Simulation-Based Study of Control Strategies for Beating of Human Cardiomyocyte Cultures

Antti-Juhana Mäki, Jussi T. Koivumäki, Jari Hyttinen, and Pasi Kallio, *Member, IEEE*

***Abstract*— Many biological systems, such as cell cultures *in vitro*, are complex, non-linear, and time varying, resulting in challenges for the development of effective control methods. Therefore, it is beneficial to develop simulation environments to understand dynamics of these biological systems and, for example, compare different control strategies. In this paper, we created a mathematical model to simulate temperature-dependency of beating human cardiomyocyte cultures. The developed system can be used for comparing different closed-loop control strategies and for studying how sensitive the beating of cardiomyocytes is for temperature variations. We employed the system for comparing the performance of fuzzy logic-based and traditional Proportional-Integral controllers and presented, how their performances varied in different cell culture cases. Our results indicate clearly, how the fuzzy logic-based controller can outperform the traditional Proportional-Integral controller when they are implemented to control the beating of cardiomyocyte cultures.**

***Note to Practitioners*—This paper was motivated by the challenge of creating controllable engineered living materials. For this reason, we created a simulation environment to help the development of these materials, thus to reduce the discovery time. As a demonstration, we showed in this paper how the developed simulation environment can be used to design and compare different controller strategies, such as the traditional Proportional-Integral-Derivate controller and controllers based on fuzzy-logic. In the paper, we implemented different controllers and cardiomyocyte environments to study how beating of cardiomyocyte cultures could be controlled in an optimal way *in vitro*. We show, how fuzzy-logic controllers outperform the traditional PI-controller when applied to a highly non-linear and time-varying biological system.**

***Index Terms*— Fuzzy logic, simulation environment, cardiomyocytes, beating rate control, temperature.**

I. Introduction

ENGINEERED living materials are biohybrid composites that integrate living and nonliving components together. The underlying idea in this emerging field of technology is to combine the advantages of both components, providing new material features and functions. For example, living robots using biohybrid actuators can be developed. Depending on the size, these living robots could be powered by, for instance, muscle cells on a larger scale, or bacteria at small dimensions [1]. As a demonstration of the potential of this approach, Fu et al. developed an artificial butterfly by patterning living cardiomyocytes on a butterfly-shaped, soft hydrogel film, resulting in a film that swinged its wings in the cell culture medium [2].

To be able to provide a controlled actuation function, the living biological component should be controlled. When working with living biological systems, several control challenges originating from their non-linear, complex, and time-varying behavior are confronted [3, p. 290]. For example, we have previously studied beating cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSCs) and showed that the beating rate of hiPSC-CMs has a non-linear response to temperature [4], [5]. The non-linear, complex and time-varying behavior limits the implementation of traditional control strategies in biological processes. Another common problem with these traditional controllers is that when the controller is tuned for performing well at a certain operating point, the same controller may destabilize the highly non-linear system at another operating point [3, p. 255]. Furthermore, there is typically a high variation in biological systems; for example, the spontaneous beating rate of hiPSC-CMs has been reported to range from 21 to 52 beats per minute [6], resulting in significant challenges to the traditional controllers. Therefore, researchers have studied fuzzy logic to control biological systems, as this approach does not require a precise mathematical model of the system [7], [8], [9], [10], [11]. Furthermore, fuzzy control allows for an easy way to eliminate the control response to the small transient-level changes by implementing a dead zone on the controller [12, p. 458]. Moreover, to develop successful engineered living materials and functional control strategies for them, it is crucial to study the cellular behaviors and controller performance under different spatiotemporally varying parameters [1]. Due to the high cost and time-consuming experiments of *in vitro* wet lab experiments especially with human cell models, computational models and computer simulations provide a fast and cost-efficient means for developing and comparing control strategies for such cellular systems as beating cardiomyocytes.

To provide functional control strategies for cardiomyocyte-based living biological actuators,

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we implemented an *in silico* simulation environment to study how to control the beating rate of hiPSC-CMs in closed-loop using temperature-based actuation. By controlling the beating rate of the cardiomyocyte, it will be possible to use the cardiomyocyte culture as a living biological actuator in the future. Furthermore, we demonstrate in this paper that the implemented simulation environment can be used for designing and comparing different control strategies, such as a fuzzy logic controller and a more traditional Proportional-Integral (PI) controller. Compared to *in vitro* cell culture experiments, our *in silico* simulation environment allows for faster and cheaper comparison of different controller methods and tuning them to be used with specific cell variants as will be demonstrated.

II. Methods

Our goal was to develop a model-based *in silico* simulation environment to investigate the beating behavior of hiPSC-CMs and to compare different strategies to control the beating rate of the hiPSC-CMs by varying the cell culture temperature.

The *in silico* simulation environment consists of four mathematical models illustrated in Fig. 2. The *Controller model* compares the set-point and the measured beating rate and creates a control signal based on the difference. The *Temperature model* provides time-dependent temperature on the cell culture. The *Cardiomyocyte beating model* estimates the beating rate of the hiPSC-CMs based on the modeled cell culture temperature and the *Measurement model* mimics the image-based measurement of the beating.

To be able to extend our research to living hiPSC-CMs in the future, the *Temperature model* and the *Measurement model* were implemented to describe our *in vitro* cell culturing system presented earlier [4] and [5]. Briefly, the system shown in Fig. 1 consists of a cell culture device with a gas supply to provide a desired gas environment for the culture, a heating plate (a 49 × 49 × 1 mm^3 glass plate coated with an electrically conductive indium tin oxide layer (ITO) on one side of the plate), a temperature sensor plate (TSP) to monitor temperature on the cell culture area, and a microscopy system, all made in-house. To measure the beating rate of the CMs (in beats per minute, BPM), a non-invasive video image-based method is used [5]. The *Cardiomyocyte beating model* estimates the beating rate of cardiomyocytes in a given temperature. It is based on previously published models that describe cardiomyocyte ion dynamics [13], [14]. More detailed information about the implantation in the simulation environment is given in Cardiomyocyte beating model section.

In the *Controller model*, we implemented three different controllers; a traditional PI-controller and two fuzzy logic controllers: *fuzzy complex* and *fuzzy simple*. A block diagram of the system using the *fuzzy complex* controller is illustrated in Fig. 2. In the block diagram, r is the set-point (in BPM) providing the desired beating rate set to the controller, e is the error (in BPM) between the set-point r and the currently measured beating rate ym (in BPM), ym_prev is the previously measured beating rate (in BPM), dy is the current beating rate change $ym - ym\_prev$, y is the real beating rate (in BPM), u is output of the controller (in °C) that describes how many

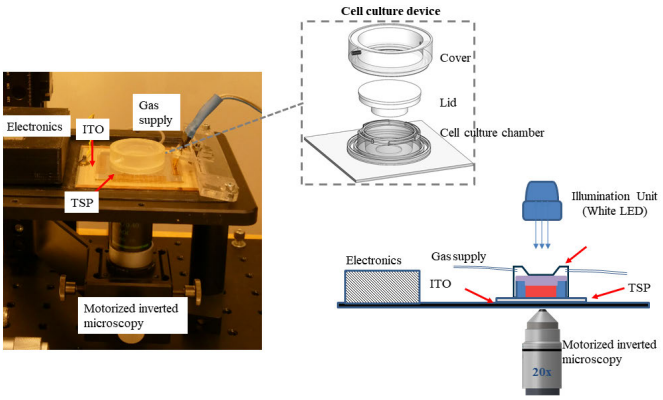

Fig. 1. A long-term cell culture system modeled in this paper (adapted from [4]).

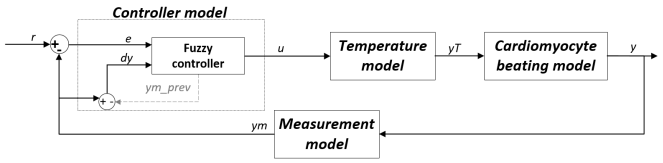

Fig. 2. A block diagram of the developed simulation environment using fuzzy controller.

degrees of Celsius the heater temperature should be changed, and yT is the current cell culture temperature (in °C). Next, each block is described in more detail.

A. Controller Model

In this study, we designed three different controllers as mentioned previously. Here, we explain in more detail each of them, starting from the designed fuzzy controllers before presenting the traditional PI-controller implemented in this paper.

In fuzzy controllers, the control strategy is based on the difference between the desired and measured beating rates, which the controller converts to the required temperature change u of the heater. For example, if the beating rate is lower than desired, the controller will increase power on the heater, as the beating rate increases with rising temperature [4]. However, as the beating rate response to the temperature change is non-linear, the controller needs to be carefully designed to perform correctly. We implemented Mamdani-type fuzzy controller [15] that includes three main parts. In the first part, which is called fuzzification, crisp information (including set-point, measurement, and error signals) is converted to fuzzy representation. Then, fuzzified information is processed using rule-based control (IF-THEN rules) and the outputs of each rule are combined into a single fuzzy set (aggregation). Finally, this information is defuzzified to crisp information based on the defuzzification method [10], [11], [16]. Two fuzzy controllers were designed. Figure 3 shows the block diagram of the *fuzzy complex* controller. Fuzzy Logic Toolbox in MATLAB and Simulink environment (Version R2018b, The MathWorks, Inc., Natick, MA, USA) were used to design the fuzzy controllers and to implement the closed-loop system simulations, respectively.

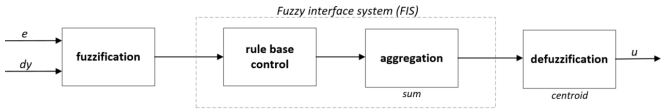

Fig. 3. A block diagram of the fuzzy controller used in this study.

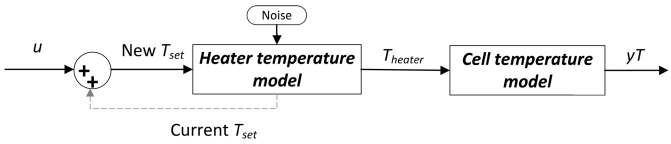

Fig. 4. Block diagram of *temperature model*.

In the *fuzzy complex* controller, two input signals, the error e and the change in the output dy, were chosen. The change in the output was used instead of the change in the error to minimize a possible derivate kick that can happen when there is an abrupt change in the reference signal [17]. For fuzzification, we used trapezoidal and triangular shapes as membership functions; the trapezoidal shape was used at the extremes, where lower sensitivity was required. For example, if the measured beating rate is far away from the desired beating rate, a larger temperature change and thus a smaller sensitivity is used. On the other hand, the triangular shape was used between the extremes to provide finer temperature tuning when the measured BPM is close to the desired value. We also implemented a dead zone in the membership function; when the measured beating rate is close ($\pm$0.5 BPM) to the desired value and the output change is close to zero, no control action is needed. With this, we minimize the control response to small transient-level changes [12, p. 458] in the beating rate, which are typical for *in vitro* hiPSC-CMs cell cultures. For aggregation and defuzzification, we used sum and centroid methods, respectively. The employed membership functions for *fuzzy complex* are presented in Fig. S1.

To study more comprehensively fuzzy control options, we also developed a simpler controller, named as *fuzzy simple*, that solely used the error signal e as an input, and where, therefore, the change in the output value did not affect the control output. The employed membership functions were similar to *fuzzy complex* shown in Fig. S1 (a) and (c). As this simpler controller requires a smaller number of tuning parameters, it could significantly shorten the time-consuming determination of the control rules [8]. With this we examined, how well the simpler controller performs compared to one with a more complex logic in different simulation conditions. Control surfaces of both fuzzy controllers are shown in Fig. S2.

For comparison, we also designed a traditional PI-controller. In this case, only the error signal e was used as an input. This controller can be mathematically presented in the time domain as

$$u(t) = K_p e(t) + K_i \int_0^t e(\tau) d\tau \tag{1}$$

where K_p and K_i are non-negative coefficients of proportional and integral terms, respectively. As the purpose of the paper is to compare different controller strategies and study how robust they are in the considered cell application, we tuned the PI-controller only for normally beating cardiomyocytes and used the obtained values, $K_p = 0.5$ and $K_i = 0.35 \times 10^{-3}$, in all simulations. Thus, the controller parameters were obtained from the response of the normal *Cardiomyocyte beating model* at 37°C (see Fig. 6 and its explanation for more detailed information). Additionally, we used an anti-windup method to reset the initial value of the integrator part to a pre-defined value if it grows too large [18].

TABLE I

STATE-SPACE MODEL VALUES FOR THE *Heater* AND THE *Cell Temperature* MODELS

Parameter	*Heater*	*Cell*
a_{11}	-0.024	-0.002
a_{12}	0.017	-0.001
a_{21}	-0.029	0.017
a_{22}	-0.037	-0.082
b_1	0.001	6.95×10^{-5}
b_2	0.002	0.005
c_1	28.510	246.8
c_2	0.200	-0.104

B. Temperature Model

The *Temperature model*, shown in Fig. 4, consists of an input signal (a sum of the controller output signal u and the current temperature set-point value T_{set} for the heater) and two temperature models: for the heater and the cell culture, respectively. The *Heater temperature model* estimates the dynamics of the heater temperature based on the current temperature and the input T_{set} value. The *Cell temperature model* estimates the temperature in the cell culture (yT) based on the current cell culture temperature and the heater temperature T_{heater} that is provided as an input to the model.

The heater temperature model and the cell temperature model are developed using the System Identification toolbox in MATLAB and previously measured temperature data [4]. Explicit, time-invariant, second-order state-space single-input, single-output, models include the state vector $\mathbf{x}(t)$, input $u(t)$, and output $y(t)$. The state-space models have the following structure

$$\begin{aligned} \dot{\mathbf{x}}(t) &= \mathbf{A}\mathbf{x}(t) + \mathbf{B}u(t) \\ y(t) &= \mathbf{C}\mathbf{x}(t) + \mathbf{D}u(t) \\ \mathbf{A} &= \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix}, \quad \mathbf{B} = \begin{bmatrix} b_1 \\ b_2 \end{bmatrix}, \quad \mathbf{C} = \begin{bmatrix} c_1 & c_2 \end{bmatrix}, \quad \mathbf{D} = \begin{bmatrix} 0 \end{bmatrix} \end{aligned} \tag{2}$$

where matrices $\mathbf{A}$, $\mathbf{B}$, $\mathbf{C}$, and $\mathbf{D}$ are a state matrix, an input-to-state matrix, a state-to-output matrix, and a feedthrough matrix, respectively [19]. The feedthrough matrix being zero implicates that there is neither direct nor linear relationship between input and output signals. Constants a_{11}, a_{12}, a_{21}, a_{22}, b_1, b_2, c_1, and c_2 were defined using System Identification toolbox and are given in Table I.

Using the parameters given in Table I, we compared the measured and simulated heater and cell culture temperatures. The accuracy of both models was over 95% with a temperature error typically smaller than 1°C. The error in the temperature model refers to how closely the model can capture the dynamics of the temperature observed in a real heat plate of a cell culture system. Results from these models are presented in

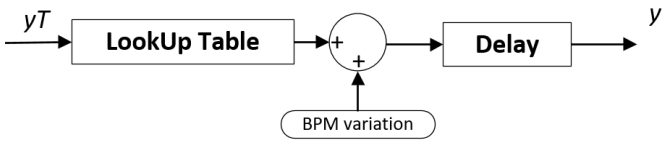

Fig. 5. Block diagram of the *Cardiomyocyte beating model*.

Fig. S3 and S4 for the heater and cell temperature models, respectively.

C. Cardiomyocyte Beating Model

The *cardiomyocyte beating model* was used for estimating the beating rate of cardiomyocytes in a given cell culture temperature. The model, shown in Fig. 5, consists of LookUp Table, delay, and randomized noise that represent a typical beating rate variation of hiPSC-CMs in a stable environment. In the simulator, we defined the typical beating rate variation to 0.5 BPM based on our previous results, where the measured variation was between 1-10% of the average beating rate at a constant temperature [4]. In addition, we estimated a delay between a temperature change in the cell environment and a change in the beating as three seconds based on previous studies of cardiac adaptation time constant [20], [21].

To obtain the LookUp Table for beating rate vs. temperature, we employed an established ordinary differential equation-based model that describes cardiomyocyte ion dynamics [13] that was more recently extended to replicate the electrophysiological phenotype specific for isolated hiPSC-CMs [14]. The hiPSC-CM model, including more than 100 differential equations, is computationally heavy to solve, and therefore it was not included in the developed Simulink model of the control environment. Instead, we first ran simulations with the hiPSC-CM model to get steady-state beating rates at different temperatures for a single cell. As our controller simulations describe an environment with cell clusters, in which the system outputs are spatial averages of single cell behaviour, we fitted curves to the simulated single cell beating rate results, using a fourth-degree polynomial function as shown in Fig. 6, and saved these fitted values at different temperatures to LookUp Tables. In our simulations employing the whole control environment, the beating rate was obtained from the specific LookUp Table for the given cell culture temperature and the used *Cardiomyocyte beating model* described next.

To study how our controller methods work with slightly differently beating cell models, in addition to the model developed in [14] (named as normal), we implemented three cell model variants: long-QT, supra-CC, and infra-CC. In the long-QT model variant, conductance of the delayed rectifier potassium current, I_{Kr}, was reduced 10% of the original value in [14]. In the supra-CC and infra-CC model variants, the maximum rate of the sodium-calcium exchanger was increased and reduced, respectively, by 10%, so that the so-called calcium clock is either supra or infra active, respectively. Relationships between the beating rate and the temperature from these four models are illustrated in Fig. 6.

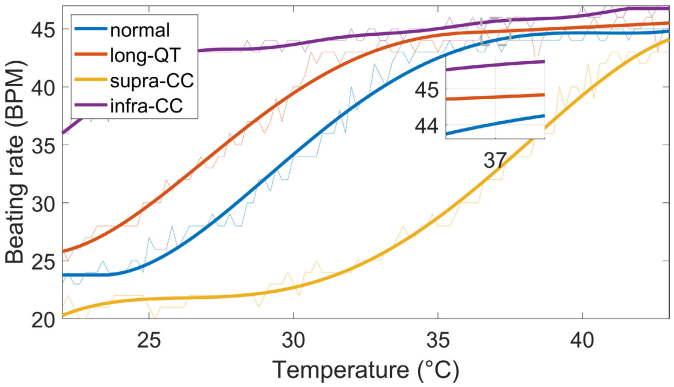

Fig. 6. Simulated stationary beating rate response to temperature with four different models; single hiPS-CM cell models (dashed lines) and their fitted values used in the simulator (solid lines). An insert marked with a dashed rectangle shows responses around 37°C.

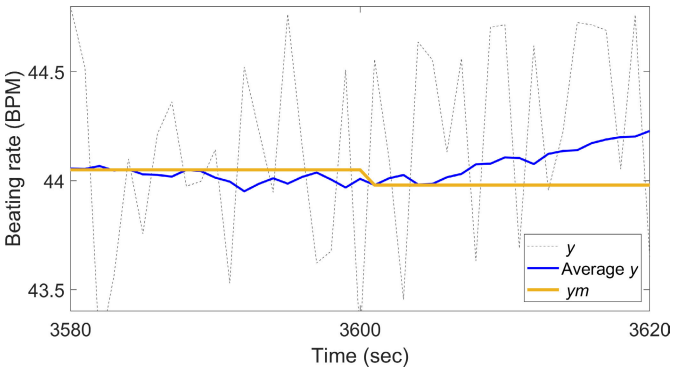

Fig. 7. An illustration of the difference between simulation outputs from the *Cardiomyocyte beating model* (y) and the *Measurement model* (ym). Measured beating rate (ym) value is kept constant during the current measurement interval and is only changed after new measurement starts, which is approximately at time 3600 seconds in this example. Average y is 30-seconds running average of y.

D. Measurement Model

To measure sufficiently precisely the average beating rate of a cell cluster, 30 to 60 seconds long videos recorded with 50 frames per seconds or faster are typically used [22]. However, *in vitro* cell cultures can experience undesired stimulations when exposed to illumination during imaging, thus limiting its usage. Furthermore, to keep the amount of data in feasible limits, the beating rate is typically measured only at certain time intervals. Accordingly, we implemented these real-life limitations for our simulation environment so that the beating rate was only measured and changed after certain time periods; every 1, 5 or 30 minutes in this paper. This was done by implementing zero-order hold to keep the measured beating rate signal ym (see Fig. 2) constant between the measurement intervals, even though the beating rate signal (y) is constantly changing. This is illustrated in Fig. 7, which includes signals y, 30-seconds running average of y, and the measured output ym. It should be noted that possible stimulations because of the illumination, for example a small change in the beating rate, are very complex and a system specific, thus are excluded from our simulator.

E. Different Simulation Cases

In this section, we present the simulation cases studied in this paper. The main purpose of the cases is to compare

controllers' performance and their robustness with four human cardiomyocyte model variants (presented in Fig. 6) using different measurement intervals (1, 5, and 30 minutes). Three measurement intervals were studied because of the limitations of optical imaging as a sensor in this application, as explained previously. For comparing the performance, we designed three regulation cases and one tracking case. As each controller (3) was simulated in each case study (4) using four differently behaving cardiomyocytes variants and three different measurement time intervals, 144 simulations ($3 \times 4 \times 4 \times 3$) were performed in total. In addition to comparing the proposed controllers, we demonstrate how the system essentially developed for controlling the beating rate of hiPS-CMs can be used to enhance the control of temperature in these cultures. Next, each simulation case is described in more detail.

1) Regulation: In three different regulation problems, we studied how well each controller maintained a constant beating rate at the typical cell culture temperature 37°C. Firstly, because outside temperature variations cannot be fully eliminated, there is always some small temperature variations in real cell culture experiments. Furthermore, there is always some variation in the beating rate of hiPSC-CM cultures. Because of these two factors, the beating rate is never exactly constant. Furthermore, external disturbances, which are described next, also affect the cell culture temperature, and thus the beating rate. Therefore, we designed temperature disturbances to mimic typical *in vitro* cell culture situations, such as temperature drop in the cell culture during the change of the cell culture medium. When these disturbances were added to our simulation environment, regulation performances of the different controllers could be studied.

In the three studies performed, one study (Regulation 1) was without, hand two studies were with external disturbances. In Regulation 2, we added a sudden 5°C temperature drop in the cell culture for a while before temperature returned to the normal 37°C. This temperature drop mimicked a situation where the cell culture chamber (see Fig. 1) is open for a while, for instance, during cell medium change. In Regulation 3, we applied beating rate disturbances to our environment, simulating a culture condition where some undesired chemical stimulation would disturb the cells. We simulated this by adding a positive 5 BPM disturbance to the beating rate (y) for 100 minutes before the disturbance was returned to zero.

2) Tracking: The second part of our simulation focuses on studying the controller performance when tracking a reference beating rate which is changing. This is useful in cases where a changing beating rate is desired. For example, the beating rate of cardiomyocytes could be used in volumetric flow control in an artificial pump created from engineered living materials. It could also be integrated with the previously mentioned artificial butterfly [2], enabling to modify the swinging speed of the wings by temperature-based beating rate control of the cardiomyocytes. To demonstrate this idea, we implemented changing set-point signals and studied how each controller tracks these signals. The set-point signals were tuned for each cell model individually with the following procedure. First, the desired beating rate was set to the beating rate of each model at 37°C. Next, the set-point was changed to the value located in the middle between the maximum and minimum beating rates (see Fig. 6), followed by changing it first to the minimum value and then to the maximum beating rate. Finally, the set-point was returned to the beating rate at 37°C. Used set-point signals for different *Cardiomyocyte beating* models are presented in Fig. S5.

TABLE II

SUMMARY OF TOTAL ERROR (*Etot*) FROM 144 SIMULATIONS WITH THREE CONTROLLERS: FUZZY COMPLEX (*FC*), FUZZY SIMPLE (*FS*), AND TRADITIONAL ONE (*PI*). FIRST 36 SIMULATIONS (ROWS) ARE REGULATION STUDIES AND LAST 12 TRACKING STUDIES. MEASUREMENT INTERVAL (*dt*) IS GIVEN IN MINUTES

Study case number and name	*FC*	*FS*	*PI*
1) Reg1, model: normal, *dt*: 1	0	0	0
2) Reg1, model: normal, *dt*: 5	0	0	0
3) Reg1, model: normal, *dt*: 30	0	0	0
4) Reg1, model: long-QT, *dt*: 1	0	0	0
5) Reg1, model: long-QT, *dt*: 5	0	0	0
6) Reg1, model: long-QT, *dt*: 30	0	0	0
7) Reg1, model: supra-CC, *dt*: 1	4	4	4
8) Reg1, model: supra-CC, *dt*: 5	4	4	4
9) Reg1, model: supra-CC, *dt*: 30	4	4	4
10) Reg1, model: infra-CC, *dt*: 1	0	0	0
11) Reg1, model: infra-CC, *dt*: 5	0	0	0
12) Reg1, model: infra-CC, *dt*: 30	0	0	0
13) Reg2, model: normal, *dt*: 1	2060	2135	6152
14) Reg2, model: normal, *dt*: 5	5417	5698	7841
15) Reg2, model: normal, *dt*: 30	13433	13285	14942
16) Reg2, model: long-QT, *dt*: 1	885	999	3963
17) Reg2, model: long-QT, *dt*: 5	2315	2457	5243
18) Reg2, model: long-QT, *dt*: 30	4292	4012	6700
19) Reg2, model: supra-CC, *dt*: 1	6019	5296	12154
20) Reg2, model: supra-CC, *dt*: 5	11819	10754	17490
21) Reg2, model: supra-CC, *dt*: 30	29019	20640	71786
22) Reg2, model: infra-CC, *dt*: 1	912	964	2878
23) Reg2, model: infra-CC, *dt*: 5	3439	2900	6527
24) Reg2, model: infra-CC, *dt*: 30	3270	3166	3562
25) Reg3, model: normal, *dt*: 1	1732	1664	3345
26) Reg3, model: normal, *dt*: 5	5705	5072	13348
27) Reg3, model: normal, *dt*: 30	25965	18670	56689
28) Reg3, model: long-QT, *dt*: 1	2175	2254	4495
29) Reg3, model: long-QT, *dt*: 5	6567	5437	15763
30) Reg3, model: long-QT, *dt*: 30	32353	27070	65214
31) Reg3, model: supra-CC, *dt*: 1	1089	972	1336
32) Reg3, model: supra-CC, *dt*: 5	3153	2802	9382
33) Reg3, model: supra-CC, *dt*: 30	16746	30306	77620
34) Reg3, model: infra-CC, *dt*: 1	4514	4501	8820
35) Reg3, model: infra-CC, *dt*: 5	10609	9196	20497
36) Reg3, model: infra-CC, *dt*: 30	30660	31726	55394
37) Track, model: normal, *dt*: 1	19447	18639	32625
38) Track, model: normal, *dt*: 5	28037	31480	40246
39) Track, model: normal, *dt*: 30	51858	61021	135723
40) Track, model: long-QT, *dt*: 1	17889	17186	33402
41) Track, model: long-QT, *dt*: 5	28108	26030	44044
42) Track, model: long-QT, *dt*: 30	51123	46174	161525
43) Track, model: supra-CC, *dt*: 1	25413	26345	41440
44) Track, model: supra-CC, *dt*: 5	32074	35640	49261
45) Track, model: supra-CC, *dt*: 30	61989	70172	155885
46) Track, model: infra-CC, *dt*: 1	9010	7907	22803
47) Track, model: infra-CC, *dt*: 5	18020	13270	37607
48) Track, model: infra-CC, *dt*: 30	45994	31412	54174

III. RESULTS

This section presents first the three regulation studies followed by the tracking study. Results from all 144 simulations are summarized in Table II and in Figs. 9 and 10.

A. Regulation Studies

The goal in all regulation studies was to maintain the beating rate constant at the typical cell culture temperature

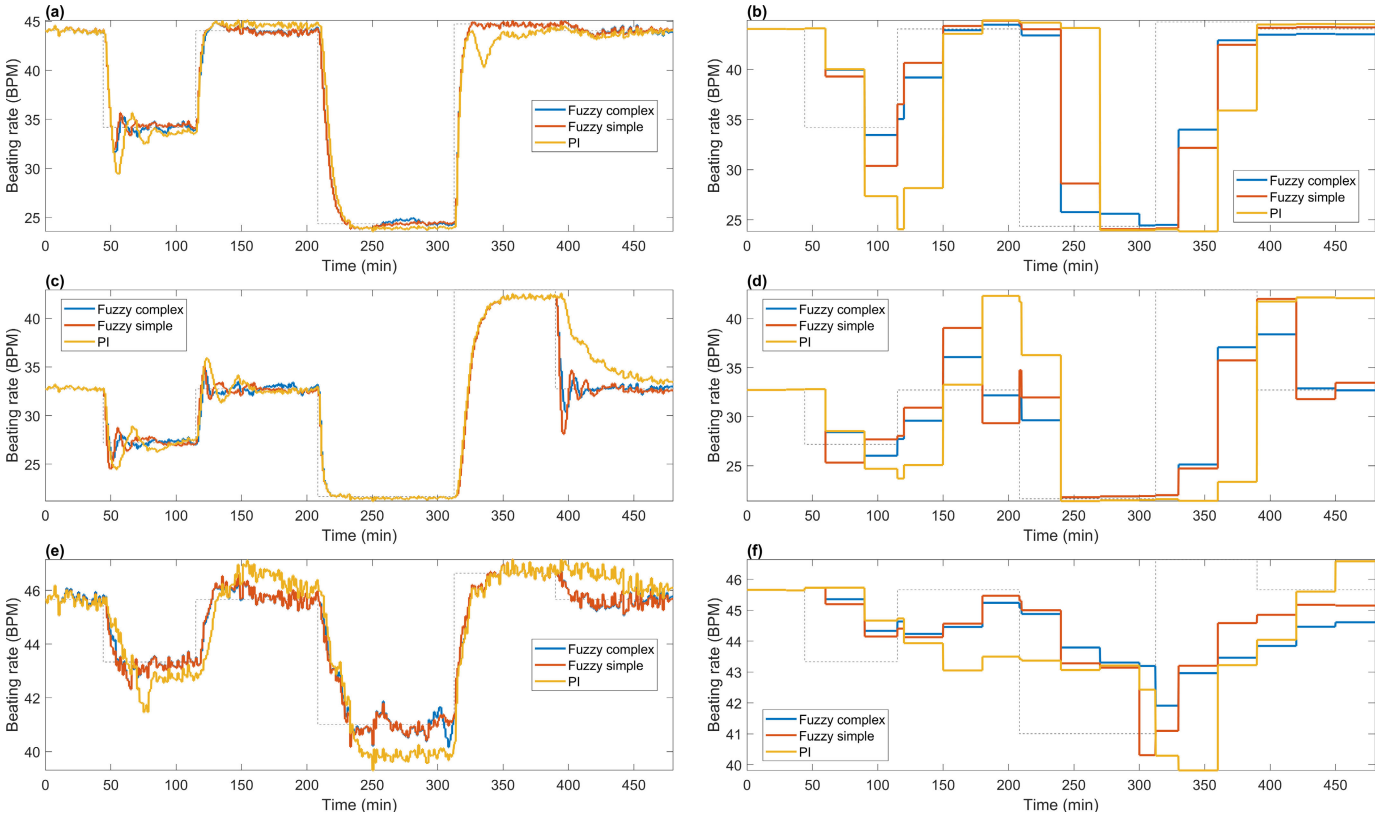

Fig. 8. Tracking study with three different *Cardiomyocyte beating* models with two measurement intervals (*dt*) 1 min and 30 min: (a) normal, $dt = 1$ min, (b) normal, $dt = 30$ min, (c) suppra-cc, $dt = 1$ min, (d) suppra-cc, $dt = 30$ min, (e) infra-cc, $dt = 1$ min, and (f) infra-cc, $dt = 30$ min.

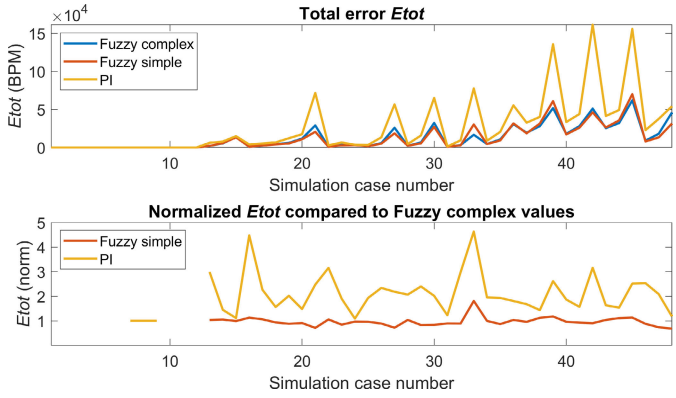

Fig. 9. Comparison of total (upper) and normalized (lower) errors *Etot*. Normalized values are compared to *Etot* values from Fuzzy complex.

37°C. As the cell cultures are different, the temperature 37°C results in different beating rate set-point values r as follows: approximately 44.0 BPM for the normal hiPSC-CM cluster, 44.8 BPM for the long-QT cluster, 32.7 BPM for the supra-CC cluster, and 45.7 BPM for the infra-CC cluster.

1) Regulation 1: In Regulation 1, no external disturbance was added and only the measurement interval (*dt*) was varied (1, 5, and 30 minutes).The results with the two fuzzy controllers and the PI-controller are given in the supplementary material (see Figs. S6 and S7). Because of the applied dead zone range, one BPM in this study, the results are nearly identical.

2) Regulation 2: In Regulation 2, we added a 5°C temperature drop in the cell culture for 60 minutes before returning the temperature back to 37°C. Results show how the fuzzy controllers outperform the PI-controller. The results and the difference between the desired and measured beating rate with different controllers, cell models, and measurement intervals are given in Figs. S8 and S9.

3) Regulation 3: In Regulation 3, we simulated a culture condition, where an undesired chemical stimulation would interfere with the cells, resulting in an increased beating rate. Results show how both fuzzy controllers again outperform the PI-controller. The results and the difference between the desired and measured beating rate with different controllers, cell models, and measurement intervals are given in Figs. S10 and S11.

B. Tracking Study

In this section, we compare the controller performance under a continuous change in the beating rate set-point. The results are shown in Fig. 8. The results indicate that the fuzzy controllers enable easier and faster control without the fear of an unstable system, which is a likely problem with the traditional PI-controller. The difference between the desired and measured beating rate with different controllers, cell models, and measurement intervals are given in Fig. S12.

C. Summary

Based on the results, we conclude that both implemented fuzzy controllers outperformed the PI-controller, especially

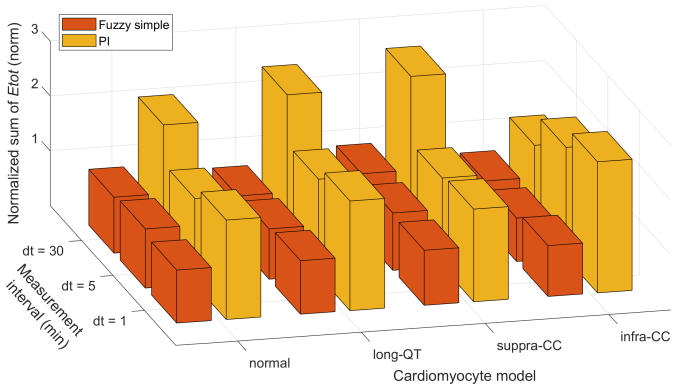

Fig. 10. Comparison of normalized *Etot* values from Fuzzy complex, when all four simulation cases (Regulation 1-3 and Tracking) are summed together.

when a longer measurement interval was used. It is evident that when the measurement interval is increased, the fuzzy controllers are more robust. The total error *Etot* was typically reduced at least 40% with the developed fuzzy controllers compared to the PI-controller. A full summary of the total error *Etot* is provided in Table II, where rows 13 to 36 are for Regulation 2 and Regulation 3 studies, and rows 37 to 48 for Tracking study. These are also compared in Figs. 9 and 10, highlighting how the both fuzzy controllers can provide more accurate temperature control compared to the PI-controller. With the PI-controller, *Etot* was typically at least two times higher when compared to the fuzzy controllers. Furthermore, as it is very easy to design the fuzzy controller with the desired dead zone range (one BPM in this study), no control action is performed when the measured beating rate is close to, but not exactly, the desired beating rate. In other words, measured small beating rate changes caused by the normal biological beating rate variations or by external disturbances such as slight changes in the laboratory room temperature, are not affecting the controller.

IV. Conclusion and Discussion

In this work, we designed and implemented a simulation environment that can be used to study dynamics of beating human cardiomyocyte cell cultures and to discover suitable controlling strategies for these highly non-linear *in vitro* systems. The resulting control strategies can be employed in several types of biological studies. For example, in co-culture studies, where cardiomyocytes are cultured with other cells such as neuronal cells or vasculature, the proposed concept can be used for changing the beating of cardiomyocytes in a precisely controlled way. This will allow to study how different beating rates influence the other cell types in these co-culture systems. Furthermore, cardiomyocytes with a controlled beating rate can be used in different heart disease studies. For instance, the effect of the beating rate on the oxygen consumption of cardiomyocytes can be studied in such a controlled *in vitro* environment in the context of hypoxia or ischemia. Cardiomyocytes with controlled beating can be used as a part of engineered living biomaterial systems, such as biohybrid robots or smart scaffolds for tissue engineering, where biological actuation is needed.

We studied how robust different closed-loop controller methods are for controlling different cardiomyocyte *in vitro* cell cultures. Our results show that both proposed fuzzy controllers can easily outperform the traditional PI-controller, especially when more non-linear beating models and longer measurement intervals were considered. As expected, the performance of the PI-controller decreased with respect to fuzzy controllers when longer time intervals were used. There are also other modern feedback control methods suitable for controlling complex, highly non-linear time-varying systems, thus having capability to work in the proposed problem as well. Especially methods, such as artificial neural networks and data-driven control, would be viable alternatives. For example, a data-driven control approach, where machine learning enables to directly determine control actions from the data without explicit modeling of the system, is a fascinating approach that should be further studied.

We noticed that a simple fuzzy controller using only difference signal and five rules generated similar results than a more complex fuzzy controller using two input signals (difference and change in output) and 17 rules. Therefore, in the future, we will first implement the proposed simple fuzzy controller to our actual *in vitro* cell culture system. We strongly believe that the developed method provides a more controlled environment, and it thus will significantly enhance *in vitro* cell culture studies, resulting better *in vivo* significance.

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