1) WM topic and description

EMF Microdosimetry:

Quantitative study of the spatial, temporal, and spectral distributions of EM fields imparted to cellular and subcellular biological structures.

2) Comprehensive review (state-of-the-art)

In the study of the biophysical mechanisms at the basis of the EM field interaction leading to biomedical applications, one needs also to know the field strength at the microscopic scale to establish a quantitative relation between the field and the observed effect [1]. Indeed, going down toward microscopic structures, the evaluation of the average exposure of tissues and organs is not sufficient since permittivity and conductivity inhomogeneity of biological structures has to be taken into account [1]. Therefore, microdosimetric techniques are needed for calculating the real distribution of the field on sub-cellular structures, such as cell membranes and organelles [2]-[5]. Many papers have been published in these last years of this topic, and a great impulse came also from the key role that microdosimetry plays in some diagnostic techniques based on dielectric spectroscopy and in the estimation of the transmembrane voltage in electroporation [6]-[9].

To approach the microdosimetric problem, two fundamental steps are needed. The first one involves the setup of a proper dielectric and geometrical cell model. Typically the biological cell is considered as a multilayered structure. The most common choice is the three-layered cell [2]–[11], where the cytoplasm, the plasma membrane, and the extracellular medium are considered as separate compartments, but some papers consider also more shells like nucleus or bound water layers [12]-[14].

As for the dielectric model main attempts are in order to dielectrically characterize the plasmatic membrane, which is generally considered one of the main EM interaction targets. The initial models adopted considered a frequency-independent behavior of the cell membrane, signifying a pure capacitive behavior [2], [10], [11], [15]. Recently dispersive models have been considered unavoidable to study CW EM fields with frequency higher than hundreds of MHz and nanopulses electric fields [3]-[5], [8], [9], [12]-[14]. Some authors pointed out also the importance of cytoplasm characterization [16]

The second step concerns the setup of appropriate EM solutions on the cell environment. Almost all authors choose a quasi static approach, indeed the theoretical problem was addressed at the single-cell level, using the assumption $\lambda \gg R$, where $\lambda$is the EM field wavelength in the tissue and R the maximum dimension of the cell; this is reasonable as, in the RF range, λ is always greater than some millimeters and R is of the order of tens of μm. the frequency dependence of the induced field, when necessary, is usually taken into account through the frequency dependence of the complex permittivity of the cellular compartments. Three main kind of solutions exist: circuit models, analytical solutions applied to simplified cell shapes, such as spherical and spheroidal multishell models, and numerical methods applied to irregular-shaped cells [2]–[18]

Some general consensus exists that up to the MHz range the cell membrane insulate quite well the cell interior with respect to the electric field; at higher frequencies such a capacitive effect decreases. One can roughly say that around 100 V/m at low frequencies and 10 kV/m at high frequencies are necessary to induce values of transmembrane voltage comparable to the ones of normal cell functions (mV). For similar reasons the dimensions of the cells are important in determining the induced transmembrane potential at low frequencies: the larger the cell the

higher the induced TMP. For what concerns the high frequency components it is possible to say that membrane dielectric values influence inversely the induced TMP: the lower the membrane permittivity, the higher the TMP. At high frequencies, the role of dielectric modelling is predominant in driving E field intensities also when dealing with realistic cell shapes: while cell morphology can influence up to 30% the final value [10], [15], the choice of permittivity can change the TMP up to ten folds [4], [9]. Realistic models are unavoidable when dealing with applications where the effect plausibly arises from the field distribution within the cell and not simply to the maximum E field value on it (this last is the case for example of electroporation). At lower frequency the cell shape becomes more important, in particular when a preferential direction is identified [5], [18]

3) Gaps and challenges

In spite of the huge work done, some open issues are still alive and represent key challenges in the development of microdosimetry.

The first one is represented by the microthermal issue.

About this point, there is a quite general consensus that microthermal heating has to be considered negligible [11]. Nevertheless, it is important to note that neither temperature mapping at single cell level, nor a reliable assessment on microthermal properties of the cell, have ever been provided in the bioelectromagnetic context. Conversely recent advances in nonstandard laboratory instrumentation, such as differential scanning calorimetric technique, or scanning thermal microscopy, permit both images of microthermal distributions and the estimation of microthermal properties [19]. Thus, the application of such techniques could in the future have the final word on this topic.

Second important point is represented by the computational/numerical difficulty to relate the microscopic field at the level of the single cell to the field distribution at the mesoscopic one were clusters of cells are to be considered. The main challenge is the problem of efficiently coupling different models at the adjacent spatial scales. A possible solution consists in the application of bridging domain coupling techniques [20] that furnish a linkage between different kinds of models coexisting inside the bridging domains where the two spatial scales overlap

Finally, the importance of molecular details and properties of the membrane like the molecular structure of the membrane bilayer, anisotropy, the presence of solvated shells, water at interface, are at the center of a wide scientific debate, lots of doubts exist whether and how they must be considered.

Major challenge is represented by the capability to validate microdosimetric results with reliable experimental work.

4) Objectives to be achieved

Main objective will be to obtain microdosimetric models able to produce reliable microdosimetric results; one fundamental step will be the clarification of the microscopic level description necessary (molecular detailed or continuum).

Second objective is the comparison of the results, properly experimentally validated, obtained for different typical signals in order to obtain conceptual rules related to EM intensities, frequency content, repetition etc. Such knowledge will be valid to extrapolate and predict EM fields at the

microscopic level to understand where and when microdosimetry is a helpful, powerful tool for efficient biomedical application development.
Last objective will be the accurate theoretical and numerical study of the relationship of microdosimetry with macroscopic fields.

5) Proposed research activities

It is possible to identify some main activities:

1) Development of an appropriate set of realistic cell models (blood, sensory and neuronal cells etc.) both from a morphological point of view and from the necessary structural details (cell interior compartments like nucleus, endoplasmatic reticulum etc.). For this activity a strong multidisciplinary work between biologists and engineers will be necessary.
2) Provide dielectric cell models as reliable as possible, on the bases of physico-chemical properties (molecular based description) and experimental data. For this activity a cooperation of theoretical and experimental work will be necessary.
3) Set up of appropriate strategies for a one-to-one comparison between experimental and theoretical determinations able to validate the microdosimetry results. Possible approaches are: electric measurements (currents and impedance) on single cells with ad hoc single cell microchambers, and more invasive labeling techniques like both potentiometric and thermal dyes. This will be mainly an experimental activity.
4) Development of an integrating strategy with the upper levels of complexity towards macroscopic dosimetry and applicators design. This will be mainly a theoretical/numerical activity.

References

1. Apollonio et al., 2013, IEEE MTT, 61, 2031-2045
2. Liu and Cleary, Bioelectromagn., vol. 16, pp. 160–171, 1995.
3. Kotnik and D. Miklavcic, Bioelectromagn., vol. 21, pp. 385–394, 2000
4. Liberti, et al., IEEE Antennas Wireless Propag. Lett., vol. 8, pp. 865–868, 2009
5. Merla et al. IEEE Trans. Microw. Theory Techn., vol. 58, no. 3, pp. 691–698, Mar. 2010
6. Smith et al. IEEE Trans. Plasma Sci., vol. 34, no. 4, pt. 2, pp. 1394–1404, Aug. 2006.
7. Joshi et al. IEEE Trans. Plasma Sci., vol. 32, no. 4, pp. 1677–1686, Aug. 2004.
8. Merla et al., IEEE Trans. Biomed. Eng, vol. 58, no. 5, pp. 1294–1302, 2011.
9. Merla et al., IEEE Trans. Biomed. Eng., vol. 49, no. 8, 2302 - 2311, 2012.
10. Martin et al. Bioelectromagn., vol. 25, pp. 631–633, 2004.
11. Foster, Schwan, Handbook of Biological Effects of Electromagnetic Fields, 1986, pp. 27–9
12. Denzi et al., J Membrane Biol., 246(10):761-767.
13. Simenova and Gimsa, Bioelectromagnetics, vol. 27, pp. 652–666, 2006
14. Kotnik and D. Miklavcic, IEEE Trans. Biomed. Eng., vol. 47, no. 8, 2000
15. Munoz et al. Phys Med Biol 48: 1649–1659, 2003
16. Simenova et al., Bioelectrochemistry, vol. 56, pp. 215–218, 2002
17. Kotnik and D. Miklavcic, *Biophys. J.*, vol. 90, pp. 480–491, 2006
18. Maswiwat et al., Bioelectrochemistry, vol. 74, pp. 130-141, 2008
19. Gill et al. J. Biomolecular Techn., vol. 21, no. 4, pp. 167–193, 2010.
20. Xiao et al. ,J. Comput. Theoret. Nanosci., vol. 5, pp. 1–10, 2008