Project's topic

Theranostic ultrasound perspectives for pediatric bone diseases
Control, monitoring and therapeutic perspectives of bone diseases in children using ultrasounds

Context and Objectives

Control and monitoring of the children skeleton growth are crucial for early diagnosis and therapy follow-up of pediatric bone pathologies. Poor skeletal health or complications, even minor ones such as a fracture, can have short-term consequences (temporary disability of activity, school, sports or leisure) and longer-term consequences (physical disabilities). An unconsolidated fracture, or a physical activity resumption too fast (after an osteogenesis apparatus treatment for example), can have irreversible consequences. Certain idiopathic juvenile diseases, cancer, genetic disorders, osteogenesis imperfecta..., must be better diagnosed and monitored. Current treatments have side effects: chemical reactions, metabolic effects, or mechanical effects (surgery). These are all elements that today require a better knowledge of the biomechanical behaviors of children's bones, the development of new diagnostic device associated to new therapeutic perspectives, and an interdisciplinary of multi-physical, medical, biomechanical, biochemical, acoustic, image and signal processing skills. This is what we propose to investigate in this project which brings together teams specialized in its fields of expertise.

New and relevant research axis are thus proposed in this project: evaluation of relevant parameters of children's bone quality in *in vivo* configurations (subject no1), development of an ultrasonic diagnostic modality for pediatric bone imaging, complementary to B-mode ultrasound, with a better characterization of bone lesion (subject no2), and the use of low-intensity ultrasound stimulation as therapeutic treatment of this lesion, thus better characterized (subject no3).

Subjects and purpose

Subject no1: Characterization of the children's cortical bone as a two-level porosity material

There is then a growing interest in the bone health of children, firstly because children are concerned by specific infantile osteopathologies (such as tumors, non-union fractures, osteogenesis apparatus treatment, physis pathologies) and secondly because the bone health during childhood will be of a great importance for bone health in adulthood. Therefore, the characterization of the children's bones and of the skeleton development is a key issue. The data collection and the development of relevant biomechanical models of bone growth are critical needs for the medical community. For pediatricians, radiologists and orthopedic surgeons, knowledge of the impact of a pathology on children bone quality remains a major goal in order to anticipate the evolution of diseases, to guide their diagnoses and then to define an optimal therapeutic strategy. Furthermore, a relevant characterization of growing bone is a key element to develop effective and adapted devices of children's bone disease diagnosis and therapy.
Over the past several years, major multi-physical and multiscale studies on non-pathological children bone samples (removed surgically from healthy zones) has been established as reference database. Analysis of diagonal stiffness coefficients in the three orthogonal bone axes has been done giving some indication of how bone anisotropy is related to age [1]–[4]. High-resolution X-ray images enable to characterize the vascular pores network in healthy children's bone and to relate morphometric parameters to mechanical properties [5], [6]. Nevertheless, the characterization of bone as an in vitro material at mesoscopic or microscopic scales is not sufficient, we need to consider the mechanical behavior of bone in the *in vivo* conditions and to identify the relevant parameters related to it. In particular, we have to study more precisely the contribution of the soft tissues around the bone (periosteum, muscle) and the fluid inside the pore networks (vascular and lacuno-canalicular) of cortical bone. The children's cortical bone is a material

with two-level porosity network: the vascular porosity (Haversian and Volkmann canals around 100µm) and the lacuno-canalicular network (1 to 10µm pores). The presence of fluid in this two-level porosity induces a viscoelastic behavior and plays a key role in the interaction mechanisms of the ultrasonic waves and bone tissue.
The vascular porosity is, generally, taken into account in terms of volume fraction without considering the spatial distribution or the shape or the organization of the pores [6]. Previous studies show that the morphometric parameters are critical to investigate the mechanical behavior of the children's bone.
The evaluation of the bone tissue permeability at lacuno-canalicular level remain a tricky and fundamental open question.
Consequently, we need to pursuit the macroscale study on *ex vivo* samples, with acoustical and biomechanical experiments, and to conduct a further study, from nano to microscale, with high-resolution images (SAM, micro- and nano-CT). We will subsequently make further progress to enhance the parametric and morphometric analysis of the children's bone and gain detailed insight into lacuno-canalicular porosity and permeability.
It is noteworthy that the lacuno-canalicular network is the place where are the osteocytes which are regarded as the pivotal cells orchestrating the biomechanical regulation of bone mass and structure. They are considered as the mechanosensory cells responsible of bone remodeling under mechanical loading [7]. Consequently, the characterization of the lacuno-canalicular porosity is an important step to analyze and understand the therapeutic effect of ultrasonic wave on bone regeneration.

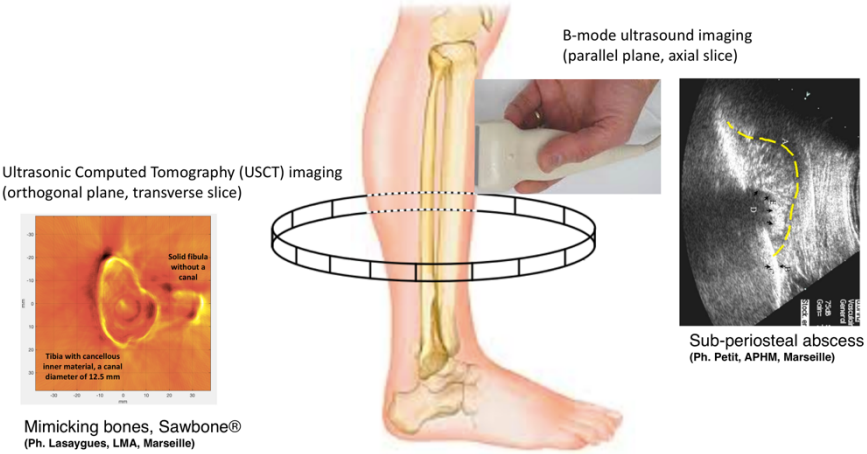
Points of interest could be
- Multi-scale behavioral law (macro- to nanoscale, lacuno-canalicular network modelling);
- porosity and permeability
- numerical modelling of behavioral laws;
- wave-cell interaction, wave-organ interaction, fluid-solid interaction;
- the contribution/effect of the soft tissues around the bone (periosteum, muscle)

Subject no2: USCT vs. B-mode Ultrasound ("echography")

For children's bone pathologies, the development of ultrasonic imaging modalities and protocols are important challenges in order to provide an alternative to other imaging techniques. Conventional modalities such as X-ray radiology or Computed Tomography (CT) must be limited due to their radiation hazard. Magnetic Resonance Imaging is a major tool but require more frequently anesthesia [8] than CT, and give poor cortical information. Nowadays, the B-mode ultrasound is the first-line examination for the diagnosis of many children's diseases. Due to its efficiency, the B-mode ultrasound is particularly dedicated to pediatric use, allowing to increase the number of procedures without harmful effects. However, B-mode ultrasound has difficulty to penetrate bone and, therefore, can only see the outer surface of bony structures, and not what lies within them. Currently, densitometry methods are the most commonly used to assess skeletal status in metabolic bone diseases. Investigations are mainly performed using Dual Energy X-ray Absorptiometry (DXA). DXA provides information on quantitative content of hydroxyapatite calcium in measured bones. Thus, DXA widely used in a clinical practice does not provide any data on bone quality features. It is well known that biomechanics competence of the skeleton is dependent both on bone mineral density (BMD) measured by DXA and quality features of bone such as elasticity or microarchitecture. Some methods may give additional data on bone tissue and among them are quantitative ultrasound (QUS) [9]. QUS used since mid-eighties shows several important advantages: ability to assess some quality features of bone tissue, relatively low costs and small sizes of equipment. QUS has also some disadvantages involving difficulty to precisely measured bone tissue features, skeletal sites limited only to peripheral skeleton Commercially available QUS machines measure several peripheral skeletal sites: calcaneus, phalanges, or patella, and there is no general solution for the others. Commonly, the scope of interest in reviews concerning QUS [9] is mainly osteoporotic populations. It's obvious that osteoporosis is the most

common metabolic bone disease but there are several other areas where the follow-up of bone status is essential, especially in children. Skeletal status changes during the whole life in physiologic (growth, involution) or pathologic states (endocrine disorders, side effects of some medications etc.). The role of children's bone quality is currently widely accepted in a clinical context. But there is an urgent need to develop ultrasonic methods able to assess not only children's bone quantity but also children's bone quality. B-mode ultrasound does not allow this characterization of the children's bone microarchitecture. The image is not quantitative; the grayscale levels are not linked to any significant physical parameters of the organs, such as speed of sound or attenuation.

For several years, tests have been conducted using the diffraction-mode ultrasonic imaging of pediatric bone with a parametric characterization of bone lesion. The developed methods are based on the ultrasonic computed tomography (USCT) principles provide images of soft medium such as breast. The acquisition geometry of the signals is no longer linear nor sectoral, as in B-mode ultrasound, but circular in the orthogonal plane, and based on a multi-channel and multi-frequency antenna. The main difficulty of the USCT applied in the case of bone imaging is the large impedance contrast between echogenic structures and the surrounding soft tissues. In that context, approximation methods commonly used for soft tissues fail to provide quantitative images, except when adapted but time consuming algorithms such as compound USCT for instance are used [10]. We have then suggested a purely numerical non-linear inversion algorithm, and the minimization procedure between the full recorded and simulated data which is solved using a conjugate-gradient method [11], [12]. The main objective of this subject is to develop a real prototype of predictive analysis and diagnosis of the pediatric skeleton based on USCT. The locks associated with an in-vivo configuration are still numerous: the number of transducers, the power and the intensity of the wave, the image resolution and the contrast in the deep zone, the accessibility of non-peripheral skeletal sites, or in between the two leg and arm bones, and the muscle and soft tissue effect in term of dispersion and attenuation of the wave.



Points of interest could be

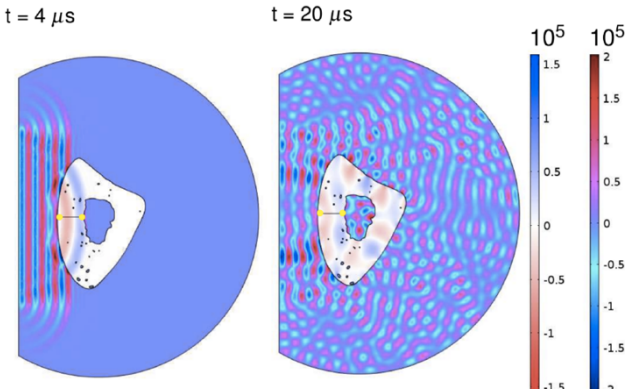
- Numerical modelling of wave-organ interaction in the case of USCT;
- the contribution/effect of the soft tissues around the bone (periosteum, muscle);
- Signal processing
- Image processing in the case of paired bone (radius and ulna / tibia and fibula)

Subject no3: Therapeutic perspectives

Ultrasonic waves can be used as a therapeutic vector in the context of bone repair. Understanding the mechanisms induced by ultrasonic wave propagation at the different scales of bone tissue, from the organs to the cells, would make it possible to take into account the main parameters of the therapeutic process and to integrate them into a protocol associated with diagnosis. Nowadays, clinical ultrasonic therapy modalities are mainly high-intensity focused ultrasound to destroy tumor tissue, or lithotrity for renal or prostatic stones. None of them is applicable to bone pathologies (especially in children) because of the high intensities of radiation. Osteo-articular diseases cause a disruption of the bone (re)modeling, generating weak bones leading to serious skeletal events (fractures, spinal compression). One solution could be to

resort to low-intensity ultrasonic stimulation (*LIUS*). In this case, low-intensity ultrasonic waves interact with surrounding tissue and cells, producing mechanical effects that promote bone regeneration. Little is known about the mechanotransduction induced by *LIUS*.

In this subject, we want to understand the process that drives bone regeneration, and in particular the mechanotransduction processes. How does the organ translate ultrasonic stress into cellular biological reaction? A first component of the project involves the development of a multi-physical, multiscale numerical modeling that integrates (*A*) wave propagation and its interaction with bone as a poro-elastic material, with soft tissues represented by viscous fluids, and the biological fluids such as marrow and blood; (*B*) fluid/structure interactions at the cellular scale. The *LIUS* method must be optimized to define the frequency, the power and/or the intensity of the ultrasonic wave to be applied to improve bone repair. This requires the implementation of cell culture protocols associated with an ultrasonic transmitted device. Understanding the ultrasonic mechanical and piezo-electric effects on biological tissues would be a major scientific breakthrough. If the ultrasonic parameters (frequency, intensity, power) useful for *LIUS* therapy can be controlled and coupled with an ultrasonic imaging system, a pediatric theranostic tool can be proposed.



Numerical modelling of LIUS wave propagation

Points of interest could be

- Lacuno-canalicular network modelling;
- Numerical modelling of fluid-solid interaction;
- Numerical modelling of the ultrasonic transducers effects (frequency, intensity, power)

Publications

[1] J.-P. Berteau, C. Baron, M. Pithioux, F. Launay, P. Chabrand, and P. Lasaygues, "In vitro ultrasonic and mechanic characterization of the modulus of elasticity of children cortical bone," *Ultrasonics*, vol. 54, no. 5, pp. 1270–1276, Jul. 2014.

[2] J.-P. Berteau *et al.*, "Ratio between mature and immature enzymatic cross-links correlates with post-yield cortical bone behavior: An insight into greenstick fractures of the child fibula," *Bone*, vol. 79, pp. 190–195, Oct. 2015.

[3] E. Lefèvre *et al.*, "Analyzing the anisotropic Hooke′s law for children′s cortical bone," *J. Mech. Behav. Biomed. Mater.*, vol. 49, pp. 370–377, Sep. 2015.

[4] E. Lefèvre, P. Lasaygues, C. Baron, C. Payan, H. Follet, and M. Pithioux, "Ultrasonic assessment of diagonal stiffness coefficients in children cortical bone," *Comput. Methods Biomech. Biomed. Engin.*, vol. 18, no. sup1, pp. 1978–1979, Oct. 2015.

[5] F. Montagner, V. Kaftandjian, D. Farlay, D. Brau, G. Boivin, and H. Follet, "Validation of a novel microradiography device for characterization of bone mineralization," *J. X-Ray Sci. Technol.*, vol. 23, no. 2, pp. 201–211, 2015.

[6] Y. Bala *et al.*, "Pore network microarchitecture influences human cortical bone elasticity during growth and aging," *J. Mech. Behav. Biomed. Mater.*, vol. 63, pp. 164–173, Oct. 2016.

[7] Y. Han, S. C. Cowin, M. B. Schaffler, and S. Weinbaum, "Mechanotransduction and strain amplification in osteocyte cell processes," *Proc. Natl. Acad. Sci.*, vol. 101, no. 47, pp. 16689–16694, Nov. 2004.

[8] P. Petit *et al.*, "Rate of Abnormal Osteoarticular Radiographic Findings in Pediatric Patients," *Am. J. Roentgenol.*, vol. 176, no. 4, pp. 987–990, Apr. 2001.

[9] P. Laugier and G. Haïat, *Bone quantitative ultrasound*. Dordrecht; New York: Springer Science, 2011.

[10] P. Lasaygues, E. Ouedraogo, J.-P. Lefebvre, M. Gindre, M. Talmant, and P. Laugier, "Progress towards in vitro quantitative imaging of human femur using compound quantitative ultrasonic tomography," *Phys. Med. Biol.*, vol. 50, no. 11, pp. 2633–2649, Jun. 2005.

[11] R. Guillermin, P. Lasaygues, and G. Rabau, "Quantitative Ultrasonic Imaging of Bones," in *The 22nd International Congress on Sound and Vibration*, Florence, Italy, 2015, pp. 1–6.

[12] S. Bernard, V. Monteiller, D. Komatitsch, and P. Lasaygues, "Ultrasonic computed tomography based on full-waveform inversion for bone quantitative imaging," *Phys. Med. Biol.*, vol. 62, no. 17, pp. 7011–7035, Aug. 2017.