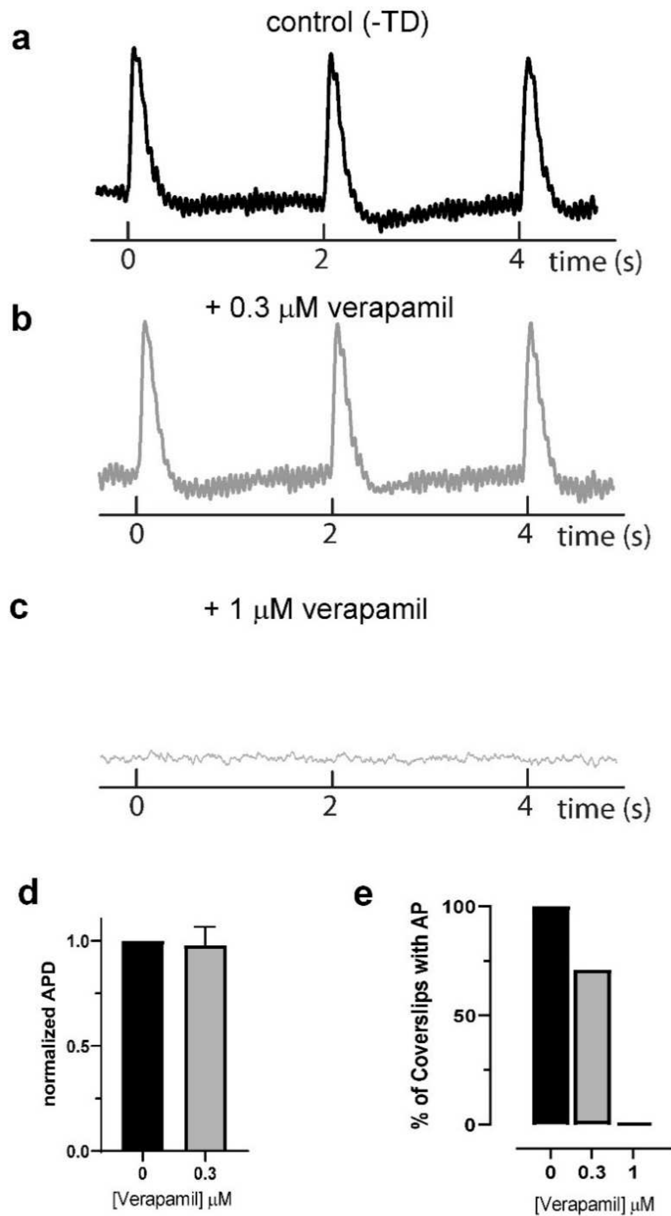

Figure 3: Lack of appropriate verapamil response in iPSC-CMs cultured without the use of TD. a) Exemplar APs of iPSC-CMs without TD paced at 1hz, prior to the addition of verapamil. b) Exemplar APs of iPSC-CMs without TD paced at 1hz following the addition of 0.3μM verapamil. c) iPSC-CM without TD paced at 1hz display a complete loss of AP signal after the addition of 1μM of verapamil. For A-C, the X axis is the time and the Y axis is the relative change in fluorescence intensity. d) iPSC-CMs without TD shows no statistical difference -/+ verapamil dose. (n=6) e) Histogram showing the percentage of iPSC-CM monolayers cultured without TD that display any signal following the addition of the indicated dose of verapamil (n=6).

of verapamil. (Fig. 3d). Virtually none of the cells displayed a signal at 1μM (Figure 3e). Taken together, this is an unexpected response to a CCBs and we believe it could indicate a lack of maturity in iPSC-CMs. Moreover, this presents a major challenge to studying $Ca_v1.2$ channels in iPSC-CMs. We therefore considered whether or not maturing the cells using TD would improve this response in any way.

Indeed, addition of TD caused a marked improvement in the response of the iPSC-CMs to verapamil. In the exemplar shown, the AP of iPSC-CM's treated with TD were shortened after a 0.3μM doses of verapamil (Fig. 4 a,b). On average, we observed a more graded decline in APD_{80} in iPSC-CMs with TD, with 1μM TD further reducing the APD_{80} (Fig. 4c). This response to verapamil dosage is aligned more with what we would expect in adult CMs. Adding to this, the fact that virtually all cells were able to initiate an AP at 1μM (Fig. 4d); *versus* none for the iPSC-CMs without TD (Fig. 3e), demonstrate a clear improvement in the iPSC-CM response to this CCB. These findings show that maturing iPSC-CMs using TD can affect their overall response to verapamil, which therefore increases their usefulness as a model system to study calcium channels.

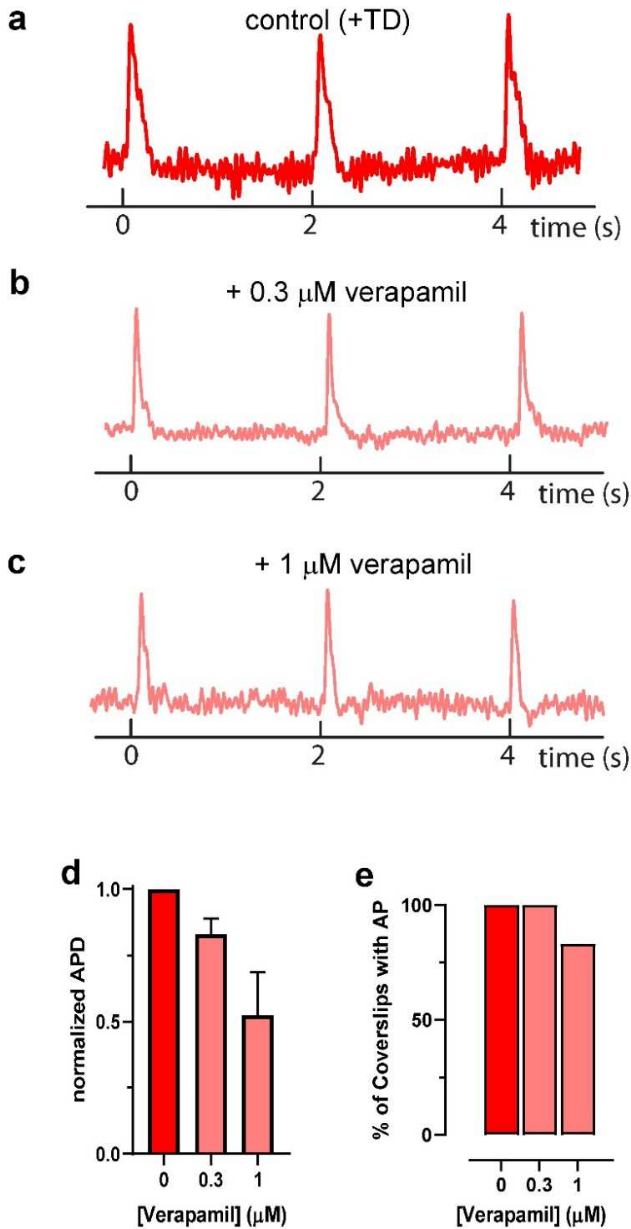

Figure 4: Rescued verapamil response in iPSC-CMs cultured with the use of TD. a) Exemplar APs of iPSC-CMs treated with TD paced at 1 Hz, prior to the addition of verapamil. b) Exemplar APs of iPSC-CMs with TD paced at 1 Hz following the addition of 0.3μM verapamil. C) iPSC-CM with TD paced at 1 Hz display a shortening of the AP after the addition of 1μM of verapamil. For A-C, the X axis is the time and the Y axis is the relative change in fluorescence intensity. d) iPSC-CMs treated with TD show a dose-dependent shortening of the APD after the addition of verapamil. (n=6) e) Histogram demonstrating that iPSC-CM monolayers cultured with TD consistently produce an AP following the addition of different doses of verapamil(n=6).

Chapter 5: Discussion/Conclusion

Proper $Ca_v1.2$ channel function is important for numerous biological processes, like hearing, learning and memory. It's also critical for heart health, as these channels play major roles in cardiac functions, such as calcium induced calcium release, EC coupling, and the regulation of gene expression. Understanding the basic mechanisms of $Ca_v1.2$ channel function and disfunction is imperative. The ability to accurately model calcium channelopathies, such as Timothy's syndrome, *in-vitro* is the first step to that end. Although Timothy's Syndrome is a rare form of LQTS it has a rather high mortality rate compared to other forms (Keating and Sanguinetti, 2001). And in general, cardiac arrhythmias are a leading cause of morbidity and mortality, accounting for nearly 15-20% of deaths worldwide (Srinivasan and Schilling, 2018). So, having an accurate model system is imperative, and any viable steps to improve that system should be considered. The addition of TD to the iPSC-CM cultures significantly improved the overall response of the cells, particularly in terms of normalizing the effect of the CCB verapamil on the cardiac AP.

Interestingly though, there is established literature demonstrating that the additional electrical properties of iPSC-CMs should change when maturation methods such as TD, and this includes an increase in conduction velocity and a decrease in APD (Pre et al., 2014; Zhu et al., 2017). So, the fact that we showed no statistically significant difference in conduction velocity or average APD_{80} between iPSC-CMs with or without TD was unexpected. But this may be because the sample size was rather small, and the differences between the two weren't large enough to make up for this.

There was, however, a difference in the response to verapamil. We found that iPSC-CMs without TD do not react to verapamil in an expected way as it was difficult to even collect data from them after a 1μM dose, which is well below the normal IC_{50} of this drug. Of course, given a high enough dose, verapamil will block any AP from CMs. But at very low concentrations the AP of CMs should shorten, and the AP of iPSC-CM without TD was unchanged at 0.3μM verapamil, yet it was eliminated by 1μM verapamil. IPSC-CMs with TD, on the other hand, illustrated a more graded response to verapamil similar to adult CMs, with nearly every coverslip displaying a signal after a 1μM dose of verapamil.

It's important to remember, that iPSC's have differing expression levels of essential proteins than those found in adult CMs. There is data that demonstrates that several methods deployed to improve the maturation of iPSC-CMs, also enhanced key cardiac structural markers, and increased ion channel expression (Lundy et al., 2013; Oh et al., 2016). So it seems that the expression levels of ion channels in iPSC-CMs change as they mature. It is possible, that the responses to verapamil in iPSC-CMs without TD, compared to the expected adult CM response, can be explained by differing expression levels of critical channels involved in an AP. Sodium, calcium, and potassium channels are primarily responsible for the initial upstroke, plateau, and repolarization phases of a cardiac AP respectively. Since it seems that the iPSC-CMs without TD are having trouble initiating an AP, a lack of the appropriate amount of sodium channels may be the culprit. Calcium channels in iPSC-CMs could be playing a larger role in initiating the AP in place of sodium channels. Therefore, when the cells are given verapamil, with even a small amount of concentration, the AP is blocked before it can even start. This is

consistent with prior studies in which maturation of iPSC-CMs increased the expression of voltage gated sodium channels. Thus, TD may be slightly affecting ion channel expression, that is leading the cells to express their proteins in a manner closer to adult CMs.

Just treating cells with TD may not be enough to push iPSC-CMs to their full potential. However, combined with methods shown in other studies that suggest using electrical or mechanical stimulation, it can be possible to further improve iPSC-CM maturity. Future aims can consider using these methods in tandem with one another. iPSC's are a useful model system that shows an exciting degree of promise. As the ability to further mature the cells is improved, their usefulness as a model system will grow.

Chapter 6: Materials and Methods

1. **iPSC differentiation and TD treatment:**

   (Protocol adapted by Deborah DiSilvestre) Day 0: Cells should be 90-95% confluent.1) Wash cells 1x with DPBS.2) Replace media with 6µM CHIR in RPMI/B27(-) w/o Insulin (ex.25 ml of media gets 15µl of10 mM CHIR). Use 3 mL per6-well well. Day 1: Do Nothing. Exactly 24 h after CHIR treatment cells may be peeling off plate. Day 2:1) Wash cells 1x with 0.5 mL DPBS.2) Feed cells with2 mL RPMI/B27(-). Day 3:1) Wash cells 1x with DPBS.2) Replace media w5 uMIWR1in RPMI/B27(-) w/o Insulin (ex.25 ml of media gets 12.5 µL of10 mM IWR1.) Use 3 mL per 6-well well. Day 4: Do nothing. Day 5:1) Wash cells with PBS.2) Feed cells with3ml RPMI/B27(-). Day 6: Do nothing. Day 7 and on:1) Wash cells PBS.2) Feed cells with 3ml of RPMI/B27(-) w/o Insulin.3) Check wells for beating cardiomyocytes. After Day 7:Feed every 2 days and watch for beating iPS-CMs. Cells were treated daily with TD after Day 20 and 22

2. **Optical mapping**

   Coverslips with beating iPSC-CMs are transferred to a petri dish and stained with 10µL of Di-4-ANNEPs mixed with ~900µL of RPMI medium. To avoid photobleaching, the cells were covered in aluminum foil and taken to a dark room to be optically mapped. Cells were left to stain for 15 minutes and then washed with Medium 199 mixed with 1mg/ml of 2,3- Butanedione monoxime to avoid movement artifacts. An *Ion Optix MyoPacer EP* field stimulator was used to control the voltage and frequency (hz) and iPSC-CMs were first stimulated at an input 20V and increased by 5 if they weren't successfully paced. The cells were

imaged using a SciMedia optical mapping system featuring a high speed MiCAM03 camera.

3. **Data collection and Statistics:**

   Graphpad was used for all graphs and statistical analysis. BV workbench by SciMedia was used to make all activation and conduction velocity maps as well as APD_{80} and average conduction velocity. APD_{80} and CV were taken as the average within a region of interest that displayed robust signal. The APs were subjected to gaussian filtering, drift removal, and mean filters prior to quantification using the BV workbench software.

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