Micro-CT analysis in the development of *in vitro* alternative method for the study of bone metastases

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**Aims**

The skeleton is the organ most frequently affected by metastases, mainly caused by cancers of the breast and of the prostate (about 80% of the metastatic bone diseases) [1]. To reduce the morbidity associated with bone metastases, it would be important to reduce their etiological factors. Although current knowledge suggests that many cancer therapies can cause bone loss and subsequent osteoporosis, no *in vitro* or *in vivo* studies have been able to establish the role of the microenvironment in the development of osteoporotic bone metastases. The purpose of this study is to evaluate whether osteoporosis can provide a conducive microenvironment for the development of bone metastases, and to assess the relationship between bone metastases and osteoporosis. The experimentation involves the use of an *in vitro* three-dimensional (3D) model, in order to re-create the tumor microenvironment present in physiological conditions.

**Method**

Bone segments from patients underwent under surgery for partial or total hip or knee replacement, caused by osteoarthritis or by pathological fractures due to osteoporosis, were used in order to create a 3D *in vitro* model. In detail, breast cancer cells (MCF-7) and prostate cancer cells (PC-3) were cultured with these human bone segments, kept in constant agitation in a pyrex tube (roller tube system) in normal and in hypoxia conditions. Before cell culture and after 7 days, the bone segments were analyzed by micro-CT system Skyscan 1172 and subsequently processed for histological evaluation in order to assess the presence of tumor cells, the degree of osteolysis, the bone resorption (TRAP) and the trabecular structure changes. Moreover, these samples were used for the evaluation of the viability (Alamar Blue) and, through immunohistochemical analysis, for the positivity of cytokeratin, vimentin, RANK. The supernatants were evaluated through immunoassays analysis for metalloproteinase 9 (MMP-9), metalloproteinase 7 (MMP-7), Tissue inhibitor of metalloproteinases (TIMP), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 11 (IL-11), alkaline phosphatase (ALP), collagen type I (CICP), osteoprotegerin (OPG), receptor activator of nuclear factor kappa-B ligand (RANKL), Transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), Parathriod Hormone Related Protein (PTHrP), Osteocalcin (OC), Insulin Growth Factor (IGF).

**Micro-CT Analysis**

All the specimens were scanned at 70 kV of source voltage and 140 μA, with a total rotation of 180° and a rotation step of 0.4°. An aluminum filter of 0.5 mm was used between the source and the sample. The image pixel size was 12 μm and the scan duration was nearly 45 minutes for every specimen (s/w Skyscan 1172 version 1.5 build 14). The reconstructions were
performed using the software NRecon (version 1.6.9.16) and the resulted jpg images had 2000X2000 pixels with a pixel size of 12 μm. Beam hardening, ring artifacts and the specific misalignment corrections were used.

The datasets of the samples before cell culture and after 7 days were co-registered in Dataviwever following the Bruker Method Notes: MN044 - Image and dataset registration in Dataviewer (Tooth), MN063 - Image and dataset registration in Dataviewer (Expanded clay) and MN048 - Advanced image coregistration in dataviewer.

BMD and TMD were also calculated through calibration by means of two cylindric phantoms with known concentration of the mineral compound calcium hydroxyapatite (CaHA) of 250 and 750 mg/ cm$^3$ and a diameter of 8 mm (Bruker Method Note MN009 - BMD calibration in CTa).

Results

After the binarization of both datasets of the same sample before cell culture and after 7 days of experimental time, the datasets were loaded in Dataviewer (v. 1.5.1.2). The results before and after co-registration are shown in Figure 1.

![Figure 1: Bone segments before (left) and after co-registration (right).](image1)

The Reference and Target co-registered datasets were loaded in CTVox in order to localize the datasets differences in the two experimental times (Figure 2 and Figure 3).
Both BMD and TMD showed a decrease after 7 days of cell culture (Figure 4).
Conclusion
One of the greatest limitations in studying cancer-bone metastasis is the lack of an appropriate ex vivo model that can be used under defined conditions mimicking the in vivo bone microenvironment in response to cancer-bone interactions. We have developed and utilized a three-dimensional cancer-bone metastasis model using free-floating living healthy and osteoporotic human bone organs in the presence of cancer cells in a roller tube system. Micro-CT analysis was used to evaluate micro-structural and densitometric changes in the trabecular bone samples of the described innovative 3D alternative method for the study of bone metastases.

References: