COST EMF - MED (Action BM1309):
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STSM Report:

DIELECTRIC PROPERTIES MEASUREMENTS OF AN IN VIVO BREAST TUMOR MODEL

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Abstract:

The advances in theranostic technologies based on the interaction of electromagnetic fields with biological tissues led to the development of dielectric spectroscopy, with particular attention to the living biological tissue. This STSM aimed to the measurements of the dielectric properties of an original *in vivo* breast model realized in a rat and simulating the actual condition of a human breast. The model accounts not only fat tissue, but fibrous, lymphatic and vascular components also, to simulate the dis-homogeneity of the human breast. Measurements of complex dielectric permittivity were conducted on *in vivo* samples with a portable setup, fully validated on reference liquids and *ex vivo* tissues, particularly suitable to conduct measurements in laboratories dedicated to the animal tests.

A. Purpose of the STSM

The knowledge of the dielectric properties of tissues is fundamental for the development of theranostic techniques based on the interaction of electromagnetic fields (EMFs) with biological tissues, possibly with the help of targeting paramagnetic nanoconstructs. Up to now, some gaps of knowledge are present in the characterization of dielectric behavior of tissues and tumors [1-6], especially regarding *in vivo* measurements. In fact it is important to investigate how the irreversible variations (cell degradation, dryness, and temperatures variation, etc.) occurring in biological tissues after their excision from the host can infer the dielectric properties behavior. In this STSM, the dielectric properties of an *in vivo* breast model developed at the Academy of Sciences of the Czech Republic by Dr. Luca Vannucci were measured in the frequency range of 500 – 3000 MHz. This breast model was developed for hyperthermia studies and it is under study for original model publication.

B. Work Description

Prior to this STSM, a portable system for the broadband measurement of dielectric properties of tissues was set up (Figure 1) in the Enea labs. The setup consists in a portable spectrum analyzer (FSH8 Rhode & Schwarz 300 kHz-8 GHz), equipped with a tracking generator to operate as a Vector Network Analyzer (VNA) connected to an open-end coaxial sensor (Keysight 85070E Dielectric Probe Kit) through a low phase variation coaxial cable. The instrument is remotely controlled by a homemade Labview code which enable the acquisition of S_{11} parameters necessary for the evaluations of complex permittivity.

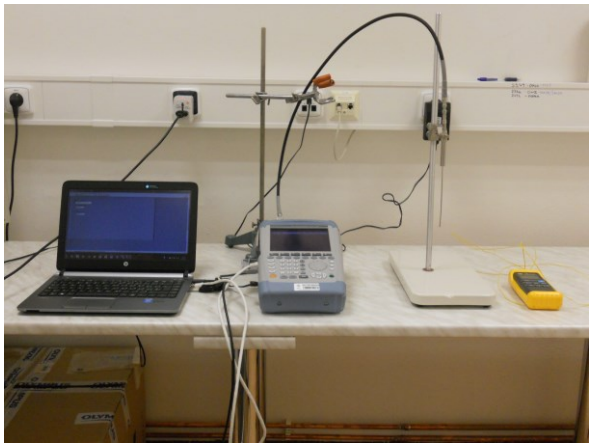

Figure 1: Portable set up for dielectric properties measurement

The measurement procedure was:

1. Calibration of the VNA in the frequency range 500 – 3000 MHz;
2. Calibration of the dielectric characterization setup by measuring the complex S_{11} of four reference conditions (i.e. open circuit, short circuit, distilled water and methanol). Temperature measurement (Fig. 1) of the liquids was taken into account in the calibration;
3. Measurement of the complex S_{11} returned by the probe when immersed into the sample under test;
4. Processing the complex S_{11} by in-house Matlab [7] routine to calculate the complex permittivity of the sample under test versus frequency.

The reliability of this procedure was proved during a short term mission performed in the framework of MiMED TD1301 COST Action [COST-STSM-ECOST-STSM-TD1301-010216-071164 Report] carried out in ENEA labs.

In this STSM, the measurements of the *in vivo* dielectric properties of both healthy and tumorous breast models developed in rats, developed at the Academy of Sciences of the Czech Republic by Prof. Luca Vannucci were scheduled. Unfortunately, at the date of STSM, only the rats with the healthy breast models were available, in fact in the rats treated with the injection of cultivated tumor cells in the breast model, the tumor regressed in few weeks.

The *in vivo* model, developed in the rat, simulates the condition of a human breast (in the following indicated as pseudo-breast - PB). The PB is a ball of subcutaneous fat tissue, with fibrous, lymphatic and vascular components to simulate the dis-homogeneity of the human breast. In order to perform the scheduled measurements, the PB was made available for testing by removal of skin flap. Temperature and size of PB were measured each time. To better avoid interferences by neighboring structures (muscle) especially for small “pseudo-breast”, a space was made free below the PB, respecting the structures and the vascularization, to allow the positioning of a histological glass. This solution, not only permitted to hold up the PB obtaining a good isolation of it from the surrounding structures but also the possibility to modify the position of the animal on the operating table with better exposition and stability of the PB during the measurements. It is worth noting that the fat tissue of PB is contained inside a light connectival capsule with microvascular net. The capsule was maintained for the first measurements and then it was locally removed giving direct access to the tissue for superficial and deeper measurements. All surfaces were naturally wet due to serum and lymphatic circulation. Not bleeding was present during the experiments but accumulation of serum/lymphatic fluid and active blood circulation (visible vessels) could be noted. After performing *in vivo* measurements the rat was sacrificed and further measurements were performed on PB in situ, about one hour after. Then, the PB was harvested and put in a Petri dish to be analyzed at about 24 hours from sacrifice.

Similarly, *in vivo* and *ex vivo* measurements were performed on abdominal fat (either from the pelvis or retroperitoneal), since it is more homogeneous and usefully used as a reference.

Six different rats were examined. If possible, different measurement points were considered, according to PB size. Five different measurements for each point were performed and average and standard deviation provided.

C. Results

In Table 1 *in vivo* results on PB are shown: for each measurement point, average and standard deviation values are reported. Red data represent the measurements performed with capsule. Following, the ex vivo results on PB are shown in Table 2 and Table 3 (1 h and 24 h from sacrifice respectively).

Table 1

In vivo	Pseudo-breast		1500 MHz		2450 MHz	
Rat	Size	Temperature	ε	σ	ε	σ
	[cm]	[°C]		[S/m]		[S/m]
#2	1.8 x 1.5 x 1.0	28.6	14.46 ± 0.92	0.34 ± 0.01	14.98 ± 0.23	0.48 ± 0.00
			14.59 ± 0.21	0.33 ± 0.00	14.09 ± 0.22	0.46 ± 0.00
#3	2.2 x 2.2 x 1.4		15.98 ± 2.58	0.37 ± 0.06	16.12 ± 1.91	0.51 ± 0.08
			16.77 ± 1.25	0.37 ± 0.03	16.09 ± 1.19	0.51 ± 0.04
			10.15 ± 0.06	0.18 ± 0.00	9.86 ± 0.05	0.24 ± 0.01
			13.73 ± 0.08	0.33 ± 0.00	13.26 ± 0.08	0.45 ± 0.00
			13.50 ± 0.28	0.21 ± 0.00	13.05 ± 0.30	0.31 ± 0.01
			10.02 ± 0.08	0.24 ± 0.01	9.62 ± 0.08	0.33 ± 0.01
#4	2.5 x 2.0 x 1.5	31.5	14.35 ± 2.26	0.35 ± 0.05	14.26 ± 2.16	0.53 ± 0.08
#5	2.2 x 2.0 x 1.0	32.7	7.24 ± 1.30	0.14 ± 0.04	7.12 ± 1.16	0.24 ± 0.05
			7.01 ± 0.45	0.13 ± 0.01	6.71 ± 0.43	0.20 ± 0.02
			8.31 ± 1.97	0.15 ± 0.05	7.97 ± 1.64	0.23 ± 0.07
#6	3.0 x 2.2 x 1.2	29.0	14.44 ± 2.05	0.33 ± 0.06	12.63 ± 4.13	0.49 ± 0.16
			7.31 ± 0.37	0.17 ± 0.01	7.30 ± 0.52	0.26 ± 0.01
			6.66 ± 0.14	0.14 ± 0.00	6.38 ± 0.13	0.21 ± 0.00
#8	1.7 x 1.8 x 1.0	29.5	8.45 ± 1.48	0.19 ± 0.04	7.77 ± 1.33	0.26 ± 0.05
			7.82 ± 0.29	0.20 ± 0.01	7.22 ± 0.49	0.26 ± 0.02

Table 2

Ex vivo 1 h	Pseudo-breast		1500 MHz		2450 MHz	
Rat	Size	Temperature	ε	σ	ε	σ
	[cm]	[°C]		[S/m]		[S/m]
#2	1.8 x 1.5 x 1.0		12.67 ± 0.12	0.26 ± 0.00	12.25 ± 0.11	0.37 ± 0.00
#3	2.2 x 2.2 x 1.4	22.3	11.96 ± 0.16	0.24 ± 0.00	11.57 ± 0.16	0.35 ± 0.01
#4	2.5 x 2.0 x 1.5	20.6	11.36 ± 0.89	0.25 ± 0.02	10.98 ± 0.86	0.36 ± 0.04
#5	2.2 x 2.0 x 1.0	20.0	9.84 ± 0.50	0.21 ± 0.01	9.46 ± 0.48	0.33 ± 0.02
#6	3.0 x 2.2 x 1.2	28.5	9.26 ± 1.62	0.19 ± 0.03	9.17 ± 1.05	0.30 ± 0.05
#8	1.7 x 1.8 x 1.0	27.6	6.23 ± 0.62	0.17 ± 0.02	5.94 ± 0.60	0.23 ± 0.03

Table 3

Ex vivo 24 h	Pseudo-breast		1500 MHz		2450 MHz	
Rat	**Size**	**Temperature**	**ε**	**σ**	**ε**	**σ**
	[cm]	[°C]		[S/m]		[S/m]
#2	1.8 x 1.5 x 1.0		9.62 ± 0.22	0.19 ± 0.00	9.02 ± 0.20	0.29 ± 0.01
#3	2.2 x 2.2 x 1.4		14.83 ± 0.43	0.36 ± 0.01	14.26 ± 0.43	0.48 ± 0.02
#4	2.5 x 2.0 x 1.5	20.3	8.63 ± 0.26	0.22 ± 0.00	8.32 ± 0.24	0.31 ± 0.01
#5	2.2 x 2.0 x 1.0	21.2	7.16 ± 1.08	0.19 ± 0.03	6.83 ± 0.75	0.27 ± 0.04
			4.85 ± 0.27	0.13 ± 0.01	4.62 ± 0.26	0.18 ± 0.01
#6	3.0 x 2.2 x 1.2	22.0	6.11 ± 0.76	0.15 ± 0.02	6.18 ± 0.69	0.23 ± 0.03
			4.77 ± 0.19	0.13 ± 0.01	4.58 ± 0.19	0.17 ± 0.01

From the tables a great variability of both permittivity and conductivity is evidenced. Lower permittivity values were measured in rat #5 and rat #8 with respect to the other PBs: in these cases, the PB were prepared only two weeks before STSM and probably they had less time to develop fibrous, lymphatic and vascular components typical of the ones realized some weeks before. On the contrary, the typical dis-homogeneity in the PBs of rat #2, #3, #4, and #6 could be the reason of the higher measured permittivity and conductivity values. However, the dis-homogeneity can usefully mimic the practical condition of a real breast evaluation.

In this very preliminary set of tests, no significant differences were evidenced between in vivo and ex vivo ranges of measurements. Even though these initial observations seem to indicate that the bio-physical phenomena occurring in PBs after the excision from the host do not infer the dielectric properties behavior, further tests are necessary for achieving definitive evidence. Finally, the obtained permittivity and conductivity values, however characterized by a great variability, resulted to be compliant with those proposed in [5] for computational electromagnetic simulations.

References

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D. Future collaboration with host institution

The reported data represent interesting but preliminary results on the *in vivo* dielectric properties of the considered PB model. A first important step in the *in vivo* setup optimization was accomplished but further measurements are necessary to fully characterize the tumor model. For this reason, the collaboration with Dr. Vannucci cannot be considered concluded. During the meeting performed during the last two days of this STSM, a working plan was set, in order to schedule the scientific activities for next months.

E. Expected Publications

Additional data on PB and new ones on tumor breast model are necessary, in order to completely characterize the Dr. Vannucci's model. When this set will be complete, a joint journal publication is foreseen to divulgate permittivity results achieved in *in vivo* and *ex vivo* measurements on rat tumor.

F. Other comments

In this STSM, the measurements of the *in vivo* dielectric properties of both healthy and tumorous breast models developed in rats were scheduled. Both models were prepared some weeks before the mission but, at the date of STSM, only rats with healthy PBs were ready. In fact in the rats treated with the injection of cultivated tumor cells in the PB, after an initial increase, the tumor regressed in few weeks, so this model was not available for the measurements. It is worth noting that the tumor breast model has been set up by Dr Vannucci on mouse, but the resulting PB samples were too small to be measured by the slim dielectric probe which requires a sample volume of about 1 cm^3. For this reason, rats were chosen for the experimental work of this STSM in order to obtain bigger PBs, but the biological response in rats to tumor injection, resulted different with respect to the response in mice.

The experimental study above described was conducted together with Laura Farina from Sapienza University of Rome. She was sponsored from the COST Action TD 1301 – MiMed with a STSM (COST-STSM-TD1301-100416-071441).

Part of materials and animals for the experimental work was supported by the Czech grant COST CZ LD 15135 developed in the frame of the COST Action MiMED TD1301.

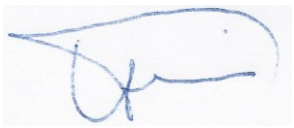
Confirmation by the host institution of the successful execution of the STSM:

We confirm that ROSANNA PINTO from ENEA in Rome has performed the research work as described above in our laboratories at INSTITUTE OF MICROBIOLOGY V.V.I. CZECH ACADEMY OF SCIENCES from APRIL 10, 2016 to APRIL 18, 2016.

Contact Person of Host Institution

Luca Vannucci

Signature



Name of researcher

Rosanna Pinto

Signature