Microstructural and material basis of bone mechanical behaviour during growth.

Summary Facing the difficulties in collecting paediatrics bone samples, little is known about the growth-related changes in bone microstructure, tissue composition, material properties and how those changes influence bone mechanical properties. Therefore, no relevant reference values exist regarding the aforementioned parameters. This limits our understanding of the consequences of juvenile bone pathologies as well as the endpoints to define a therapy as successful. This proposal aims to accurately depict the age related changes in bone mineralization and bone microarchitecture and study their impact on local (i.e., tissue scale) and apparent mechanical behaviour. The research plan will include two aims: 1) the characterization of the changes in mineralization, morphology and tissue composition during growth and their impact on micromechanical behaviour *in vitro* in a collection of peri-operative samples (*i.e.*, MALICE project) and 2) study the relative role of these material / microstructural properties and the geometry and architecture in samples from the aim 1 and *ex vivo* in bone samples from an anthropologic collection of juvenile skeleton using multiscale biomechanical simulation.

Research Plan

Aim 1: In vitro characterization of the growth-related changes in tissue composition, morphology and material properties and their impact on micromechanical properties (Figure 1).

Based on the existing literature it is known that in the early stages of bone growth, cortical bone tissue is composed of a mixture of primary lamellar bone, woven bone, osteonal bone and later of interstitial bone [1-3]. We hypothesize that with age the relative proportion of each kind of tissues will vary, the percentage of bone volume occupied by primary lamellar bone and woven bone will decrease in favour of an increase in the proportion of osteonal bone. Therefore, total bone volume will be characterized by an equation including the respective proportion of each tissue. This will be assessed by histomorphometry on 8 µm-thick section from bone samples embedded in MMA. On the adjacent 100 µm-thick section, quantitative microradiography will be performed to assess the age-related changes in the tissue mineral density and its distribution at the tissue level (i.e., for the whole tissue volume) and in a second instance in regard of the different type of tissue. This later approach will be possible by correlating the images obtained by histomorphometry and by quantitative microradiography. Microradiographic images will also used to measure cortical porosity. Age of the donor will be used to implement a temporal dimension in the description of the outcomes [4, 5].

Images obtained from microradiography will be then used to estimate the age-related changes in bone stiffness, using analytical models based on the concept of multiscale continuum micromechanics. These models take the hierarchical organization of bone into account, and are based on the volume fractions of different *elementary bone constituents* such as mineral, collagen and water, their mechanical properties, morphology and their mechanical interactions. These micromechanical models have been validated based on statistically independent experimental data across different species, ages and anatomical locations [6]. This model is presented in details in the next section. This part of the study will be performed in collaboration with Professor Christian Hellmich (Institute for mechanics of materials and structures, Vienna University of Technology, Austria) and A/Professor Peter Pivonka

(Northwest Academic Center, The University of Melbourne, Australia). This approach will provide mechanical outcomes as the mean stiffness and distribution. We will statistically explain how the variance in mechanical outcomes is temporally impacted by the changes in the structural and material properties aforementioned.

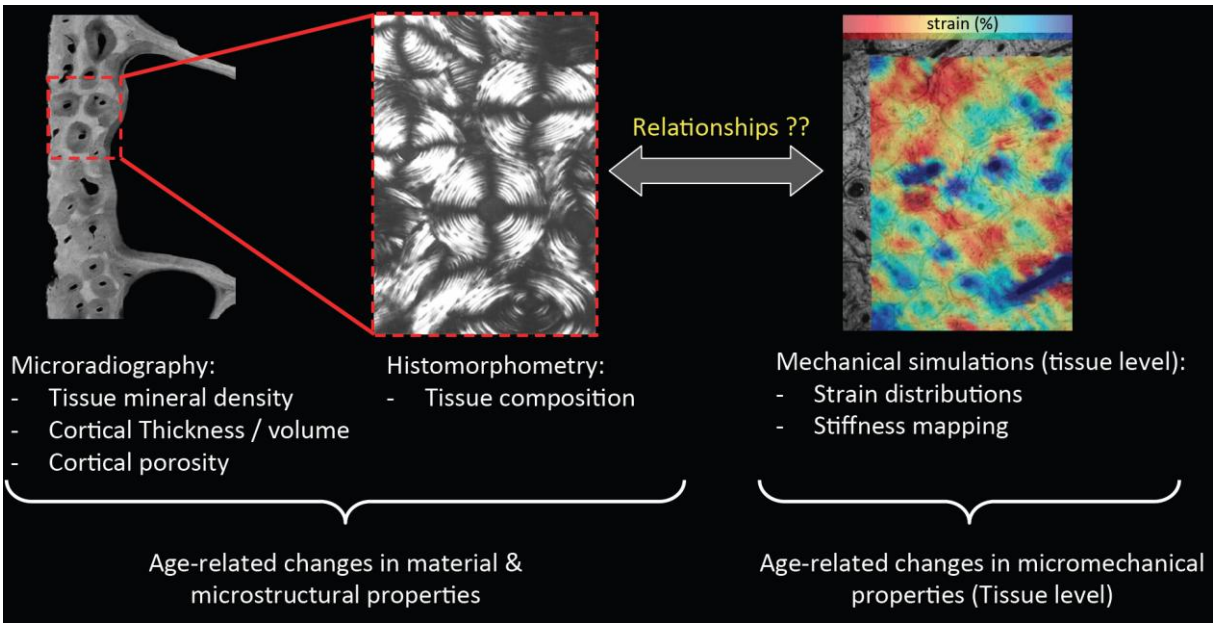

Figure 2: Schematic description of the aim 1.

Aim 2: Relative contribution of growth-related changes in geometry / architecture and material properties in the apparent strength (Figure 2).

We proposed that the aim 1 will provide quantitative surrogates of bone strength at the tissue level. However, the relative role of those local properties (material and tissue composition) and geometry / microarchitecture on the apparent bone strength is not known. To do so, we will apply in 3D volumes of the samples used in the aim 1 a computational model. The homogenization model to be used aimed at deriving the variation of the elastic coefficients of bone tissue as a function of the radial and longitudinal position from the distribution of porosity and tissue mineral density. Below the organ (i.e., macro-scale), the method consider 3 scales (see figure 2), corresponding to the 3 step of homogenization. The elastic properties and the volume fraction of elementary constituents of bone matrix (mineral, collagen and water) and the porosity of bone tissue assessed by microradiography constitute the input data of the method. The different volume fractions are deduced from the following equations (HA: hydroxyapatite / coll: collagen / W: water):

$$fHA + fcoll + fw = 1$$

Where fx is the volume fraction of the element x. The estimation of fHA is obtained using:

$$fHA = \frac{Tissue\ mineral\ density}{\rho_{HA}}$$

Where ρ_{HA} is the mass density of hydroxyapatite obtained. To determine volume fractions of collagen and water with used the following equation :

$$\frac{f_{coll}}{f_w} = 0.36 + 0.084\, e^{f_{HA}}$$

Which is an empirical relation obtained through measurements of volume fractions in demineralized bone [7, 8]. For details about the homogenization steps refer to [9, 10].

The application of this method is therefore based on the calibration of the imaging system to know the relation between the grey levels and the tissue mineral density, i.e., the degree of mineralization [11]. Therefore the approach is feasible either in data obtained using microradiography or micro-CT as our group has calibrated the assessment of tissue mineral density with these 2 methods. In the *Aim 2*, age related changes in architecture and tissue mineral density in 3D volumes will be assessed either in the collection of sample from MALICE project but also in a collection of anthropologic juvenile skeleton held by Dr. Craig Cunningham and Prof. Sue Black from Centre for Anatomy and Human Identification University of Dundee, UK. They are the curators of the Scheuer collection, the only collection in the world to encompass 120 sub-adult entire or partial skeletons [2, 12]. The latter alternative will permit to confirm the results in a larger and more robust population, it will also permit to test wether the relative contribution of material and architectural properties in bone strength is similar in weight-bearing and non weight-bearing bones.

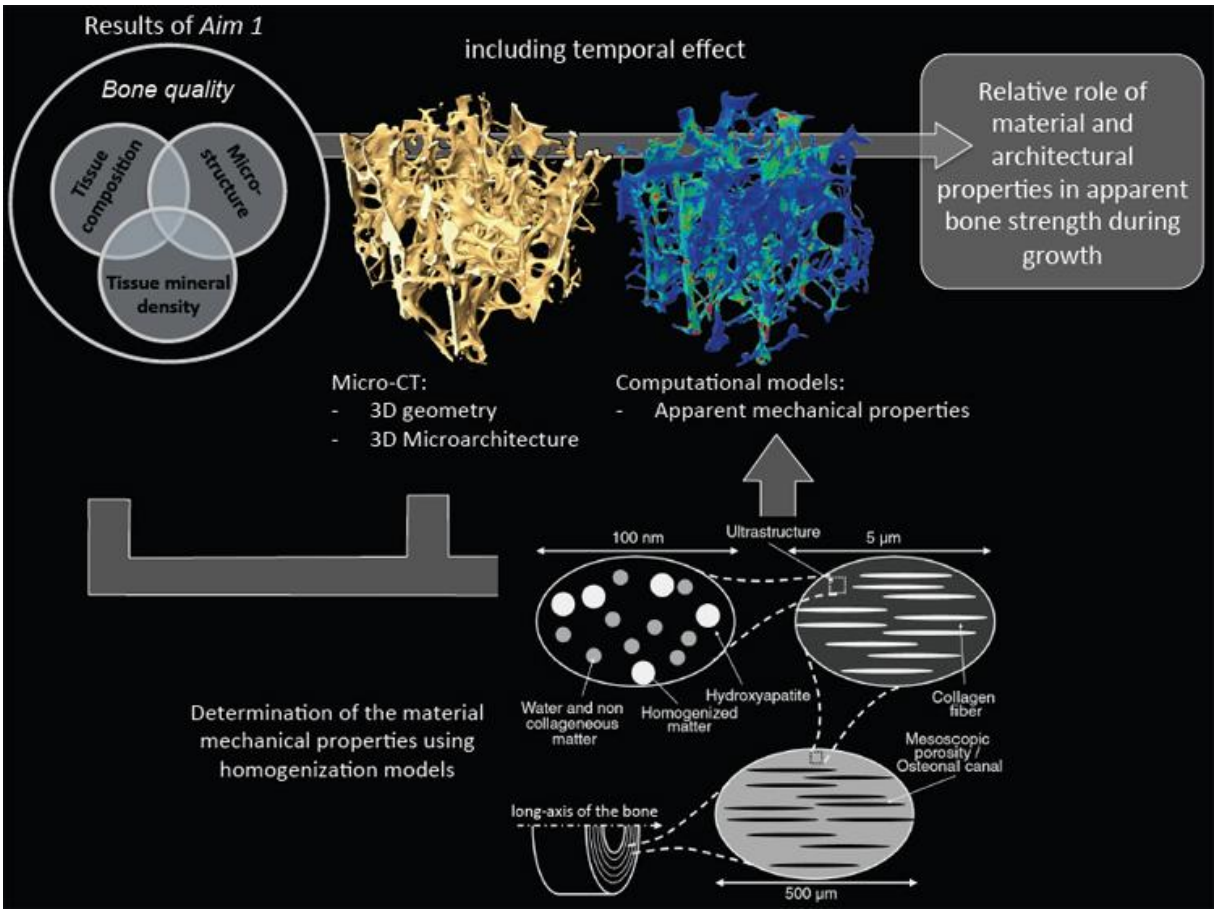

Figure 2: Schematic description of the aim 2.

Significance of the study This study will accurately and temporally describe the relationship between bone material properties, bone macro- and microstructure during growth. This will provide new insight for understanding of the mechanism of juvenile bone affections leading to fragility. Moreover based on two unique resources of bone samples, we propose to provide models that would permit to simulate therapeutic strategies according to their expected effect at the tissue level. For instance the impact of bisphosphonates, a drug used in the treatment of *osteogenesis imperfecta*, on material is well characterized. Therefore, the outcomes of this project would help to simulate the results on mechanical behaviour of such a treatment to determine wether the treatment will be beneficial or another strategy should be preferred in function of the baseline characteristics of the bone assessed *in vivo*.

References:

[1] Rauch F, Travers R, Glorieux FH. Intracortical remodeling during human bone development--a histomorphometric study. Bone 2007;40: 274-80.

[2] Scheuer L, Black S. The Juvenile Skeleton. Amsterdam: Elsevier Academic Press; 2004.

[3] Akkus O, Polyakova-Akkus A, Adar F, Schaffler MB. Aging of microstructural compartments in human compact bone. J Bone Miner Res 2003;18: 1012-9.

[4] Roschger P, Rinnerthaler S, Yates J, Rodan GA, Fratzl P, Klaushofer K. Alendronate increases degree and uniformity of mineralization in cancellous bone and decreases the porosity in cortical bone of osteoporotic women. Bone 2001;29: 185-91.

[5] Boivin G, Bala Y, Doublier A, Farlay D, Ste-Marie LG, Meunier PJ, Delmas PD. The role of mineralization and organic matrix in the microhardness of bone tissue from controls and osteoporotic patients. Bone 2008;43: 532-8.

[6] Vuong J, Hellmich C. Bone fibrillogenesis and mineralization: quantitative analysis and implications for tissue elasticity. J Theor Biol 2011;287: 115-30.

[7] Broz JJ, Simske SJ, Greenberg AR. Material and compositional properties of selectively demineralized cortical bone. J Biomech 1995;28: 1357-68.

[8] Hellmich C, Ulm FJ, Dormieux L. Can the diverse elastic properties of trabecular and cortical bone be attributed to only a few tissue-independent phase properties and their interactions? Arguments from a multiscale approach. Biomech Model Mechanobiol 2004;2: 219-38.

[9] Sansalone V, Naili S, Bousson V, Bergot C, Peyrin F, Zarka J, Laredo JD, Haiat G. Determination of the heterogeneous anisotropic elastic properties of human femoral bone: from nanoscopic to organ scale. J Biomech 2010;43: 1857-63.

[10] Sansalone V, Bousson V, Naili S, Bergot C, Peyrin F, Laredo JD, Haiat G. Anatomical distribution of the degree of mineralization of bone tissue in human femoral neck: impact on biomechanical properties. Bone 2012;50: 876-84.

[11] Hellmich C, Kober C, Erdmann B. Micromechanics-based conversion of CT data into anisotropic elasticity tensors, applied to FE simulations of a mandible. Ann Biomed Eng 2008;36: 108-22.

[12] Cunningham CA, Black SM. Development of the fetal ilium--challenging concepts of bipedality. J Anat 2009;214: 91-9.