COST EMF - MED (Action BM1309):
European network for innovative uses of EMFs in biomedical applications

STSM Report:

EM Study of the Role of Microtubule Cytoskeleton in Dielectric Properties of Cells

Researcher: Ondřej Kučera. email: kucerao@ufe.cz

Home Institution: Institute of Photonics and Electronics, The Czech Academy of Sciences.
Contact: Michal Cifra. email: cifra@ufe.cz

Host Institution: Instituto de Salud Carlos III. Contact: Victoria Ramos Gonzáles

STSM Reference: ECOST-STSM-BM1309-010416-071319

STSM dates: FROM 1thApril 2016 TO 25th April 2016

Abstract:

Microtubules, a part of the cytoskeleton in eukaryotic cells, play several important roles in the physiology of a cell. Thanks to that they are targets of therapeutic strategies against cancer. Since microtubules are electrically polar, they can be moved and oriented both in static and alternating electric fields. The goal of this Short Term Scientific Mission was to bring experimental data about the role of microtubule cytoskeleton in electromagnetic response of cell or organism models such as human fibroblasts or zebrafish embryos, respectively. Series of experiments combining biological, biochemical, engineering and physical know-how was performed in order to identify the perspective avenues of research on contribution of microtubules to biological systems' response to non-ionizing electromagnetic exposure.

A. Purpose of the STSM

The purpose of this Short Term Scientific Mission was to (i) bring experimental data about the role of microtubule cytoskeleton in electromagnetic response of cell models and to (ii) help to establish cooperation between involved parties in the field of biomedical use of electromagnetic fields.

While several works proposed that the interaction of electromagnetic field with microtubules could provide an explanation of observed effects, the mechanistic study on the effects of electromagnetic fields on cytoskeleton was missing. Better understanding of the contribution of cytoskeleton to dielectric response of cells is, therefore, highly needed for a general biophysical insight as well as for the development of novel diagnostic methods and therapeutic strategies. The scientific purpose of this Short Term Scientific Mission was to provide preliminary data for assessment of the role of microtubules in the response of observed biological samples to external electromagnetic fields.

Besides its scientific goals, the Short Term Scientific Mission was aimed on sharing of the experience end establishing cooperation between the host and home institutions. Specifically, the applicant aimed to (i) learn the procedure of preparation of Zebrafish embryos and human fibroblasts and (ii) acquire practical experience with electro-rotational and magnetic field exposure setups. The host groups aimed to benefit from applicant's experience with design and use of planar wave-guide sensors for dielectric characterization of biological samples. Preliminary data gained during the Short Term Scientific Mission should serve as a basis for development of collaboration between involved research groups in the form of joint research projects and scientific mobility projects.

B. Work Description

Three types of experiments were performed. Firstly, zebrafish embryos were exposed to chemical agents disintegrating microtubules and their dielectric response was compared to controls with intact cytoskeleton. Secondly, fibroblasts were exposed to magnetic fields and the difference in the morphology of microtubule network was compared with controls. Thirdly, the dosimetric study was performed in order to assess the intensities of the electromagnetic field from radio-communication devices acting on microtubules in humans.

Zebrafish embryos were collected in the early stage of their maturity and were exposed to nocodazole, which is a cytotoxic agent responsible for cytoskeleton disintegration, at late blastula stage. The eggs were examined for dielectric changes in an electrorotational study. The main idea is that cells with disintegrated microtubular cytoskeleton should have different electrical polarity and different mechanical properties because cytoskeleton mechanically supports the developing egg. The response to external force of electrical character should then differ from intact cells. Two groups of embryos were analyzed after their treatment with 10 μg/ml nocodazole during 30 minutes (first group) or 1 hour (second group). The action of the agent is expected to block the development of the embryos. After washing with the buffer, the embryos were tested for dielectric response in rotating electric field in a solution of defined conductivity. The speed of their rotation was recorded and the morphological changes were tracked.

Fibroblasts and their β-tubulin were labeled using appropriate immunoassay in order to visualize a potential morphological change after exposure to magnetic field. After incubation, fibroblasts were exposed to alternating magnetic field (MF) with stationary component for 9, resp. 24 hours. The proliferative response of cells to magnetic field was measured as a difference of viable cells by means of Trypan blue exclusion analysis. Also, visual inspection was performed to test for changes of morphology in response to magnetic fields. The β-tubulin expression was analyzed and quantitated by immunocytochemistry through computer-assisted image-analysis.

Dosimetric study was performed using standard radio-frequency electromagnetic field dosimeters in order to assess the absorption of high-frequency electromagnetic energy in human body and the shielding effect of human body in exposure studies. The purpose was to estimate the intensity of electromagnetic field acting on microtubule network in cells in human body. The experiments were carried out in defined field structure in both anechoic and echoic environments.

C. Results

The results obtained demonstrated a proof-of-concept that microtubule network is a good candidate for studying morphologic changes in cells exposed to electromagnetic fields. However, the contribution of microtubules to response of cells is probably insignificant for common intensities of the field.

Electrorotational studies using zebrafish embryos shown that exposure to nocodazole has unspecific impact on the morphology of an embryo. The impact on dielectric properties is observable yet also unspecific. In general words, the morphology of embryos is rather disorganized after the exposure and the dielectric response follows the same avenue. More experimental effort is needed to assess and distinguish contribution from mere disorganization and the lack of strong electrically polar components (microtubules) in embryos. We expect that detailed fluorescent studies may shed more light on this issue.

The effect of magnetic field on the proliferative response was not significant. However, a tendency of increase in the expression of β-tubulin was observed. This MF effect is being evaluated with other analysis, that are underway. We tried control experiment using highly diving cell models in order to observe some changes in the proliferation and/or morphology and the results were insignificant. We may conclude that the exposure to magnetic field has a potential impact in cytoskeleton but this does not translate to substantial proliferative and morphologiacal changes.

Dosimetric studies have shown that the absorption in the body effectively shields the electromagnetic field and that the intensities recorded by dosimeters are directionally dependent leading to underestimation of the exposure level for scenarios where the dosimeter is in the shadow of the body. This has a direct impact on the intensities expected to act on cytoskeleton and microtubule network in cells in human body. We may state that the higher levels of exposure suggested by dosimetric studies should be taken as a reference for studying this issue.

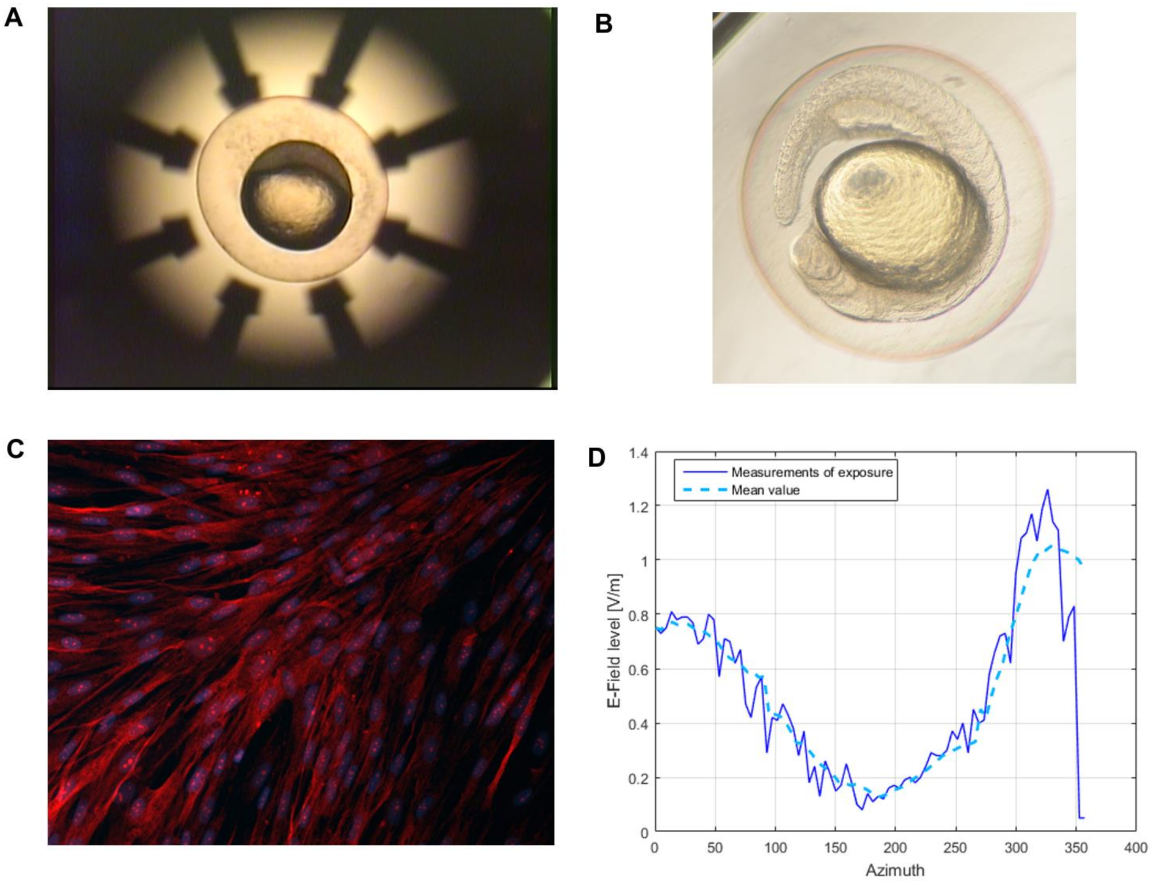

Fig. 1 – Zebrafish embryo during experiment in the electro-rotation setup (A), development of the embryo studied after magnetic field exposure (B), β-tubulin labeled in red in fibroblasts after exposure to magnetic field (C), and the directional dependence of the intensity of the electric field showing the shadow effect of human body (D).

We expect more detailed results to be published using channels specified in the following.

D. Future collaboration with host institution

The collaboration established during this Short Term Scientific Mission is foreseen to continue in future. We plan to use the framework of both national and European funding schemes for future collaboration. The unique combination of expertise of the home and the host institution lays solid grounds for preparation of joint projects aimed on investigation of the interaction of electromagnetic fields with cellular and subcellular systems.

E. Expected Publications

The preliminary results shown here need further work in order to be suitable for journal publication. Nevertheless, we expect to present the obtained results during upcoming scientific conferences. A journal paper is foreseen in case of successful continuation of the collaboration established during this Short Term Scientific Mission. This is highly desirable, however, it depends on the success of future research and mobility projects proposals.

Confirmation by the host institution of the successful execution of the STSM:

We confirm that Ondřej Kučera has performed the research work as described above.

Victoria Ramos González	Ondřej Kučera
Signature	Signature

Hospital Universitario Ramón y Cajal
SaludMadrid
Comunidad de Madrid