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A digital hardware system for real-time biorealistic stimulation on *in vitro* cardiomyocytes

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Abstract: Every year, cardiovascular disease causes millions of deaths worldwide. These diseases involve complex mechanisms that are difficult to study. To remedy this problem, we propose to develop a heart-brain platform capable of reproducing the mechanisms involved in generating the heartbeat. The platform will be designed to operate in real-time, with the most economical and integrated design possible. To achieve this, we are implementing highly biologically coherent cellular models on FPGA, which we interconnect with *in vitro* cell cultures. In our case, we are using the Maltsev-Lakatta cell model, which describes the behavior of the pacemaker cells responsible for the heart rhythm, to stimulate a cardiomyocyte culture.

Keywords: Sinoatrial Node, real-time, FPGA, biomimetic, *in vitro* stimulation

1. INTRODUCTION

According to WHO data, in 2019, 32% of deaths worldwide were caused by cardiovascular disease[1]. As populations age, this number will rise in the future years. Yet the mechanisms involved in these diseases remain poorly understood due to the interactions' complexity.

In reality, heart disease investigation is often complex because it involves not only the heart but also other organs, to which it is related, in the first place, the nervous system. This is the reason why it is essential to study the brain-heart system to obtain a realistic overview of the disease[2].

In this paper, we are focusing more specifically on diseases affecting the generation of cardiac rhythm (Fig. 1). This affects the sinoatrial (SA) node, a cluster of pacemaking cells located in the right atrium of the heart, linked with the autonomic nervous system.

The usual treatment for these diseases ranges from drugs to transplants and the implantation of assistive devices. Each of these treatments could induce difficulties, as they usually involve a loss of quality of life, with significant effects on lifestyle, as well as a substantial cost for society. These treatments target only the heart, while heart disease involves also other organs. If we can obtain a wider overview of all the relationships between these organs, especially in the case of heart disease, then it would be possible to propose more accurate treatment solutions.

For all these reasons, further research in this field is essential. However, research is focused mainly on the use of animal models, which have the advantage of being faithful to the system under study, but are also very costly, time-consuming and have ethical issues. *In vitro* neurocardiac co-culture simplifies *in vivo* investigation but remains complex to be achieved and limited to the interaction between sympathetic neurons and cardiomyocytes[3]. In both cases, artificial control of the biological unit is complicated. On the other side, biorealistic models on computers have the advantage of reducing the time consumption and the cost to repro-

duce experiments but need to maintain heavy infrastructure and the specialized skills associated while being unable to process models in real-time[4].

Here, we propose to develop a biohybrid platform that reproduces artificially the heart-brain system to overcome these limits (Fig. 2). It starts by recording the activity of *in vitro* neuron culture mimicking the measure of the blood flow.

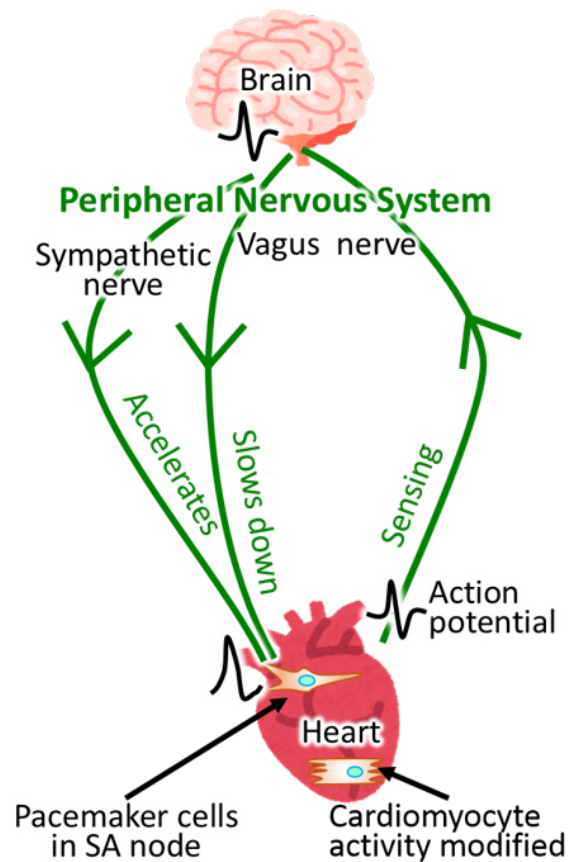


Fig. 1. Overview of the heart-brain interaction system.

[†] Pierre-Marie Faure is the presenter of this paper.

Then, this activity will be processed by a Spiking Neural Network (SNN)[5, ?, ?] that would speed up or slow down the sinoatrial node through parasympathetic and sympathetic stimulation. This output signal will be put on an *in vitro* cardiomyocyte culture to assess its efficiency, to prepare for a closed-loop system[8].

In this article, we focus on the heart part of the platform, by emulating the cells that make it up the sinoatrial node while reducing the drawbacks of common methods. Notably, we aim to use the most biorealistic approach, to insert this work into a complete platform reproducing the whole heart system, while processing the needed calculation in real-time[9]. In addition, we add the constraint of minimizing hardware resources, power consumption and physical footprint. For this purpose, we target a hardware digital implementation (FPGA): a system capable of combining all these criteria while retaining a certain degree of flexibility[10].

2. PACEMAKING MODELIZATION

We aim to adopt a model capable of describing the behavior of sinoatrial node cardiomyocytes. Two main categories of models exist, those opting for a systems approach to the heartbeat, which describes it through automatic behavior, and those advocating a biological approach, which starts from the

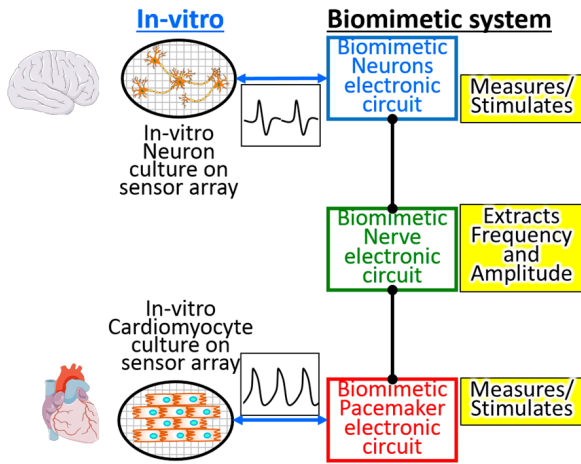


Fig. 2. Architecture of the complete platform for reproducing the heart-brain system. *In vitro* neuron culture drives the input of our platform that processed it in three stages, reproducing biological logic then, stimulating *in vitro* cardiomyocyte culture.

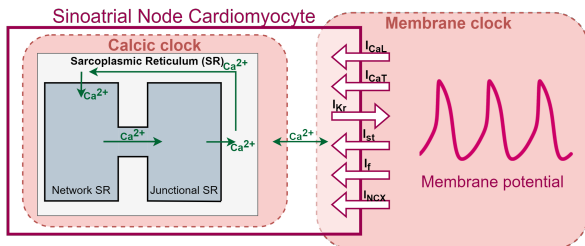


Fig. 3. Overview of the coupling of calcic clock and membrane clock in Maltsev-Lakatta model

phenomena at play and reassembles the cardiac rhythm at a cellular level.

In this second category, which interests us because we want to be as faithful to reality as possible, there are two main types of models: those with a calcium clock and those with a membrane clock[11]. The former describes the chemical circuit of calcium ions through the cell, considering that the beat came from within the cell itself, while the latter takes up the Hodgkin-Huxley approach[12], a conductive-based model, and used membrane ionic currents as the source of the signal oscillation. This approach takes biological phenomena and transposes them into the electrical domain. This enables a realistic description of these phenomena while using the tools of electrical engineering.

Today, the latest models have resolved this controversy by reconciling the two points of view. The combination of the two explains the robustness of the heartbeat and its wide frequency range.

As we aim to get as close as possible to the real system, we implement the Maltsev-Lakatta model[13], which describes the cardiomyocytes of the sinoatrial node of a healthy rabbit heart. It behaves like an oscillator requiring no external stimulation. In this model (Fig. 3), each clock is associated with a parameter. This couple of values determines the beating frequency of the transmembrane voltage generated by the model. This feature ensures a simplified modification of the model frequency without changing the equations themselves.

3. RESULTS

3.1. Digital Implementation

The model resolution is implemented on a low-cost, compact and flexible digital circuit (FPGA) compatible with our requirements. This resolution is pipelined so that with each clock stroke, a complete system is resolved (Fig. 4).

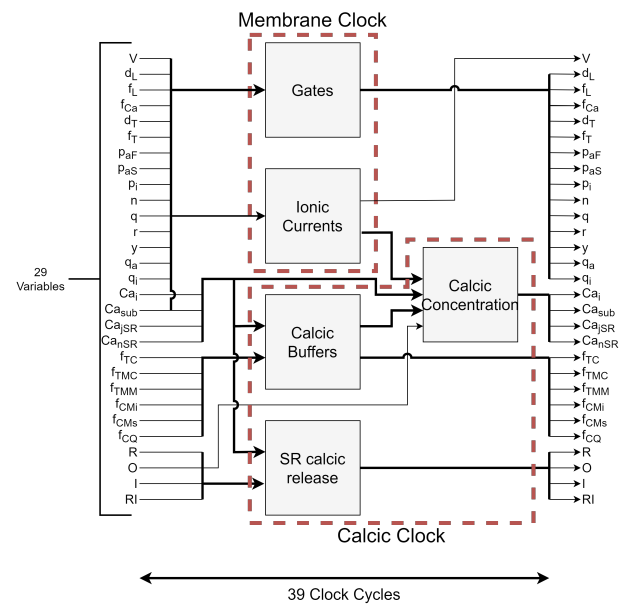


Fig. 4. Architecture of the model on FPGA

The implementation is designed as closely as possible to the specific features of the FPGA. Multiplication uses a 25x18-bit format to match the size of the Digital Signal Processor (DSP) inputs. In addition, this constraint requires the use of fixed-point notation. A great deal of work has gone into minimizing the loss of precision associated with these two constraints.

To implement a solver of the Maltsev-Lakatta model on FPGA, we rewrite their equations to reduce the number of operations used. For the same reason, heavy-cost operations as exponential or division, are preprocessed into a BRAM component to make a look-up table. For gate equations, we proceed with the following method. We start with the classical formula:

$$\frac{dx}{dt} = \frac{x_{\infty} - x}{\tau_x} \quad (1)$$

As we are using the Forward Euler method to solve this equation, we can rewrite it:

$$\frac{x[n+1] - x[n]}{dt} = \frac{x_{\infty} - x[n]}{\tau_x} \quad (2)$$

This gives us the next iteration for a gate variable :

$$\begin{aligned} \frac{x[n+1] - x[n]}{dt} &= \frac{x_{\infty} - x[n]}{\tau_x} \\ A_x &= 1 - \frac{dt}{\tau_x} \\ B_x &= x_{\infty} \cdot \frac{dt}{\tau_x} \end{aligned} \quad (3)$$

As τ_x and x_{∞} only depend on the transmembrane potential (V) variable, we preprocess their values into a BRAM holding 1024 (2^{10}) values to diminish our resources consumption. With these modifications, for processing one gate, we use 2 ROMs and only 1 DSP.

With the same philosophy, we modify ionic currents which are generally written under the following format :

$$I_x = x_0 \cdot x_1 \cdot x_2 \cdot g_x \cdot (V - E_x) \quad (4)$$

Where x_0 , x_1 and x_2 are gates variables of the ionic current which can use between 0 and 3 of them. As they will be summed and integrated, we can rewrite them:

$$\begin{aligned} I_x^* &= x_0 \cdot x_1 \cdot x_2 \cdot (A_x \cdot V - B_x) \\ A_x &= dt \cdot g_x \\ B_x &= -dt \cdot g_x \cdot E_x \end{aligned} \quad (5)$$

These values are hard-coded. The introduction of the integration time step at the earliest point allows us to save resources.

For other parts of the model, I_{NaK} , I_{NCX} and calcic clock equations, we processed with the same philosophy. The exponential and division operations are obtained through the look-up table implemented and we modify equations to eliminate all the redundancy to reduce resource consumption. In the end, we obtained a real-time solver with a resolution step of 10 μ s and a clock rate of 100MHz. This system can solve up to 1000 independent sinoatrial node cardiomyocytes in parallel in real-time thanks to our pipelined architecture, with a latency of 39 clock cycles (Table 1).

After the implementation (Fig. 5), we compared our implementation on FPGA with our simulation on a computer

using the same method of solving without all the previous simplification and using double precision variables instead of fixed-point values (Table 2).

3.2. Biological Experimentation

Before experimenting with the previously implemented system, we tested the most appropriate stimuli for controlling the beating of a cardiomyocyte culture *in vitro*. We determined that the conditions for activating a mechanical beat are a negative voltage stimulation of the order of one volt. The resulting beat is expected to have the same frequency as the stimulation signal.

For these experiments, cardiomyocytes were isolated from neonatal mice mouse hearts and dissociated into single-cell suspension that can be used for the cultivation of beating cardiomyocytes. Neonatal hearts were first enzymatically digested using the neonatal heart dissociation kit for mice from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany). [14]

To adapt our output signal of the FPGA to the culture stimulation, we use a Digital-Analog Converter (DAC) and an electronic board that inverts the analog signal and controls

Table 1. Resources used by the VHDL implementation of 1000 cardiomyocytes based on the Maltsev-Lakatta model on a Zynq-7020.

Resource	Utilization	Available
LUT	30.8%	53200
LUTRAM	79.9%	17400
Flip-Flop	7.6%	106400
BRAM	15.4%	140
DSP	55%	220

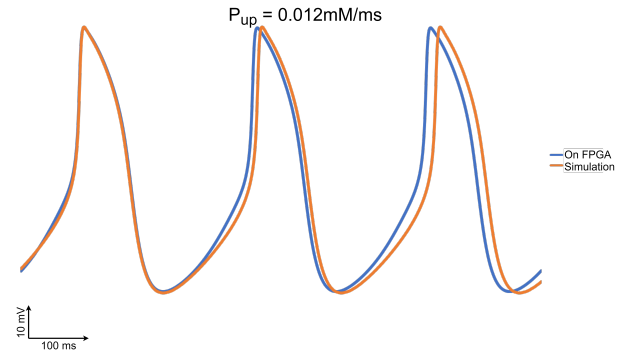


Fig. 5. Comparison of Maltsev-Lakatta model between computer simulation and FPGA for the nominative value of P_{up} (0.012mM/ms) on established oscillation

Table 2. Frequency comparison between simulation and our implementation on FPGA for different values of P_{up}

P_{up} (mM/ms)	Simulation (Hz)	FPGA (Hz)	Error
0	-	2.073	-
$4 \cdot 10^{-3}$	2.180	2.393	8.90%
$8 \cdot 10^{-3}$	2.626	2.679	1.98%
$12 \cdot 10^{-3}$	3.005	2.924	2.70%

the signal amplitude (Fig. 6). This stimulation is applied on cardiomyocytes cultivated on a MicroElectrodes Array (MEA) [15]. We record the beating through a microscope to check if our system works (Fig. 7). In total, we used four *in vitro* cultures of mouse cells on which we applied the stimulation according to the following sequence: 20s with natural activity, 20s of stimulation and 20s of resting.

We note that, during stimulation, the beating of the cells follows the frequency of the stimulation (Table 3), whereas as soon as the stimulation stops, the cells stop beating and resume their spontaneous activity. However, there is an exception to this principle with culture 3, which stops during stimulation.

We suppose that the amplitude of the stimulation is not enough as these experiments are carried out in the open air, meaning that the culture medium is cooling down, reducing the reactivity of cardiomyocytes.

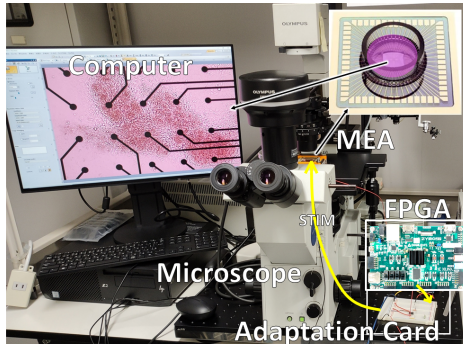


Fig. 6. Setup for stimulation of *in vitro* cardiomyocytes by the Maltsev-Lakatta model implemented on FPGA.

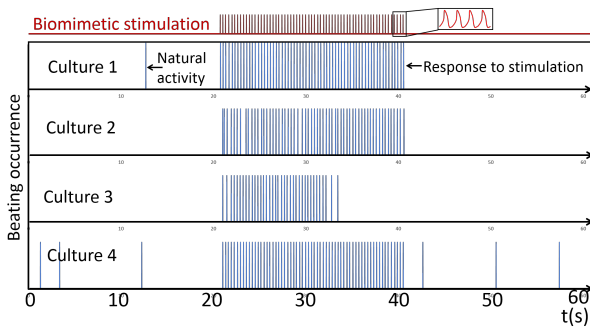


Fig. 7. Activity of *in vitro* cardiomyocytes recorded before, during and after biomimetic stimulation.

Table 3. Result of the stimulation on the four cardiomyocyte cultures with a frequency of 3.1Hz.

	Frequency (Hz)	Relative Error
Stimulation	3.1	-
Culture 1	3.15	1.61%
Culture 2	3.06	1.29%
Culture 3	3.01	2.90%
Culture 4	3.14	1.29%

4. CONCLUSION

As we saw previously, we implemented a biorealistic model of sinoatrial node cardiomyocytes reproducing its pacemaking functionality in real-time on an FPGA. This signal was used to stimulate cardiomyocytes cultured *in vitro*. This stimulation triggered beatings equivalent to the frequency of the model signal showing its ability to control the rhythm of the *in vitro* cardiomyocyte culture. In this way, we were able to reimplement the heart's pacemaker functions in real-time.

The cardiomyocyte beating was responding to the stimulation produced by our system. Then, if we attain to modify the frequency of the stimulation in a biomimetic way, we would be able to reproduce the interface between the heart and the nervous system implicated in the generation of pacemaking signal.

Some simplifications of the chosen model are responsible for a lack of precision in the generation of the signal. By increasing the precision of operations of the model, that problem could be resolved.

To investigate the stability of the system composed of biomimetic stimulation and cell culture, the effect of long-term stimulation will be investigated. Finally, a more compact recording system is under development to have access to real-time information more easily.

In the next step, we want to connect this part to the autonomic nervous system to biomimetically control the beating rhythm, paving the way for fully biomimetic heart rate generation in real-time on a small system with a low energy footprint.

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