# Towards the Development of an *in Silico* Model for the Zebrafish Action Potential

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## Abstract

*In the last decades, the use of zebrafish in different fields has significantly grown. This increasing interest is related to its characteristics, which make it very similar to humans in many aspects, especially a similar electrophysiology owing large percentage of orthologues of human genes. Thus, zebrafish has been proposed as a pharmacological and genetic screening model. Developing a numerical AP model seems very important to study pathologies and drug administration to understand the ionic mechanisms involved. Thanks to this knowledge, reducing the number of animals used for experimental studies will also be possible. This work represents the first approach toward the development of a numerical model for the adult zebrafish AP. The developed model uses the TP04 formulation of the action potential for human cardiomyocytes as a base model. Starting from this model, the main currents have been reparametrized to adapt them to the zebrafish while extending the model to account for the T-type calcium current present in the zebrafish and deleting the $I_{to}$ current, which is not found to be present instead. Moreover, AP recordings from the ventricle of adult zebrafish in isolated hearts were collected to validate the numerical model. Preliminary results showed an AP morphology in good agreement with experimental data and correct restitution curves behaviors.*

## 1. Introduction

In recent years, the zebrafish (*Danio rerio*) has been increasingly considered a potential animal model for applications ranging from embryonic development to oncology, or cardiac electrophysiology. For what concern the latter, in particular, the zebrafish action potential (AP) results remarkably similar to the human AP due to the presence of approximately 69% of human genes orthologues [1]. This provides to the zebrafish cardiomyocyte a functional similarity in cardiac ion channels, shape, and duration of the AP with respect to humans [2]. This has made the zebrafish very attractive for pharmacological studies and as a genetic screening model for studies of cardiotoxicity. Despite this great interest, no efforts have been conducted on developing a mathematical model of the zebrafish AP.

Developing such a mathematical model will open new potential applications, such as studying the underlying ionic mechanisms behind known pathologies or the response of a new drug. Further, this will also help in reducing the number of animals used for experimental studies and refine the experimental procedures. This work aims at developing a mathematical model of the zebrafish AP, which can adequately reproduce the main characteristics of the action potential and restitution properties.

## 2. Methods

An electrophysiological detailed AP ventricular model of the zebrafish is developed in this study, starting from the preexisting Ten Tusscher and Panfilov (TP04) formulation for human cardiomyocytes [3][4]. Thanks to the already cited similarities between zebrafish and humans, it seemed reasonable to start with a human model as a base model, particularly the use of the TP04 model due to its extremely computationally cost-effectiveness.

Moreover, experimental data at different stimulation protocols were collected in order to be compared with the numerical model obtained.

### 2.1. Numerical model

The approach consists in reparametrizing the gating variables and the time constants of the main currents of the preexisting human model to adapt them to the zebrafish while removing currents whose presence was not discovered in the zebrafish or introducing new currents based on formulations used in other models of the AP (e.g., formulation for rabbit sinoatrial node [5][6]) and parametrized to the zebrafish. To achieve this goal, the most recent experimental data, i.e., patch-clamp data

([7][8][9][10][11][12][13]), regarding the main currents present in the zebrafish were considered.

With respect to human, the zebrafish does not express the transient potassium current, $I_{to}$, and therefore has been removed from the original TP04 model, but it expresses the T-type calcium current that has been added in the present formulation by adapting the model proposed in [6]. The currents of the sarcolemma present in the proposed model are: i) fast sodium current, $I_{Na}$, T-type calcium current, $I_{CaT}$, L-type calcium current, $I_{CaL}$, slow delay rectifying current, $I_{Ks}$, rapid delayed rectifying current, $I_{Kr}$, the inward rectifier potassium current, $I_{K1}$, the sodium-calcium exchanger, $I_{NaCa}$, the sodium-potassium pump, $I_{NaK}$, the calcium pump, $I_{pCa}$, and the background calcium and sodium currents $I_{bCa}$ and $I_{bNa}$. The mathematical model of the zebrafish action potential is then expressed as:

$$C_m \frac{dV}{dt} = -I_{ion} - I_{stim} \quad (1)$$

where $C_m$ is the cell membrane capacitance per unit surface area, $V$ is the transmembrane voltage, $t$ is time, $I_{stim}$ is the externally applied stimulation current, and $I_{ion}$ is the sum of the ionic currents

$$I_{ion} = I_{Na} + I_{CaT} + I_{CaL} + I_{Ks} + I_{Kr} + I_{K1} + I_{NaCa} + I_{NaK} + I_{pCa} + I_{bCa} + I_{bNa} \quad (2)$$

Similarly, a 1D cable of cardiac cells can be modeled as a continuum system with the following partial differential equation

$$C_m \frac{\partial V}{\partial t} = \sigma \frac{\partial^2 V}{\partial x^2} - I_{ion} - I_{stim}, \quad (3)$$

where $\sigma$ is the cable conductance.

The model has been implemented in MATLAB R2022a (MathWorks Inc.). For 1D computations, the forward Euler method was used to integrate (2). A space discretization of $\Delta x = 0.01$ mm and a time step of $\Delta t = 0.02$ ms were used, with $\sigma = 0.249$ μS to obtain a conduction velocity of 11.47 mm/s as found in the experiments performed by our group. To integrate the Hodgkin-Huxley type equations for the gating variables of the various time-dependent currents ($m$, $h$, and $j$ for $I_{Na}$, $x_{r1}$, and $x_{r2}$ for $I_{Kr}$, $x_s$ for $I_{Ks}$, $d_L$, $f_L$, and $f_{Ca}$ for $I_{CaL}$, and $d_T$ and $f_T$ for $I_{CaT}$) the Rush and Larsen scheme was used.

AP duration (APD) is defined as AP duration at 90% repolarization (APD90). Two different protocols were used to validate the model with tissue experiments: i) a steady state protocol, and ii) the S1-S2 restitution protocol. The steady state stimulation protocol consisted in stimulating the model with a trend of 300 stimulus at a frequency of 2 Hz. The model was then checked for absence of alternants in the last three APs and peacemaking behavior after interrupting the stimulation. The APD of the last beat was compared with experimental recording. The S1-S2 restitution protocol, typically used in experiments, consists of 10 S1 stimuli at a BCL of 500 ms followed by a S2 extra stimulus delivered at some diastolic interval (DI) after the AP generated by the last S1 stimulus. The APD restitution curve is generated by decreasing DI and plotting APD generated by the S2 stimulus against DI.

Moreover, a parameters sensitivity analysis was conducted by varying the maximum conductances and the time constants by a ±15% and shifting the gating variables activation and inactivation curves by ±5 mV.

## 2.2. Experimental Data

In order to validate the newly developed AP model, AP recordings with sharp electrode from the ventricle of adult zebrafish isolated hearts were collected. The hearts were maintained in 28°C HEPES-buffered saline solution (i.e., NaCL 142 mM, KCl 4.7 mM, HEPES 10 mM, glucose 10 mM, $MgCl_2$ 1 mM, and $CaCl_2$ 1.8 mM) and paced from the ventricular apex. The recordings were made on 6 different hearts and for each of them data from 3 different cells were collected, giving a total number of 18 different recordings. For each of them, two different stimulation protocol were used, namely: i) steady state protocol at a BCL of 500 ms consisting of 300 stimuli, and ii) S1S2 protocol with 10 stimuli at a BCL of 500 ms (i.e., S1) followed by the S2 stimulus at different DI intervals.

## 3. Results

The developed numerical model resulted in a stable AP that didn't show alternants or pacemaking activity after interrupting the stimulation and with AP morphology in good agreement with experimental AP recordings. Figure 1 shows the comparison between experimental and numerical recordings of the action potential for the specific BCLs stimulation protocol in the case of BCL = 500 ms.

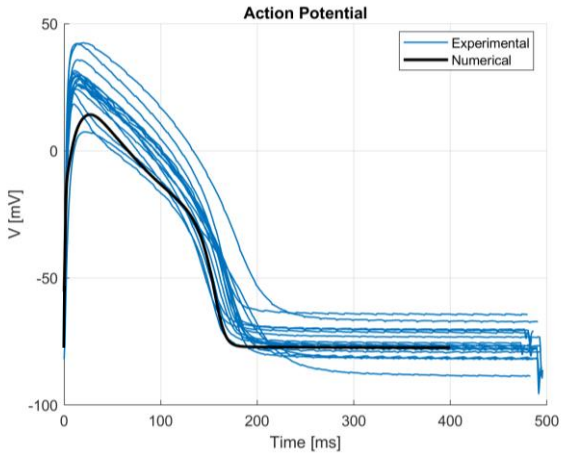


Figure 1. Comparison of the experimental and numerical AP recordings for the specific BCLs protocol with BCL = 500 ms.

A more quantitative analysis of the different AP marker

characterizing the modeled AP is given in Table 1. The experimental data were reported with the standard deviation (± SD). It was found the shape of the AP to be in good agreement with experimental recording, closer to the lower bound of the experimental records.

Table 1. Comparison of AP characteristics between model and experimental recordings for the specific BCLs stimulation protocol (BCL = 500 ms). Experimental data were reported with the corresponding standard deviation (± SD).

| AP marker | Model | Experiment (n=18) |
|---|---|---|
| $APD_{90}$ [ms] | 154.12 | 178.47 ± 16.76 |
| $APD_{80}$ [ms] | 148.71 | 168.45 ± 13.62 |
| $APD_{50}$ [ms] | 131.61 | 137.82 ± 10.84 |
| $APD_{20}$ [ms] | 73.44 | 74.11 ± 13.00 |
| APA [mV] | 90.35 | 104.45 ± 4.09 |
| $V_{max}$ [mV] | 13.13 | 27.23 ± 6.98 |
| $dV/dt_{max}$ [mV/ms] | 20.09 | 21.61 ± 3.15 |
| RMP [mV] | -77.20 | -77.22 ± 4.89 |

Figure 2 shows the APD and upstroke restitution curves for the S1S2 protocol together with experimental results, with the bars representing the standard deviation of the population (n=18).

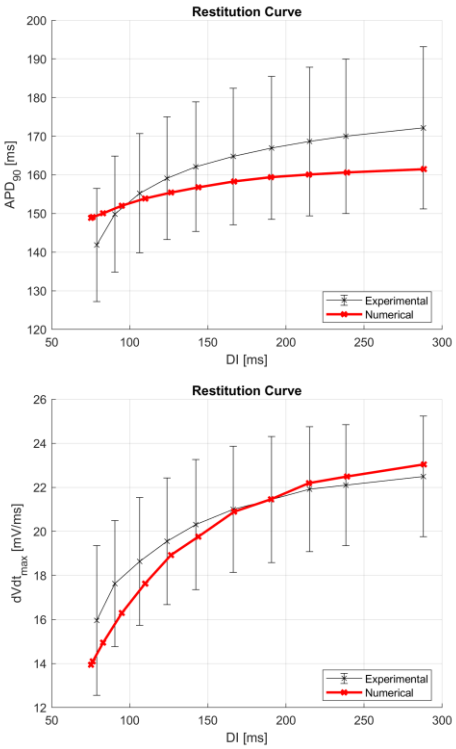


Figure 2. Experimental and numerical $APD_{90}$ (top) and $dV/dt_{max}$ (bottom) restitution behavior for the S1S2 stimulation protocol. Experimental data were reported with the corresponding standard deviation (± SD).

The restitution curve of the model falls within the experimental range but results smoother for the APD than the average experimental records. For the upstroke restitution curve of the model, in a wide range closely matches the average of the experimental records.

Figure 3 shows a table with the sensitivity analysis of the main ionic current conductance on the principal AP characteristics listed in Table 1. Results indicate that APD is particularly sensitive to the maximum conductances of all currents involved in repolarization, in particular $G_{CaL}$, $G_{Ks}$, and $G_{K1}$, with the sodium potassium pump, $G_{NaK}$ and the exchanger, $G_{NaCa}$, playing an important role as well. On the contrary, $G_{Kr}$ and $G_{CaT}$ are found to play a minor role in the APD. However, $G_{Kr}$ is found to affect AP triangulation (results not shown).

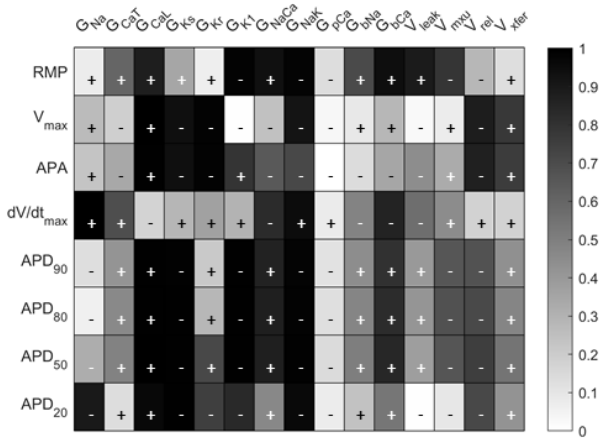


Figure 3 Sensitivity analysis of the main ionic current conductance on the AP morphology.

## 4. Discussion

Starting from the reparametrization of the well-known human action potential model, the TP04 model, this work represents the first development of a mathematical model of the zebrafish AP. The model accounts for the most important transmembrane currents that characterize the zebrafish together with the intracellular ion concentration dynamics. One of the main characteristics of this model lies in the fact that model calibration and verification has been performed using tissue simulations rather than isolated cell simulations to mimic as close as possible the experimental conditions. From the analysis of the results, it emerged that the numerical model well describes the AP waveform (as shown in Figure 1) and its morphology which are found to be within the experimental range, with the exception of the peak of the action potential, $V_{max}$, and the action potential amplitude, APA, which appears underestimated. However, this may be associated with the fact that recordings, and simulations, have been performed in tissue where a significant variability in the conduction velocity was found (results not shown). In this regard, reducing the conduction velocity in the tissue simulations,

increased the peak of the simulated action potential. Further model refinement will also manage the slightly biphasic effect of the upstroke that can be addressed to the different calcium dynamics of the zebrafish with respect to humans. Regarding the restitution properties, the model shows a smoother APD adaptation with respect to experiments, still within the experimental range, in particular for small values of the diastolic interval but also in this case additional investigation of the calcium dynamics will be needed. The sensitivity analysis showed that by changing the parameters it is possible to cause a shifting of the restitution curve in the vertical direction, increasing or decreasing the AP characteristic under evaluation but without evident changes in the shape of the curve (i.e., slope of the restitution curve). For what concern APD, for example, from Figure 3 the model shows a significant effect of the APD with respect most of the repolarization currents. Analogous consideration can be done for the effect of the gating variables and the times constants where the $I_{CaL}$ and $I_{K1}$ seemed to be the two currents that most affect the APD (results not shown). The only exception is represented by $I_{Kr}$ and this can be associated to the different current density in the zebrafish with respect to humans.

All the results have to be considered as preliminary although promising. For this reason, further investigation and a tuning of the parameters of the numerical model will be needed.

## 5. Conclusion and future developments

This paper presents for the first time an electrophysiologically detailed model of the action potential model for the adult zebrafish able to reproduce the AP morphology, its waveform, and also the restitution behavior in different stimulation protocols. However, the model is not exempt from limitations and further improvements and investigations are required. First of all, the response to drugs has to be investigated to determine the validity of the proposed model. Additionally, the model will be implemented in a 3D model of the zebrafish [14] for a more complete analysis.

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