Paleo-NMR: micro-imaging of skeletal and odontoskeletal remains

S. Capuani[1,2]
[1]Physics Department Sapienza University of Rome, Rome, Italy, [2]CNR IPCF UOS Roma, Rome, Italy

Introduction:
Archeological bones can tell us all about lifestyle, habits, diseases and death causes of our distant ancestors. During the last decade a number of analytical tools has been developed and applied to fossil and sub fossil human remains in order to estimate variables related to life history[1,2].
In particularly as brain size, lifespan and other life-history traits correlate tightly with dental development[2] the detection of specific variables related to dentine growth, represents one of the most challenging and promising tasks. Nevertheless, the task of using this record to document ancient population and help understand these complexities has barely begun. Moreover, because of taphonomic dynamics and diagenetic events, signals from this record are often noisy. Some investigations, such as the histological approach, are highly destructive, but actually represent the golden reference standard method. Since few years a new approach of virtual histology based on x-rays (CT or Synchrotron Light modality) allows to obtain similar information in a fully non destructive manner. Due to the unique ability of MR techniques to detect porous systems characteristics, a virtual histology approach based on MR is expected to provide quantitative measures from fossilized remains constituted by porous tissues, such as dentinal tissue from teeth and bone tissue. Research activity for developing new MR protocols to study anthropological collection of human remains is another fascinating application of NMR. Here I'll illustrate some examples of these type of studies.

Materials and methods:
Bones: Archeological bone specimens (dated back to the 1st-3rd Century A.C.) obtained from different locations of human tibiae coming from tombs sited in the Etruscan archeological sites of *San Donato* and *Crocevia dei Missionari* (Urbino, Italy) [2] were cleaned and put into NMR tubes filled with distilled water. A first qualitative examination of these bones by anthropologists indicated the presence of periostitic lesions, taphonomy damage, and different kind of fractures. Moreover Optical and SEM images were obtained from each specimens.
T1, T2, ADC parameters were measured and SE decay were collected to extract internal gradients (Gi). MSME (Multi Slice Multi Echo) imaging sequences at various TEs and at various TRs were used to obtain T1w and T2w images in selected slices (slice thickness =250 μm) characterized by an in plane resolution of 60μm. Pulsed Field Gradient Stimulated Echo (PGSTE) imaging sequences with diffusion gradient pulses duration δ=2ms at different diffusion gradient strengths g applied along the x axis were also used to obtain ADC (eight b-values from 650 to 15000 s/mm^2) to perform experiments in bone specimens. Moreover various TEs from 1,7 ms to 70 ms were used to perform SE experiments.
Teeth: Neolithic human tooth obtained from Sudan, Gravettian teeth of humans lived between 200.000 and 11.000 BC and actual damaged teeth were immersed in NMR tubes filled of distilled water to be investigated. The temperature of each sample was fixed to 291 K. MSME sequences (TE=3.1ms, 4.6ms or 6.6ms, TR=250, 450 and 1200 ms, ns=512, matrix 256x256 and 512x512; FOV=1.5x1.5 cm^2, slice thickness 200 μm) were obtained characterized by an in plane resolution of 60μm (matrix 256x256) or of 30μm (matrix 512x512). MicroCT images were also performed for all the samples.
All measurements were performed on a Bruker 9.4T Avance system, operating with a micro-imaging probe (10 mm internal diameter bore) and equipped with a gradient unit characterized by a maximum gradient strength of 1200 mT/m, and a rise time of 100 μs.

Results:
Bones: Multi-parametric microimaging investigations of healthy and periostitic bone samples highlighted that the bone density immediately under the surface of the periostitic cortical bone is higher than that of healthy bone specimens; i.e. in a thin slice of approximately 250 μm, periostitic bone is characterized by smaller pores compared to the healthy one. Moreover high resolution images provide an interesting tool to study microstructural trabecular organization to discriminate signs due to diagenetic events from signs linked to a specific pathology.

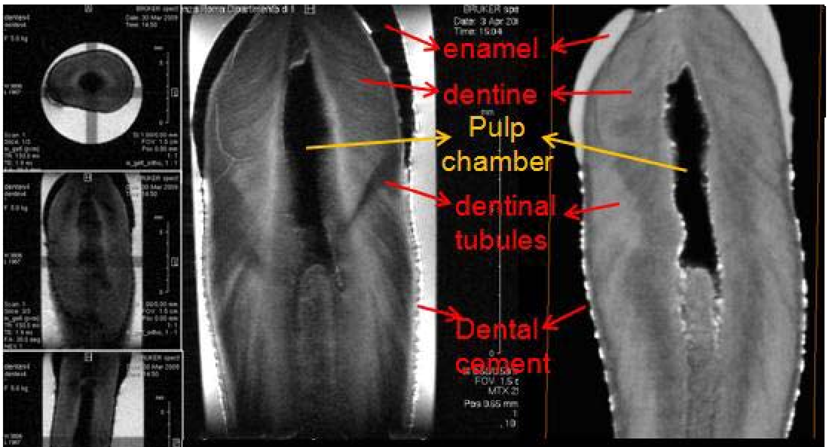

Fig.1 micro-NMR and micro-CT images of a Neolithic tooth are illustrated and specific tooth zones are indicated in both images. However micro damages in dentine and in dentin-enamel junction are visible in the NMR image only. In the left side the three conventional positioning images are showed.

Teeth: In Fig. 1, micro-NMR and micro-CT images of a Neolithic tooth are showed. T2- weighted images provide interesting information about dentin pores distribution and dimension while T1-weighted images show important anatomical details such as dentinal tubules and Andersen lines in archeological teeth which sometimes are not turned out in a microTC investigation. Different kinds of dentine (primary, secondary dentine) dentine necrosis and several other dentine microstructure can be identified in the NMR image of an archeological tooth. In particular micro-cracks and micro damages in the dentin-enamel junction are well indicated in NMR images but not in micro-CT.

Discussion and conclusion
Experimental data demonstrate that virtual histology of ancient bones and archeological teeth, based on micro-NMR images, can highlight some details that are not detectable with other non destructive techniques.
As a consequence the investigation proposed here may result a decisive support to paleo-pathological research and anthropological studies. Moreover, the development of new protocols for the virtual histology of bone remains and archeological teeth may be of high impact on cultural heritage-anthropological field, due to the large collection of fossil humans remains which will be preserved from the destruction.

References:
[1] Chhem RK and Brothwell Don R, "PaleoRadiology, Imaging Mummies and Fossils", Springer 2008. [2] Dean CM, Proc R Soc B 2006:273:2799-2808. [3] Leonetti M, Capuani S et al. Appl Phys Lett 94 (10), art. no. 101101.