## **3D Bone Architecture**

G.Taton, T.Rok, E. Rokita, Z. Tabor, M. Karwala-Szytula, F. Beckmann<sup>1</sup>, T. Donath<sup>1</sup>, J. Fischer<sup>2</sup>

Jagiellonian University Medical School, Department of Biophysics, Grzegorzecka 16a, Cracow, Poland 

<sup>1</sup>GKSS-Research Center, Max-Planck-Straße 1, 21502 Geesthacht, Germany

<sup>2</sup>Hannover Medical School, Anna-von-Borries-Str. 1-7, 30625 Hannover, Germany

The bone mineralization process and its alterations is one of the fields of interest for contemporary medicine. There are open questions concerning it onset, dynamics and the mechanism of being influenced by different physical or chemical factors. The aim of the project is to investigate the influence of the extremely low frequency magnetic field (ELFMF) on the bone mineralization process [1]. We decided to rely on the three-dimensional (3D) bone architecture investigations with the use of microtomography. The 3D micro-tomographic data allow the calculations of bone architectural parameters describing its mechanical properties. It is believed that such parameters are more precise and reliable for the description of bone changes than the other parameters [2,3].

The rat bone model was used. Pregnant female rats were taken into consideration. One group was treated with ELFMF while the second stayed untreated as a control group. Magnetic field parameters typical for magnetotherapy in humans were utilized (frequency 15Hz, intensity 1mT). The rats breed was treated also with ELFMF after the labour. The young treated and control rats were killed in different age (10, 20 and 30 days). Their femoral bones were dissected and investigated.

12 bone samples dissected in different animals age were scanned with the microtomography with the use of synchrotron radiation. Only the femoral head was scanned due to the lack of trabeculae in the bone shaft. The beamline BW2 equipped with microtomographic scanning system was utilized. The energy of 18 or 24 keV was applied depending on sample sizes. Achieved image resolution was 6  $\mu m$  - 10  $\mu m$  depending on the sample size. The 3D scanning of single sample takes 5 to 13 hours depending on the sample size.

A set of parameters characterising the trabecular bone was calculated, e.g.: tissue volume (TV), bone volume (BV), trabecular thickness (TT), trabecular number (TN), trabecular separation (TS) and BV/TV. Also the trabecular thickness distribution and trabecular separation distribution were calculated.

The former project stage was concentrated on the best architectural parameters choice allowing the observations of subtle bone structure changes [4]. The ELFMF influenced and osteoporotic bones were investigated. Because of the large time-consumption of 3D microtomographic measurements only few bone samples were investigated: 20 and 30 days old animals treated with ELFMF and their control counterparts (4 samples) and two samples connected to the osteoporotic investigations. Since that the proper statistical analysis was not possible. Comparing the architectural parameters driven for investigated samples it was stated that some simple parameters could be taken into consideration [4] assuming that the parameter variability for particular individuals (individual variability –IV) is not higher than the differences caused by the investigated factors. In order to estimate IV additional bone samples treated with ELFMF (and their control counterparts) were scanned in the last project stage.

Despite more samples (12) were finally measured the proper statistical analysis is still impossible. The conclusions can be driven only on the basis of some simple results comparisons. As the estimator of IV the maximal observed parameter difference in the control or treated group (two samples in all cases) was taken. As the estimator of the parameter change (PCh) caused by the ELFMF the mean parameter values difference for both groups was taken (see formulas defined below):

IV = max (|T1-T2|, |C1-C2|); PCh=|(T1+T2)/2-(C1+C2)/2|

where: T1, T2 – samples treated with ELFMF for particular age groups, C1, C2 – control samples. The calculated IV and PCh for three chosen architecture parameters are shown in Table 1. The results of structural parameters calculations are presented in Fig 1.

Table 1. The calculated estimators for individual parameter variability (IV) and parameter change (PCh) caused by the ELFMF for three chosen bone structure parameters. See text for abbreviations.

Age [d]		BV/TV	TT [µm]	TS [μm]
10	IV	0,052	6,75	24,78
	PCh	0,033	0,69	6,45
20	IV	0,026	4,49	8,69
	PCh	0,053	3,64	5,83
30	IV	0,075	27,18	31,68
	PCh	0,026	9,40	15,44

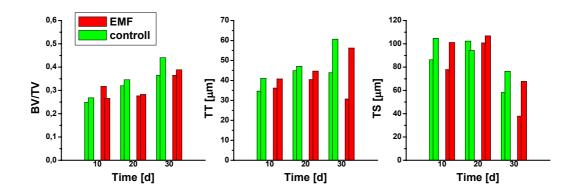


Fig. 1. Chosen bone architectural parameters calculated from 3D microtomographic measurements (see text for abbreviations).

As one can see in Table 1 the observed IV is in most cases larger than the parameter changes caused by the investigated physical factor. Also there is no clear dependence between the investigated parameter value changes and the influence of ELFMF (fig.1). Performed investigations did not confirm the influence of ELFMF on the trabecular bone structure.

It is possible that the influence of ELFMF could be observed if the larger data sets would be involved. The more sophisticated statistical analysis probably would discover logical behaviour of calculated parameters in larger populations allowing to minimize the IV importance. Such solution regards the changes in measurements procedure allowing faster sample scanning.

## References

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