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The allocation of bone fluid in bovine cortical bone utilizing a multi-technique analysis

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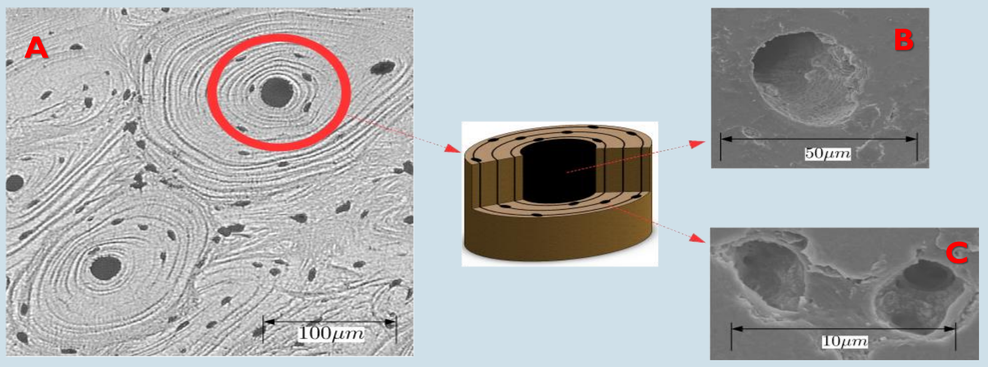
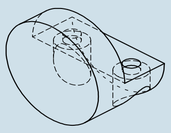
Motivation

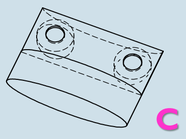
It is well accepted that the mechanical properties of bone depend crucially on the materials composition in terms of mineral and collagen content, as well as the hierarchical organization of those components [3]. What is less known, is the role of the third major structural component in bone, which is water. It is partitioned in three levels of pore spaces, ranging from the nanometer to the millimeter level. The porosity of cortical bone (**A**) includes the Vascular Porosity (**B**), the Lacunae Porosity(**C**) , and the Ultra-structure Porosity. Besides the hierarchical organization, the quantitative aspects of this partitions remains largely unknown. The present work elucidates this issue, by refining the landmark protocol of Lees and coworkers [2], and performing simultaneous investigations with the light microscope LM.



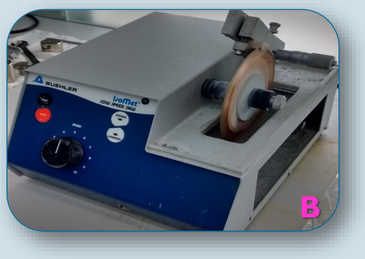
Material and Experimental Methods

Sample Preparation

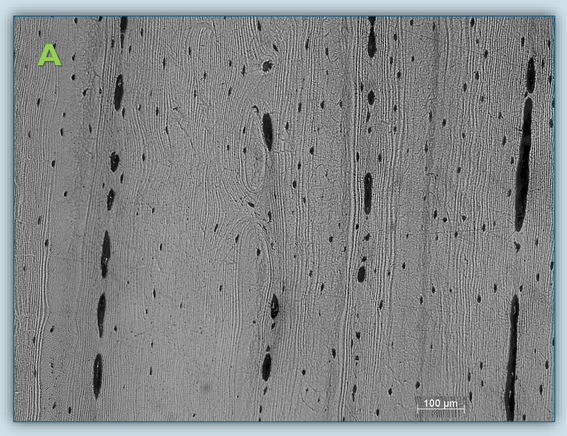


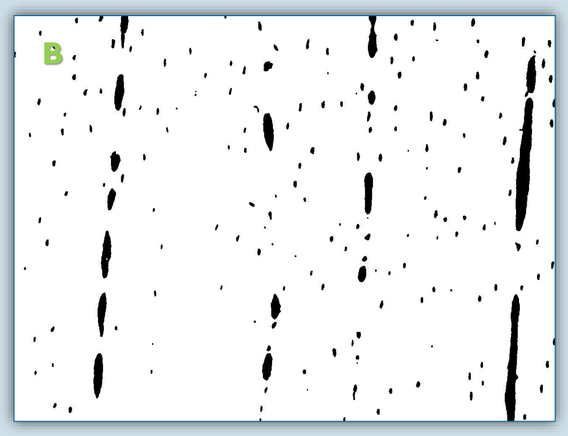


The 24 bone samples were harvested from a healthy bovine femur (**A**). The bone samples were first cut into smaller pieces using a diamond blade band saw. The smaller pieces were cleaned from the remaining soft tissue. The samples were later cut into the individual sample sizes with a low speed saw (**B**). The samples were subsequently attached to a custom-made steel holder (**C**) , and polished in a two-step polishing process using a rotating polishing system (**D**).



Porosity Determination





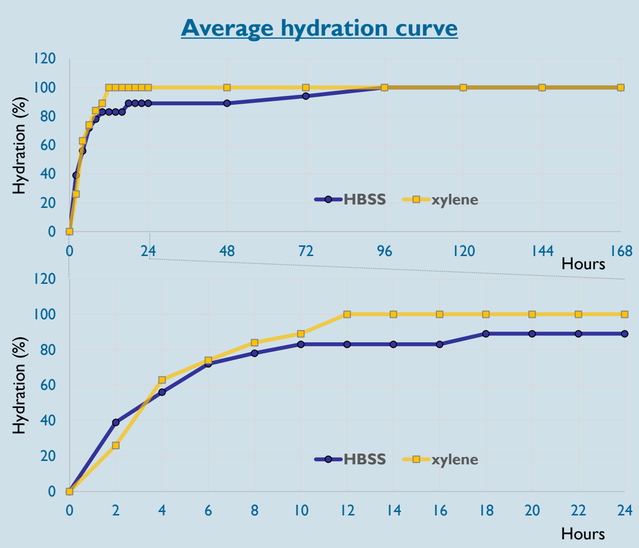
Each image obtained under the LM (**A**) was converted into an 8-bit image and filtered manually (**B**). The image segmentation was performed by a two-level threshold method. The pores were divided into Vascular pores and Lacunae pores by setting an user-defined second threshold condition.

Dehydrated State

The samples was dehydrated under vacuum alongside with an orange silica gel desiccant. The mass from each of the samples was monitored in a precision balance until no change was noticed. At this point, the samples are considered to be dehydrate, *i.e.* the bone samples are dry in equilibrium with a water-free environment and at room temperature.

Hydrated State

From the twenty-four dehydrated samples, eighteen samples were immersed into a Hank's Balanced Salt Solution (**HBSS**) with pH 7.4, and six samples were immersed in **Xylene**. The weight from each immersed sample was registered every two hours for a twenty-four hour period. After the twenty-four-hour period, the weight from each immersed sample was measured every day for a six-day period. At this point the samples are considered to be hydrated. Subsequently, the volumes were calculated using the Archimedes´ Principle.



Demineralized State

The twenty-four samples were demineralized using a 0.5 M EDTA solution with a pH 7.5. The solutions of the three immersions from each sample were examined in an Atomic Absorption Spectrometer (AAS) in order to detect the presence of Calcium, Magnesium, or Phosphorous. After not detecting any of the before-mentioned minerals in the last 0.5 M EDTA solutions, the samples are considered to be demineralized at this point.

Results & Discussion

Weight Fractions and Density

Density:

$$\rho = \frac{Mass_{total}}{Volume_{total}}$$

The mass of the bone contents are normally given in terms of the content weight fraction.

Weight Fraction:

$$WF_i^j = \frac{Mass_i^j}{Mass_{total}}$$

with:
$i = water$ for water content
$i = HA$ for mineral content
$i = org$ for organic content

Sample	ρ (g/cc)	WF_{org}	WF_{HA}
HBSS	1,99	0,2063	0,6808
Xylene	1,90	0,1949	0,6680

Sample	WF_{water}	$WF_{water}^{vascular}$	$WF_{water}^{Lacunae}$	WF_{water}^{ultra}
HBSS	0,1129	0,0161	0,0098	0,0870
Xylene	0,1383	0,0145	0,0079	0,0942

Volume Fractions

Volume Fraction:

$$f_i^j = \frac{volume_i^j}{Volume_{total}}$$

with:
$i = water$ for water content
$i = HA$ for mineral content
$i = org$ for organic content

Sample	f_{water}	f_{org}	f_{HA}
HBSS	0,2227	0,3263	0,4510
Xylene	0,2893	0,2883	0,4224

Sample	$f_{water}^{vascular}$	$f_{water}^{lacunae}$	f_{water}^{ultra}
HBSS	0,0320	0,0195	0,1711
Xylene	0,0312	0,0169	0,1906

Discussion

The results provide a significant addition and validation to previous experiments performed by [1] and [2]. Finally, with the results of the weight fractions and volume fractions we revealed the partition of bone fluid at the micro-structural and ultra-structural scale. Being bone a composite material, the allocation and quantification of fluid, along with the mineral and organic contents, are essential to a better understanding of the reaction of bones toward forces,

References:
[1] Robinson and Elliot (1957), *The Journal of Bone and Joint Surgery* 39(1):167–188.
[2] Lees et al., (1979) *Calcified Tissue International* 29(1):107–117.
[3] Fritsch and Hellmich (2007), *Journal of Theoretical Biology* 244(4):597–620.

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