TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

INSTITUTE FOR MECHANICS OF MATERIALS AND STRUCTURES
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Variances and in-variances in composition and microstructure, across femoral tissues from bovine, equine, ranine, porcine, leporine, and struthionine samples.

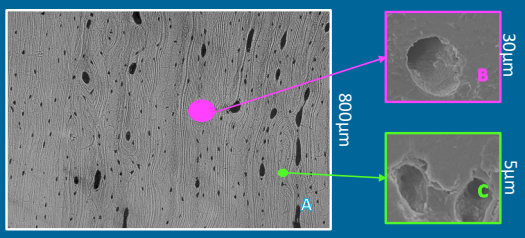
Luis Zelaya-Lainez[1], Hawraa Kariem[1], Winfried Nischkauer[2], Andreas Limbeck[2], Christian Hellmich[1]

[1]Institute for Mechanics of Materials and Structures, Vienna University of Technology.
[2]Institute of Chemical Technologies and Analytics, Vienna University of Technology.

Motivation

There is a long tradition in studying the chemical composition [1] or the micro-morphological features of bone from different species [2], but these activities were never performed simultaneously.
May such a simultaneous investigation lead to the discovery of interesting patterns and invariant properties among several vertebrates?

Overview

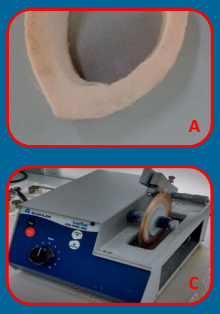


The mechanical properties of bone depend on the amount of mineral content, organic contents (90% is collagen), and water content (within the different porosities). As well as on their organization across the hierarchical structure of bone [3].
The porosity of cortical bone (A) includes the vascular porosity (B), the lacunar porosity (C) , and the ultra-structure porosity.

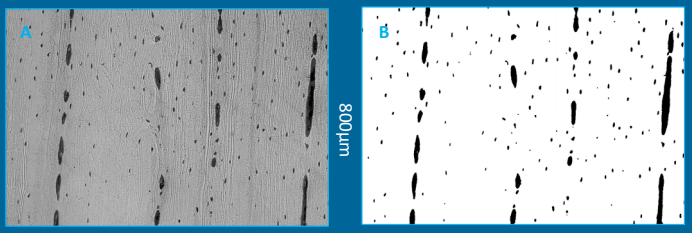
Material & Methods

Sample preparation

The 112 bone samples from 7 different species were harvested from healthy femora (A). The millimeter sample sizes were obtained by employing a diamond blade band (B), a low speed saw (C) or a scalpel. The samples were subsequently attached to a custom-made steel holder (D), and polished using a rotating polishing system (E) or an ultra-miller with a diamond tip (F).



Porosity determination



The images from the polished surfaces of each sample obtained under a light microscope (A) were converted into 8-bit images and filtered manually (B). The image segmentation was performed by a two-level threshold method. Later, the pores were classified into vascular pores and lacunae pores by setting an user-defined second threshold condition.

Dehydration and hydration protocols

To access the total water content, i.e. lacunar, vascular and ultrastructural porosity, the samples were first dehydrated under vacuum alongside with an orange silica gel desiccant. Subsequently, the samples were hydrated with HBSS until no weight difference was noticed.

Demineralization procedure

The samples were demineralized using a 0.5 M EDTA solution with a pH 7.5 (A). The solutions of the three immersions from each sample were examined using inductively coupled plasma atomic emission spectroscopy (ICP-OES) (B) 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, volume fractions, and densities at the ultrastructure

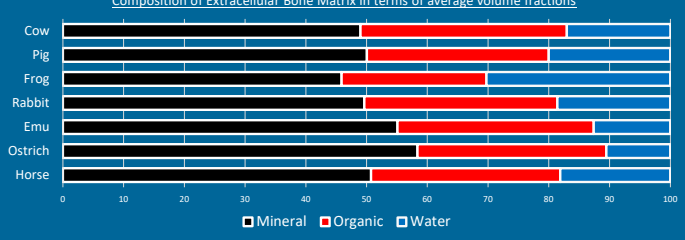
Weight Fraction:
$$WF_i = \frac{mass_i}{mass_{total}}$$

Volume Fraction:
$$f^i = \frac{volume^i}{volume^{total}}$$

Density:
$$\rho = \frac{mass_{total}}{volume^{total}}$$

The mass and volume of the bone contents are normally given in terms of the content weight and volume fractions.

Composition of Extracellular Bone Matrix in terms of average volume fractions



Species	Horse	Ostrich	Emu	Pig	Rabbit	Frog	Cow
Horse	✓	✓	✗	✓	✓	✓	✓
Ostrich	✓	✓	✓	✗	✓	✓	✓
Emu	✗	✓	✓	✗	✓	✗	✗
Pig	✓	✗	✗	✓	✗	✓	✓
Rabbit	✓	✓	✓	✗	✓	✓	✓
Frog	✓	✓	✗	✓	✓	✓	✓
Cow	✓	✓	✗	✓	✓	✓	✓

Species	Horse	Ostrich	Emu	Pig	Rabbit	Frog	Cow
Horse	✓	✓	✓	✓	✓	✗	✓
Ostrich	✓	✓	✓	✓	✓	✗	✓
Emu	✓	✓	✓	✓	✓	✗	✓
Pig	✓	✓	✓	✓	✓	✓	✓
Rabbit	✓	✓	✓	✓	✓	✓	✓
Frog	✗	✗	✗	✓	✓	✓	✗
Cow	✓	✓	✓	✓	✓	✗	✓

Species	Horse	Ostrich	Emu	Pig	Rabbit	Frog	Cow
Horse	✓	✗	✓	✓	✓	✗	✓
Ostrich	✗	✓	✓	✗	✗	✗	✓
Emu	✓	✓	✓	✗	✓	✗	✓
Pig	✓	✗	✗	✓	✓	✗	✓
Rabbit	✓	✗	✓	✓	✓	✗	✓
Frog	✗	✗	✗	✗	✗	✗	✗
Cow	✓	✓	✓	✓	✗	✓	✓

Discussion

We discovered an **invariance** of the *chemical composition* at the ultrastructural level across all the *mature mammals*, i.e. bovine, equine, and leporine specimens.

The comparison of the *"big porosities"*, i.e. vascular and lacunar porosities, among the different vertebrates shows a considerable **interspecies variation**.

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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