In Silico Electrical Modeling of Cell Aggregates

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The paper deals with two different approaches to model cell aggregates submitted to an electric stimulation, namely the equivalent circuit approach and the theoretical homogenization. For each approach, the effective impedance of the cell aggregate is given, enabling a comparison between the different models. Regarding the circuit approach, a variability in the electric parameters of the circuit in series is known to provide anomalous relaxation similar to a constant phase element model. For lognormal distribution of the parameters, a new link between the effective impedance and both arithmetic and geometric means is given. The second approach deals with the theoretical –but periodic– homogenization approach. The idea is to consider the sample as a periodic aggregate composed of a large number of cells. In each cell the electric potential is governed by the electroquasistatic model. The formal two-scale analysis leads to the so-called bidomain model, enabling a novel definition of the tissue impedance, generalizing the Maxwell-Garnett formula to cells with any geometrical configuration and without any dilution assumption. Interestingly, the microscale cell organization is shown to impact the effective impedance of the sample, linking the cell and the tissue properties.

***Index Terms*—Electrical Modeling of Cell Aggregates, Cell Networks, Homogenization, Numerical Characterization of Cell Spheroids, Multiscale Modeling**

I. Introduction

THE response of tissues and cell aggregates to electric stimulation still suffers from a lack of accurate modeling, even though the phenomenon is quite well described by the physics at the cell scale, from the linear regime (low amplitude pulse excitation) to the electroporation phenomenon [5], [4], [3], [10]. With the constantly increasing interest for bioimpedancemetry, in particular for cancer detection and treatment, such a lack of knowledge has to be overcome. Interestingly, 3D multicellular spheroids are mesoscale biological models – between the cell and the tissue scales – that mimic the tissue organization. They enable to overcome the 2D restriction of standard cell cultures and they provide an *in vitro* tissue model, which is very interesting especially in biological oncology. Understanding the electrical properties of 3D spheroids is a crucial challenge to provide a better understanding of the influence of electric field on tissues. 3D multicellular spheroids raise important theoretical and computational challenges, from theoretical homogenization approaches to high performance computing since they are made of several tens of thousands of cells.

The aim of the present paper is to compare cell circuits and homogenization approaches, and to provide a generalized impedance formula for cell aggregates. Section II presents the simple equivalent circuit approach, which provides a rough understanding of the electrical of cell assemblies with an explanation of the anomalous relaxation measured by bioimpedancemetry. As shown below, the anomalous relaxation is linked to a variability of the electrical properties of the circuit. The rigorous homogenization approach is then presented in Section III to exhibit the global behavior of the cell aggregates, accounting for the geometry of the microstructure – *i.e* the cell geometry – and thus the tissue anisotropy. A new homogenized formula of tissue impedance is proposed, generalizing the so-called Maxwell-Garnett formula to cells with any geometrical configurations and without any dilution assumption. The paper proposes thus a complementing approach to the full numerical approach previously proposed in [8], [11]. It is worth noting that this study is restricted to the linear regime.

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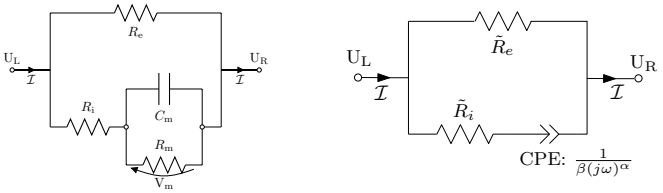
II. The equivalent circuit approach

A. Equivalent circuit of single cell

Equivalent circuits are often used to model the electrical properties of biological cell (see for instance [5], [4]). The membrane is seen as a capacitance in parallel with a resistance. The cell cytoplasm is a conductive medium modeled by a resistance in series with the membrane. This cell circuit is set in parallel with the extracellular domain, seen as a conductive medium (see Fig 1a). Using standard electric circuit laws, the



(a) Unit cell $\mathcal{C}$ (b) CPE for cell aggregate

Fig. 1. (a): Equivalent circuit of the unit cell $\mathcal{C}$ composed of a cell and its surrounding ambiant medium. (b): Equivalent circuit for cell aggregates including the Constant Phase Element (CPE) to account for cell membranes and variability.

transmembrane voltage (TMV) –the difference of potential U across the membrane– satisfies the following ordinary differential equation (ODE):

$$C_{\rm m}\partial_t {\rm V_m} + \left(\frac{1}{R_{\rm m}} + \frac{1}{R_{\rm i}}\right){\rm V_m} = \frac{1}{R_{\rm i}}({\rm U_L} - {\rm U_R}). \quad (1)$$

In time-harmonic regime, the effective admittance of the whole circuit $Y_{\mathcal{C}} := \mathcal{I}/(\mathrm{U_L} - \mathrm{U_R})$ (see Fig. 1a) depends on the pulsation ω as

$$Y_{\mathcal{C}}(\omega) = \frac{1}{R_\mathrm{e}} + \frac{1}{R_\mathrm{i}} \frac{1}{1 + Z_\mathrm{m}(\omega)/R_\mathrm{i}}, \tag{2}$$

with $Z_\mathrm{m}(\omega) = R_\mathrm{m}/(1+j\omega R_\mathrm{m} C_\mathrm{m})$, the membrane impedance. At low frequency, the cell membrane impacts the admittance, while the membrane is transparent at high frequency:

$$Y_{\mathcal{C}} \underset{\omega\to 0}{\sim} \frac{1}{R_\mathrm{e}} + \frac{1}{R_\mathrm{i} + R_\mathrm{m}} \sim \frac{1}{R_\mathrm{e}}, \tag{3a}$$

$$Y_{\mathcal{C}} \underset{\omega\to \infty}{\sim} \frac{1}{R_\mathrm{e}} + \frac{1}{R_\mathrm{i}}, \tag{3b}$$

since the membrane resistance is very high ($R_\mathrm{m} \gg R_\mathrm{i}, R_\mathrm{e}$).

B. Cell circuit networks in series

Recent investigations have addressed the micro / macroscopic relation thanks to equivalent circuit approaches to understand the anomalous relaxation measured in the experiments of tissue sample or cell aggregates impedancemetry. The idea consists in considering the spheroid (or the tissue) as a set of $N \gg 1$ unit cells (U.Cs) denoted by $(\mathcal{C}_k)_{k=1,\cdots,N}$, whose dielectric parameters are $(R_\mathrm{e}^k, R_\mathrm{i}^k, Z_\mathrm{m}^k(\omega))$. The cells are set in series, each U.C. being described by the equivalent circuit of Fig. 1a. The equivalent impedance Z_eq of such assemblies is a complex-value function of the pulsation ω given by

$$Z_\mathrm{eq}(\omega) = \frac{1}{N} \sum_{k=1}^{N} \frac{1}{\dfrac{1}{R_\mathrm{e}^k} + \dfrac{1}{R_\mathrm{i}^k + Z_\mathrm{m}^k(\omega)}}. \tag{4}$$

The factor $1/N$ is set to keep the size of the tissue sample constant. Indeed, adding more details –thus more circuits– in the tissue description should not increase the global size of the sample. If all the cells are identical, the equivalent impedance of the sample is nothing but the inverse of the U.C. admittance (2). As mentioned by Zorn [14], adding a variability in the electrical parameters leads to a global anomalous relaxation phenomenon, showing that the sample can be modeled as a Constant Phase Element (CPE) with parameters α and β, see Fig. 1b. Joris' thesis [9] investigated the link between large circuits made of slightly varying cell parameters with CPE. Here we show that for R_e and R_i following a lognormal distribution in the same range, the arithmetic mean of R_e gives the low frequency behavior of the sample while the geometric means of R_e and R_i are linked to the high frequency limit of the sample impedance. More precisely, Fig. 3a–3b shows a simulation with a circuit made of $N =$1 million of U.Cs $\mathcal{C}$ in series. Lognormal distributions with mean value and standard deviation of $(60k\Omega, 80\%)$, $(40k\Omega, 50\%)$ and $(200\mathrm{pF}, 50\%)$ are chosen for R_e, R_i and C_m respectively. Due to the high value of membrane resistance R_m, its influence is neglected. The numerical results show that the low frequency impedance is linked to the arithmetic mean of R_e^k while the high frequency impedance amplitude is given by the geometric mean of the parameters. Therefore, from the knowledge of the resistances distribution, one can obtain the formula for the equivalent outer and inner resistances $\widetilde{R_\mathrm{e}}$ and $\widetilde{R_\mathrm{i}}$ thanks to the arithmetic and geometric mean operators denoted by $\mathcal{M}_a$ and $\mathcal{M}_g$. From (3), $\widetilde{R_\mathrm{e}} = \mathcal{M}_a\left((R_\mathrm{e}^k)_{k=1}^N\right)$ and

$$\frac{1}{\widetilde{R_\mathrm{i}}} = \frac{1}{\mathcal{M}_g\left((R_\mathrm{i}^k)_{k=1}^N\right)} + \frac{1}{\mathcal{M}_g\left((R_\mathrm{e}^k)_{k=1}^N\right)} - \frac{1}{\widetilde{R_\mathrm{e}}}. \tag{5}$$

Then the corresponding CPE circuit of Fig. 1b with $\alpha = 0.91$, and $\beta = 5.9 \times 10^{-10}$ fits with the circuit in series for the lognormal distribution of the U.C parameters.

Letting N going to infinity, and assuming that the electrical parameters of the circuit $(R_\mathrm{e}, R_\mathrm{i}, R_\mathrm{m}, C_\mathrm{m})$ are characterized by specific probabilistic distribution functions denoted respectively by $\psi_\mathrm{e}, \psi_\mathrm{i}, \psi_\mathrm{m}^\mathrm{r}, \psi_\mathrm{m}^\mathrm{c}$ and mean values $\overline{R_\mathrm{e}}, \overline{R_\mathrm{i}}, \overline{R_\mathrm{m}}, \overline{C_\mathrm{m}}$, we infer the following expression of the tissue impedance:

$$Z_\mathrm{eq}(\omega) = \int_{\mathbb{R}_+^4} \frac{\Psi(\mathbf{s})}{\dfrac{1}{\overline{R_\mathrm{e}} s_1} + \dfrac{1}{\overline{R_\mathrm{i}} s_2 + \dfrac{\overline{R_\mathrm{m}} s_3}{1 + j\omega \overline{R_\mathrm{m} C_\mathrm{m}} s_4}}}\, d\mathbf{s}, \tag{6}$$

where $\Psi : \mathbf{s} \in \mathbb{R}_+^4 \mapsto \psi_\mathrm{e}(s_1)\psi_\mathrm{i}(s_2)\psi_\mathrm{m}^\mathrm{r}(s_3)\psi_\mathrm{m}^\mathrm{c}(s_4)$. The above formula is an extension of the approach proposed by Zorn [14] to explain the anomalous relaxation of simpler RC circuits. Note that the link between α and β of the CPE model and the distribution probabilities is still an open question.

III. Impedance of cell spheroids

As shown above, the cell circuit approach enables accounting for the cell variability of the tissue, but the geometrical aspects are not considered. Therefore these 0D models cannot describe the tissue anisotropy, and partial differential equations of the electric potential have to be considered.

A. Membrane voltage of 3D–cell in time-harmonic regime

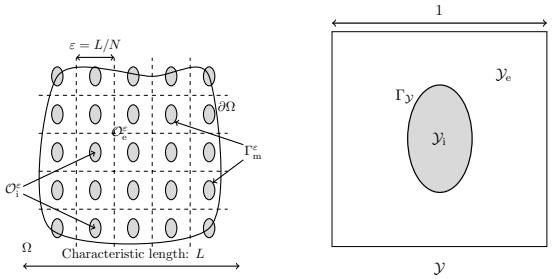
Consider now the 3D model of a single cell whose membrane is denoted by Γ_m. The membrane capacitance and conductance per surface unit are denoted by κ_m and γ_m. Let σ_i and σ_e be the (scalar) conductivities of the cell interior denoted by $\mathcal{O}_\mathrm{i}$ and the extracellular medium $\mathcal{O}_\mathrm{e}$ respectively. The TMV is denoted by $[\![U]\!]_{|\Gamma_\mathrm{m}} = U|_{\Gamma_\mathrm{m}^+} - U|_{\Gamma_\mathrm{m}^-}$. It satisfies the following equations set on Γ_m in the time-harmonic regime:

$$(\mathrm{j}\omega\kappa_\mathrm{m} + \gamma_\mathrm{m})\,[\![U]\!]_{|\Gamma_\mathrm{m}} = \sigma_\mathrm{i}\partial_\mathbf{n} U|_{\Gamma_\mathrm{m}^-} = \sigma_\mathrm{e}\partial_\mathbf{n} U|_{\Gamma_\mathrm{m}^+}, \tag{7}$$

where where ω is the pulsation, U is a complex-valued harmonic function in $\mathcal{O}_\mathrm{i}$ and $\mathcal{O}_\mathrm{e}$, and $\mathbf{n}$ the normal vector to Γ_m outwardly directed. Note that the impedance corresponding to the PDE model given by (7) is a pseudo-differential operator which links Dirichlet and Neumann traces on the membrane.

B. Periodic homogenization

Two-scale analysis [1] is a powerful tool to derive the homogenized electrical model of a tissue of characteristic size L composed of $N \gg 1$ small U.C.s, each composed of a biological cell embedded in an ambient medium, as schematised in Fig. 2. Roughly speaking, the idea of homogenization consists in transposing the microstructure model – here the cell scale model given by (7))– to the macroscopic



(a) (b)

Fig. 2. (a): The cell aggregate sample Ω composed of $N \gg 1$ unit cells. (b): the rescaled unit cell, $\mathcal{Y}$.

scale thanks to series expansion of the solution in term of the scaling parameter $\varepsilon = L/N$. From 1kHz to several MHz, the rescaled complex membrane conductivity σ_m^* defined by $\sigma_m^*(\omega) = L\,(\mathrm{j}\omega\kappa_\mathrm{m} + \gamma_\mathrm{m})$, is such that $\varepsilon\sigma_m^* = O(1)$.

Several papers have been devoted to the homogenization for syncytial tissues, a problem close to our interest, but with the scaling $\sigma_m^* = O(\varepsilon)$ in [2], [6], [12]. Such scaling is relevant for cardiac application, but it fails to describe the impedance of a biological tissue in the frequency range 1kHz to 10MHz. Following the same theoretical approach, the solution of (7) set in the domain Ω presents a spatial oscillating profile due to the alternation of extracellular and intracellular medium. The key-point of the two-scale analysis consists in splitting between the rapidly oscillating variable and the "slow" variable, thanks to the following expansion of U^ε solution to (7) in formal series:

$$U^\varepsilon(\mathbf{x}) = \begin{cases} \sum_{k\geq 0} \varepsilon^k U_\mathrm{e}^k(\mathbf{x}, \mathbf{x}/\varepsilon), & \forall \mathbf{x} \in \mathcal{O}_\mathrm{e}^\varepsilon, \\ \sum_{k\geq 0} \varepsilon^k U_\mathrm{i}^k(\mathbf{x}, \mathbf{x}/\varepsilon), & \forall \mathbf{x} \in \mathcal{O}_\mathrm{i}^\varepsilon, \end{cases} \tag{8}$$

where for any $k \geq 0$, the functions U_e^k and U_i^k are defined for $(\mathbf{x}, \mathbf{y}) \in \Omega \times \mathcal{Y}$, and are periodic in $\mathbf{y} = \mathbf{x}/\varepsilon$. Any derivative in $\mathbf{x}$ makes appear a sum of terms in power of ε, which have to be combined with the formal series. For instance in $\mathcal{O}_\mathrm{e}$,

$$\nabla_\mathbf{x} U_e^\varepsilon = \sum_{k\geq 0} \varepsilon^{k-1} \nabla_\mathbf{y} U_\mathrm{e}^k + \varepsilon^k \nabla_\mathbf{x} U_\mathrm{e}^k. \tag{9}$$

Identifying the terms with the same power in ε leads to elementary problems, which make appear the influence of the microstructure. The reader may refer to [7] for more details.

1) Effective complex conductivity of the sample

The identification process leads to the first-order correctors $\boldsymbol{W}_\mathbf{e}^\mathbf{1}$ and $\boldsymbol{W}_\mathbf{i}^\mathbf{1}$, which are harmonic vector-fields defined in $\mathcal{Y}_\mathrm{e}$ and $\mathcal{Y}_\mathrm{i}$ respectively, with vanishing mean value in $\Gamma_\mathcal{Y}$ –to ensure their uniqueness– and such that they satisfy on $\Gamma_\mathcal{Y}$

$$\left(\sigma_e \boldsymbol{\nabla_y W}_\mathbf{e}^\mathbf{1}{}_{|\Gamma_\mathcal{Y}}^{+} - \sigma_i \boldsymbol{\nabla_y W}_\mathbf{i}^\mathbf{1}{}_{|\Gamma_\mathcal{Y}}^{-}\right) \cdot \mathbf{n} = (\sigma_i - \sigma_e)\mathbf{n}, \tag{10a}$$

$$\varepsilon\sigma_m^*(\omega) [\![\boldsymbol{W}]\!]_{|\Gamma_\mathcal{Y}} = \sigma_i \left(\boldsymbol{\nabla_y W}_\mathbf{i}^\mathbf{1}{}_{|\Gamma_\mathcal{Y}}^{-} \cdot \mathbf{n} + \mathbf{n}\right). \tag{10b}$$

Periodic conditions are imposed on $\partial\mathcal{Y}$. The above correctors are used to obtain the effective conductivities of the cell and the extracellular phases denoted respectively by $\boldsymbol{\sigma}_\mathbf{e}^\sharp$ and $\boldsymbol{\sigma}_\mathbf{i}^\sharp$:

$$\boldsymbol{\sigma}_\mathbf{e}^\sharp = \sigma_e |\mathcal{Y}_\mathrm{e}| \left(\mathbf{Id} + \frac{1}{|\mathcal{Y}_e|} \int_{\mathcal{Y}_\mathrm{e}} \nabla_y \boldsymbol{W}_\mathbf{e}^\mathbf{1}\, dy\right), \tag{11a}$$

$$\boldsymbol{\sigma}_\mathbf{i}^\sharp = \sigma_\mathrm{i} |\mathcal{Y}_\mathrm{i}| \left(\mathbf{Id} + \frac{1}{|\mathcal{Y}_\mathrm{i}|} \int_{\mathcal{Y}_\mathrm{i}} \nabla_y \boldsymbol{W}_\mathbf{i}^\mathbf{1}\, dy\right). \tag{11b}$$

Note that even though the conductivities σ_e and σ_i are scalar, anisotropy of the effective conductivity may appear due to the geometry of the microstructure. Then, the homogenized problem in the limit "ε goes to 0" reads as a two-phase problem $(U_\mathrm{e}^\sharp, U_\mathrm{i}^\sharp)$ solution of the coupled problem

$$\nabla \cdot \left(\boldsymbol{\sigma}_\mathbf{e}^\sharp \nabla U_\mathrm{e}^\sharp + \boldsymbol{\sigma}_\mathbf{i}^\sharp \nabla U_\mathrm{i}^\sharp\right) = 0, \tag{12a}$$

$$\begin{aligned} \sigma_m^\sharp(\omega) v^\sharp = \varepsilon\sigma_m^\sharp(\omega) \Big(& \boldsymbol{W}_\mathbf{e}^\mathbf{1}(x/\varepsilon) \nabla U_\mathrm{e}^\sharp \\ & - \boldsymbol{W}_\mathbf{i}^\mathbf{1}(x/\varepsilon) \nabla U_\mathrm{i}^\sharp \Big) - \varepsilon \nabla \cdot \left(\boldsymbol{\sigma}_\mathbf{i}^\sharp \nabla U_\mathrm{i}^\sharp\right), \end{aligned} \tag{12b}$$

where $v^\sharp = U_\mathrm{e}^\sharp - U_\mathrm{i}^\sharp$ is the homogenized membrane potential and the complex conductivity of the membranes given by $\sigma_m^\sharp(\omega) = \sigma_m^*(\omega)|\Gamma_\mathcal{Y}|$. Boundary conditions similar to the exact problem are imposed on $\partial\Omega$. Interestingly, from (12a) one defines the effective conductivity of the tissue $\boldsymbol{\mathcal{E}}^\sharp$ as

$$\boldsymbol{\mathcal{E}}^\sharp = \sigma_e \Big(\mathbf{Id} + \mathbf{M_e} + |\mathcal{Y}_i| \left(\sigma_i \mathbf{M_i}/\sigma_e - \mathbf{Id} - \mathbf{M_e}\right)\Big), \tag{13}$$

where $\mathbf{M_{e,i}} = \frac{1}{|\mathcal{Y}_{e,i}|} \int_{\mathcal{Y}_{e,i}} \nabla_y \boldsymbol{W_{e,i}}\, dy$ and the effective admittance of the sample is then given by $\boldsymbol{\mathcal{Y}}^\sharp(\omega) = \frac{S}{\ell} \boldsymbol{\mathcal{E}}^\sharp(\omega)$, where the ratio S/ℓ is form factor, S being the surface of one electrode and ℓ the distance between the two electrodes. The formula is a geometrical generalization of (4), in the case of identical cells where the anisotropy is taken into account.

2) Link with the Maxwell-Garnett formula

Maxwell-Garnett formula has been derived under the assumption of dilute suspension of spherical balls of conductivity σ^*, without accounting for the thin membrane. The well-known formula for effective conductivity is

$$\sigma^* = \sigma_e(1 + M + M^2), \quad M = d|\mathcal{Y}_\mathrm{i}| \frac{\sigma^* - \sigma_e}{\sigma^* + (d-1)\sigma_e}, \tag{14}$$

for $d = 2, 3$. This formula has been used for spherical cells by replacing σ^* by a pseudo effective cell complex conductivity $1/(1/(\sigma_m^*) + 1/\sigma_i)$, however this approach is not rigorous due to the jump of the potential across the membrane. Indeed, the effective cell conductivtiy is a Fourier multiplier and not just a scalar (see for more details [7]). Note that if the membrane becomes very conductive, the TMV is very small and the first approximation of the above formula under dilution assumption is the Maxwell-Garnett formula.

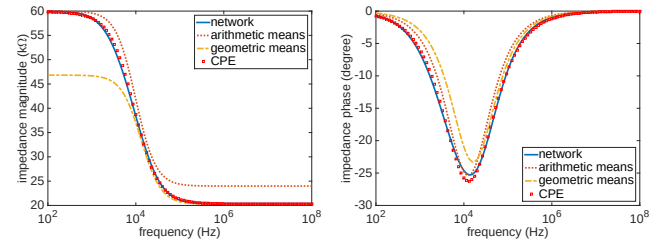
3) Numerical simulations

The parameters for the numerical simulations are given in Table I. These parameters are set in accordance with the literature [4], [5], [9]. Using (13), the effective impedance is computed for spherical cells with volume fractions $\phi_i := |\mathcal{Y}_i| = 0.12$ and 0.37. We compare the numerical results with the standard Maxwell-Garnett formula in 2D and 3D. At high dilution ($\phi_i = 0.12$) Maxwell-Garnett formula provides barely an approximation of the sample impedance,

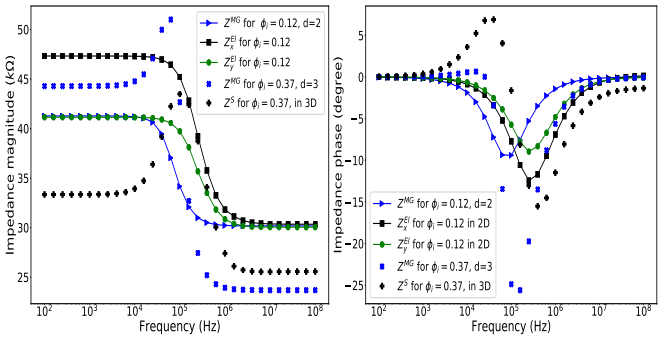
Variable	Symbol	Value	Unit	References
Cytoplasm conductivity	σ_i	1.30	S/m	[13]
Extracellular conductivity	σ_e	0.6	S/m	
Membrane capacitance	κ_m	0.01	F/m^2	[10]
Membrane conductance	γ_m	1.9	S/m^2	[10]
Characteristic length	L	1	cm	
Form factor	S/ℓ	5	cm	
small scale	ε	0.01		

TABLE I
PARAMETERS USED TO COMPUTE THE IMPEDANCE OF THE TISSUE WITH THE HOMOGENIZATION APPROACH IN FIG. III-B3 (BOTTOM).

but without accounting for the anisotropy. At lower dilution, the discrepancy between standard Maxwell-Garnett formula, which have been derived for homogeneous small inclusions and our approach is quite high as shown in Fig. 3c–3d. The impedance phases and amplitudes are in the same range as the results of section II, thus confirming the analogy between the homogenized admittance (13) and the circuit impedance (4).



(a) RC circuit (b) RC circuit



(c) Homogenization (d) Homogenization

Fig. 3. Top: Amplitude (3a) and phase (3b) of the equivalent impedance $Z_{\rm eq}$ given by (4), and comparison with the CPE of parameter $\alpha = 0.91$, $\beta = 5.9\times 10^{-10}$. Bottom: Amplitude (3c) and phase (3d) of $\mathbf{Z}^\sharp = (1/\mathbf{Y}^\sharp\alpha)_{\alpha=\mathrm{x,y,z}}$ for the cell ellipse in 2D (Z_x^{El}, Z_y^{El}) of volume fraction $\phi_i = |\mathcal{Y}_i|/|\mathcal{Y}| = 0.12$ and a 3D sphere of volume fraction $\phi_i = |\mathcal{Y}_i|/|\mathcal{Y}| = 0.37$ denoted by Z^S. Comparison with the corresponding Maxwell-Garnett formula (14) in blue.

IV. CONCLUSION

Two different approaches to model cell aggregates have been presented above. The equivalent circuit approach has been shown to exhibit the main electrical features of the cell aggregates, including the anomalous relaxation. Interestingly, for lognormal distributions, the low frequency limit of the aggregate impedance is the mean of the resistances $R_{\rm e}^k$, while the high frequency limit involves the geometric mean of both $R_{\rm e}^k$ and $R_{\rm i}^k$. The main interest of this approach lies in its simplicity. In particular, due to the small number of parameters, the identification of the CPE model parameters is feasible. Therefore, CPE models are interesting for the calibration with experimental measurement. However the geometry of the microstructure cannot be obtained.

The main result of this paper consists in deriving a new mixture formula for cells, thanks to the rigorous homogenization approach accounting for cell geometry and thus describing the tissue anisotropy. Interestingly, our formula is a generalized Maxwell-Garnett formula type for cell aggregates in any geometrical configuration and without any dilution assumption. The current main drawback of the proposed homogenization lies in the assumption of periodicity, which prevents accounting for cell variability. Forthcoming investigation will consist in deriving a stochastic homogenization approach to account for the cell variability, and thus for the anomalous relaxation of the cell aggregates. In addition, under hypothesis of dilution, the explicit formula of the solution to (10) will be derived and compared to data measurements in a future work.

These two approaches have to be complemented with a purely numerical approach, which consists in solving the cell scale PDE (7) in a large number of cells. A first impressive result has been obtained by Mistani *et al.* in the context of electroporation [11], where the time-dependent nonlinear model of Leguèbe *et al.* [10] has been simulated on 30 thousands of cells.

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