COST-ACTION BM1309

European network for innovative uses of

non-ionizing electromagnetic fields

in

biomedical applications (EMF-MED)

WORKING MODULE PROPOSAL

Zebrafish embryo model: from theory to experimental interaction with EMF

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1. WM Description

Nowadays, the zebrafish (ZF) model is becoming more and more important in medical, diagnosis, biotechnological, toxicological research areas. It has proven to be a powerful research tool for genetics, developmental biology, toxicology, neuroscience, oncology, drug discovery, cardiovascular disease and function, and many others. However, only few works has been published related to zebrafish and electromagnetic field exposure.

ZF is genetically similar to humans and easier to house and care for than rodents. Externally fertilized, zebrafish has around 200-300 offspring compared to the 5-10 obtained from rodents. Zebrafish embryos (ZFe) grow and develop very quickly, and both embryo and larvae are transparent, making them accessible to observation and manipulation at all stages of their development facilitating experimental techniques.

Therefore, our proposal is to present the zebrafish as a powerful model for getting new insights into the biological mechanisms underlying the interaction between the electromagnetic field and the living organisms. Moreover, the zebrafish embryo model can complement rodent or cell culture studies, making it an invaluable tool for risk assessment of EMF as well as in this research network on biomedical applications of EMF.

2. Comprehensive review (state of the art)

A deeper understanding of the non-thermal biological effects resulting from human exposition at (low level) electromagnetic fields (EMFs) regarding to the development of cancer and non-cancer applications are the main objective of this Action. However, interaction mechanisms are not well understood and the synergy of theoretical, in silico, in vitro, animal and clinical studies, associating physics and engineering with medicine and biology, is essential to achieve the goal. Then, from this synergy is also expected to get new insights into the effects of human chronic exposure to EMFs.

Regarding to human health risk, the World Health Organization (WHO) pointed out in its Research Agenda several recommendations about human exposure to extremely low frequency and radiofrequency EMFs – respectively, in 2007 and 2010 [1, 2]-. WHO Research Agendas recommended, with different priorities, the following studies:

- effects of early-life and prenatal EMF (ELF and RF) exposures on development, behavior and cognitive functions,
- effects of RF exposure on ageing and neurodegenerative diseases,
- development of an experimental animal model relevant to analyze the association of EMF exposure with childhood leukaemia,
- evaluation of co-carcinogenic effects of ELF fields,
- effects of ELF magnetic field (MF) exposure on immune and haematopoiesis systems on development in juvenile animals.

When these research needs cannot be achieved by means of human studies, animal models are essential. Frequently, rodents have been used for assessing possible effects of EMF on reproduction, development, cognitive functions, neurodegeneration and cancer, and also rabbits, worms and flies [3]. Few works have paid attention to ZFe for risk assessment or potential effects of EMF. These studies of EMFs (ELF-MF and RF) effects on several aspects of reproduction and embryonic development suggest the ZFe as a reliable model to investigate the biological effects of EMF [4].

The investigation of a huge number of disorders or diseases is being also studied with the zebrafish model. For example, the zebrafish is becoming an increasingly popular model for the investigation of Alzheimer. The zebrafish has complemented studies using other models, helping to complete the understanding of the disease [5]. Many others diseases regarding to possible innovative applications of EMFs in EMF-MED have been studied using the zebrafish model:

1. Cancer issue [6-8]
2. Tissue regeneration [9]
3. Electric stimulation [10-13]

Dosimetric studies have also a critical role for designing and interpreting experimental studies. Anatomically accurate whole body human models developed for dosimetric studies made possible the risk assessment of human exposure to EMFs, including several gestational stages [14-17]. Despite of the great potential and improvement of these human numerical models [18], only animal studies make practical to perform experimental studies of pre- and post-natal effects of EMF exposure on development, cognitive or other effects, thus demanding also anatomical models for animals of experimentation. So, several rodents developed by IT'IS Foundation –both male and female, pregnant females, rodents with tumors– have been used for *in vivo* and dosimetry studies [19-21].

According to the advantages of the ZFe presented, we propose the development of a precise numerical model of the zebrafish embryo that could provide a new way for studying the interaction of EMF with living organisms: *in vivo* studies with the ZF embryo and the assessment of dosimetric quantities, analysis of behavioral or cognitive patterns in adult period of the ZF embryo exposed, as well as other relevant information related to other diseases of interest and applications expected from EMF in the framework of this COST Action.

3. Gaps and challenges

The zebrafish embryo model is proposed as a potential "new model" for studying the interaction of EMF with living organisms, which could provide new insights into risk assessment for chronic human exposure to EMFs and into the evaluation of potential biomedical applications of EMF. The synergy among interdisciplinary fields through the Working Groups is needed.

Zebrafish embryo develops quickly and important biological changes occur only in 24 hours. Then, the advantage of a fast development becomes a disadvantage when dielectric characterization and/or numerical modelling of the embryogenesis is to be met. Then, one of the challenges is to follow the rapid changes and/or establishing the dielectric characterization of the valuable stages of development needed as accurately as possible.

The size of the ZFe is between 1 and 1.2 mm so numerical modelling must be supported for structural models accordingly to the resolution needed.

Eventually, developing *lab-on-a-chip* devices for observing dielectric changes in ZFe exposed to toxic agents or monitoring other parameters of interest regarding to diagnostic, drug discovery, nanoparticle interaction, etc, represents an engineering challenge.

4. Objectives to be achieved

According to the proposal, the objectives to be achieved in this WM are:

1. Dielectric characterization of zebrafish embryo model:
   - selecting the development stages of the ZFe of interest for an specified study
   - measurement techniques:
     electrokinetic techniques (EK), electrical impedance spectroscopy (EIS), electrical impedance tomography (EIT)

2. Development of *lab-on-a-chip* devices for ZFe:
   - detecting changes in dielectric properties during the ZFe development, after exposition to physical/chemical agents
   - array of sensors for high-throughput screening protocols with ZFe
   - monitoring/imaging ZFe for studies of interest regarding to biomedical applications

3. Development of numerical models of zebrafish embryo at selected stages:
   - getting an accurate structural description and developing models of the EM response both at low frequency and RF and microwaves.

4. Exploitation of the numerical model of the ZFe:
   - interpretation and assessment of *in vivo* studies of exposed ZFe to EMFs
   - studies of pre-natal and post-natal studies of EMF exposure through ZFe
   - behavioral studies of adult ZFe exposed
   - other applications regarding to expected applications suggested through EMF-MED

5. Proposed research activities

Accordingly to the objectives the proposed research activities in this WM are:

1. Development of numerical models of zebrafish embryo
   - Establishing the structural accurate models of the ZFe at selected stages, from optical (…) or MR imaging techniques
   - Developing multi-compartment dielectric models of the electromagnetic behavior, using FEM, BEM or FDTD

2. Dielectric characterization of the zebrafish embryo model
   - Experimental dielectric measurements:
     Dielectric spectroscopy of zebrafish embryos suspensions, and individual ZFe at frequencies ranging from kHz to GHz.
     Electrorotation and dielectrophoresis of individual ZFe
     Electrical tomography impedance of individual ZFe
   - Obtaining the dielectric properties of ZFe through characterized structural model and experimental measurements

3. Study of the potential effects of low level electromagnetic fields on the development and physiology of zebrafish embryos and adults, regarding to risk assessment and/or

multiple beneficial applications of EMFs suggested within the framework of this EMF-MED COST Action.

- Effects from multiple-frequency EMF exposure
- Effects from co-exposures of EMF and other physical and chemical agents

6. References

1. *WHO research agenda for extremely low frequency fields.* 2007.
2. *WHO research agenda for radiofrequency fields.* 2010.
3. SCENIHR, *Scientific Committee on Emerging and Newly Identified Health Risks. Potential health effects of exposure to electromagnetic fields (EMF).* 2013.
4. Li, Y., et al., *Extremely low-frequency magnetic fields induce developmental toxicity and apoptosis in zebrafish (Danio rerio) embryos.* Biol Trace Elem Res, 2014. **162**(1-3): p. 324-32.
5. Newman, M., E. Ebrahimie, and M. Lardelli, *Using the zebrafish model for Alzheimer's disease research.* Frontiers in Genetics, 2014. **5**: p. 189.
6. Feitsma, H. and E. Cuppen, *Zebrafish as a Cancer Model.* Molecular Cancer Research, 2008. **6**(5): p. 685-694.
7. de Jong, M., J. Essers, and W.M. van Weerden, *Imaging preclinical tumour models: improving translational power.* Nat Rev Cancer, 2014. **14**(7): p. 481-493.
8. Kiesel, M., et al., *Swelling-activated pathways in human T-lymphocytes studied by cell volumetry and electrorotation.* Biophysical Journal, 2006. **90**(12): p. 4720-4729.
9. Gemberling, M., et al., *The zebrafish as a model for complex tissue regeneration.* Trends Genet, 2013. **29**(11): p. 611-20.
10. Pineda, R., C.E. Beattie, and C.W. Hall, *Closed-loop neural stimulation for pentylenetetrazole-induced seizures in zebrafish.* Dis Model Mech, 2013. **6**(1): p. 64-71.
11. Kalueff, A.V., A.M. Stewart, and R. Gerlai, *Zebrafish as an emerging model for studying complex brain disorders.* Trends Pharmacol Sci, 2014. **35**(2): p. 63-75.
12. Mussulini, B.H., et al., *Seizures induced by pentylenetetrazole in the adult zebrafish: a detailed behavioral characterization.* PLoS One, 2013. **8**(1): p. e54515.
13. Ward, B.K., et al., *Strong Static Magnetic Fields Elicit Swimming Behaviors Consistent with Direct Vestibular Stimulation in Adult Zebrafish.* PLoS ONE, 2014. **9**(3): p. e92109.
14. Cabot, E., et al., *Quantification of RF-exposure of the fetus using anatomical CAD-models in three different gestational stages.* Health Phys, 2014. **107**(5): p. 369-81.
15. Fiocchi, S., et al., *SAR exposure from UHF RFID reader in adult, child, pregnant woman, and fetus anatomical models.* Bioelectromagnetics, 2013. **34**(6): p. 443-52.
16. Nagaoka, T., et al., *An anatomically realistic voxel model of the pregnant woman and numerical dosimetry for a whole-body exposure to RF electromagnetic fields.* Conf Proc IEEE Eng Med Biol Soc, 2006. **1**: p. 5463-7.
17. Liorni, I., et al., *Dosimetric study of fetal exposure to uniform magnetic fields at 50 Hz.* Bioelectromagnetics, 2014. **35**(8): p. 580-597.
18. Marie-Christine, G., et al., *Development of a new generation of high-resolution anatomical models for medical device evaluation: the Virtual Population 3.0.* Physics in Medicine and Biology, 2014. **59**(18): p. 5287.
19. Kuster, N., et al., *Methodology of detailed dosimetry and treatment of uncertainty and variations for in vivo studies.* Bioelectromagnetics, 2006. **27**(5): p. 378-391.
20. Oberto, G., et al., *Carcinogenicity Study of 217 Hz Pulsed 900 MHz Electromagnetic Fields in Pim1 Transgenic Mice.* Radiation Research, 2007. **168**(3): p. 316-326.
21. Schönborn, F., K. Poković, and N. Kuster, *Dosimetric analysis of the carousel setup for the exposure of rats at 1.62 GHz.* Bioelectromagnetics, 2004. **25**(1): p. 16-26.