Assessing Biomechanics of Skeletal Tissue During Growth
A multimodal approach of bone and cartilage

Jean-Philippe Paul BERTEAU, PT-PhD
Assistant Professor CSI CUNY & Physical Therapy Dept

09.07.2014
Who am I?

PT, Physical Therapist (2002)
thesis: Physical Therapy after scoliosis surgery
Physical Therapy Practitioner during 7 years
(Private office and Bordeaux University Hospital)
for this period:
University Diploma of Posturology
University Diploma of Clinical Research
Who am I?

- **PT**, Physical Therapist *(2002)*
  Physical Therapy Practitioner for 7 years
  (Private office and Bordeaux University Hospital)

- **M.Sc**, Biomechanics *(2008)*
  thesis: Optimization of scoliosis brace
  CNRS and Aix Marseille University
  Institute of Movement Sciences (ISM)
  » numerical brace, Cbrace
Who am I?

- **PT**, Physical Therapist *(2002)*
  - Practitioner during 7 years
  - (private office and Bordeaux University Hospital)

- **M.Sc., Biomechanics** *(2008)*
  - CNRS and Aix Marseille University
  - Institute of Movement Sciences (ISM)
  - » Cbrace

- **PhD, Biomechanics & Biomedical Engineering** *(2011)*
  - Thesis: Multimodal Characterization of Biomechanical Properties of Human Growing Cortical Bone
  - CNRS and Aix Marseille University
  - » Original values on AIS ribs and physiological fibula, children fracture
Who am I?

- **PT**, Physical Therapist *(2002)*
  - Practitioner during 7 years
  - (private office and Bordeaux University Hospital)

- **M.Sc., Biomechanics (2008)**
  - CNRS and Aix Marseille University
  - Institute of Movement Sciences (ISM)
  - » Cbrace

- **PhD, Biomechanics & BME (2011)**
  - CNRS and Aix Marseille University -
    Laboratory of Mechanics and Acoustics (LMA) and Institute of Movement Sciences (ISM)
  - » Original values on AIS ribs, fibula, children fracture

- **Young European Investigator** Biomechanics of the spine *(2012)*
  - European Project SpineFX Marie Curie Action
  - Hamburg University of Technology TUHH
  - » Original values on facet joint and optimization of spine treatment
Who am I?

PT, Physical Therapist (2002)
practionner during 7 years
(private office and Bordeaux University Hospital)

M.Sc, Biomechanics (2008)
CNRS and Aix Marseille University
Institute of Movement Sciences (ISM)

PhD, Biomechanics & Biomedical eng. (2011)
CNRS and Aix Marseille University -
Laboratory of Mechanics and Acoustics (LMA)
Institute of Movement Sciences (ISM)

Young European Investigator Biomechanics of the spine (2012)
European Project SpineFX Marie Curie Action
Hamburg University of Technology TUHH

Senior PostDoc-Lab Manager Biomechanics of the cartilage (2013)
Shefelbine Lab - Northeastern University

Original permeability and elasticity values of cartilage during growth
Assessing Biomechanics of Skeletal Tissue During Growth

A multimodal approach of bone and cartilage

Jean-Philippe Paul BERTEAU, PT-PhD

Assistant Professor CSI CUNY & Physical Therapy Dept

09.07.2014
A pre-question: What is a skeletal tissue? (bone and cartilage)

- **Physical Therapy view:**
  a piece of the body structure, a place of orthopedics pathology, healing, chronic pain
A pre-question: What is a skeletal tissue? (bone and cartilage)

- Physical Therapy view:
  a piece of the body structure, a place of orthopedics pathology, healing, chronic pain

- Medical-Biological view:
  organized tissue protein, calcium reserves

- Mechanical and Acoustical view:
  material with mechanical properties

- Chemical view:
  polymer with crystal/crosslinks
Can be characterized with a multimodal approach

- *in vivo*

Medical Imaging (US - MRI - CT)
Can be characterized with a multimodal approach

- *in vivo*
  
  Medical Imaging (US - MRI - CT)

- *in vitro*
  
  Mechanics
  Acoustics
  Histology
  Biochemistry
Orthopedic Physical Therapists diagnose, manage, and treat disorders and injuries of the musculoskeletal system. Adults and children cannot be treated similarly.
Biomechanics point of view:
PT controls the load applied on the skeletal tissue during recovery process or during treatment

Apply load to stimulate or to correct
Prothesis

Osteoporosis

Osteoarthritis

Green stick

Scoliosis

Pathologies of growth
**Question 1:** What is the mechanical difference between adult and children bone?

*The green stick fracture model*
**Question 1**: What is the mechanical difference between adult and children bone?

*The green stick fracture model*

![Image of green stick fracture and brittle fracture](image.png)

**Theoretical hypothesis** *(Irwin and Mertz, 1997)*

Other experimental results must be taken with caution
**Question 1**: What is the mechanical difference between adult and children bone?

*The green stick fracture model*

---

**Theoretical hypothesis** *(Irwin and Mertz, 1997)*

Other experimental results must be taken with caution.
Direct bone >> cortical
Indirect bone : cartilage >> trabecular
Two Processes: Mineralization and Collagen Maturation

1. Mineralization

![Graph showing height velocity (cm/year) vs age (years)](image)
Two Processes: mineralization and collagen maturation

Mineralization

0 - 2 yrs
Lefèbvre and Kofmann
(elbow end knee)

2 yrs – end of growth
Greulich and Pyle
(atlas: wrist epiphysis)

end of growth
RISSER TEST
Two Processes: Mineralization and Collagen Maturation

2. Collagen cross links
Mature (in adult bone) and Immature (in children bone)
Cross links amount: depends on age (Saito et al., 2010)

Two Processes: Mineralization and Collagen Maturation

2. Collagen cross links
Mature (in adult bone) and Immature (in children bone)
Cross links amount: depends on age (Saito et al., 2010)
Bone Sample Preparation

1. FIBULA DURING SURGERY
2. LAW SPEED DIAMOND SAW
3. PBS and dry ice
4. manual cutting
5. 15 fibula

L : 10 - 30 mm
l : 8 - 15 mm
e : 1 - 3 mm
Multimodal Protocol : Fibula

1. **ELASTICITY**
   - Ultrasound Propagation

2. **ENERGY**
   - Three-Point Bending

3. **BIO ORGANIZATION**
   - High Pressure Liquid Chromatography
     - Mature
     - Immature
     - Ratio
     - % Collagen
1. Elasticity, Experimental US Device

video from PZFlex

transducers: micrometric displacements

IN WATER

IN BONE
1. Elasticity, Experimental US Device

ELASTICITY

longitudinal : direct
and transverse : rotation

IN WATER

IN BONE
1. Elasticity, Ultrasound propagation

Law for non porous (or low level of porosity) material: (Ho Ba Tho et al., 1992; Pithioux et al., 2002)

\[
\Delta t = t_{AB'} - t_{AB} = \frac{AB'}{V_{eau}} - \frac{AB}{V}
\]

\[
n = \frac{V_{water}}{V} = \frac{\sin i}{\sin r}
\]

\[
V \approx \frac{V_{water}}{\sqrt{1 + \frac{V_{water} \Delta t}{e} \left( \frac{V_{water} \Delta t}{e} - 2 \cos i \right)}}
\]
1. Experimental US device

- transducers 7 and 10 MHz
- 3 times per sample: $V_l$ and $V_t$
- propagation law: $E_a$ and $\nu$

\[
E_a = \rho \cdot \frac{V_t^2 (3V_l^2 - 4C_t^2)}{(VV_l - V_t^2)}
\]

\[
\nu = \frac{(V_l^2 - 2V_t^2)}{2(V_l^2 - V_t^2)}
\]
2. Energy, Micro Three-Point Bending

![Image of micro three-point bending experiment]
2. Energy, Micro Three-Point Bending
2. Energy, Micro Three-Point Bending

Young’s modulus: \( E_m = \frac{F}{d} \times \frac{L^3}{48*I} \)
2. Energy, Micro Three-Point Bending
2. Energy, Micro Three-Point Bending

Energy: $\omega_{rup} = \omega_e + \omega_p$
2. Micro three-points bending

\[ \text{stress} : \sigma = \frac{F \cdot L \cdot e}{4 \cdot l} \]

\[ \text{strain} : \epsilon = \frac{12 \cdot e \cdot d}{L^2} \]

Young’s modulus: \( E_m = \frac{F}{d} \cdot \frac{L^3}{48 \cdot l} \)

Energy: \( \omega_{rup} = \omega_e + \omega_p \)
1 & 2 acoustics and mechanics

Acoustics velocities: \( Vl \) & \( Vt \)

Modulus of elasticity: \( E_{msta} \) & \( E_{adyn} \)

Poisson ratio: \( \nu \)

Energies: \( \omega_{rup} \) & \( \omega_{e} \) & \( \omega_{p} \)
3. Bio Organization, Biochemistry HPLC

- Grinding
- Demineralization
- Freeze-drying

![Graph showing HPLC analysis](image.png)
3. Bio Organization, Biochemistry HPLC
3. Bio Organization, Biochemistry HPLC

**Enzymatic cross-links**

**Intracellular**

- Telopeptidyl Hyl
- Telopeptidyl Lys

**Lysine Hydroxylase**

- Lysyl oxidase (LOX)

**Hydroxylysine**

+ Helical Hyl
+ Helical Lys

**Allysine**

+ Helical Lys

**deH-DHLNL**

+ Hydroxyallisine
+ Helical Hyl
+ Allisine

**Pyridinoline (PYD)**

**Pyrrololine (PYL)**

**deH-HLNL**

+ Hydroxyallisine
+ Allisine

**Deoxy-pyridinoline (DPD)**

**Deoxy-pyrololine (DPL)**

**Non-enzymatic cross-links**

- Lys or Hyl in helical domain

  + glucose, pentose

  Glycation
  Glycoxidation
  Oxidative stress

**Schiff base**

hexosyl-Lys + Lys or Arg

**Amadori product**

**The senescent divalent cross-links**

- Non-crosslink type AGEs (Carboxyl methyl lysine CML)

  Intermolecular cross-links (pentosidine, vesperlysine)

**Advanced glycation end products (AGEs)**
3. Bio Organization, Biochemistry HPLC

**Enzymatic cross-links**

- Intracellular
  - Telopeptidyl Hyl
  - Telopeptidyl Lys
- Lysine Hydroxylase
- Lysyl oxidase (LOX)
- Hydroxylysine
- Allysine

**The immature divalent cross-links**
- deH-DHNLNL
- + Helical Hyl
- + Helical Lys

**The mature trivalent cross-links**
- Pyridinoline (PYD)
- + Hydroxylysine
- + Allisine
- Pyrrololine (PYL)
- + Allisine
- Deoxy-pyridinoline (DPD)
- + Hydroxylysine
- + Allisine
- Deoxy-pyrololine (DPL)
- + Allisine

**Non-enzymatic cross-links**

- Lys or Hyl in helical domain
- + glucose, pentose
- Glycation
- Glycoxidation
- Oxidative stress

**Schiff base**

- hexosyl-Lys
- + Lys or Arg

**Amadori product**

**The senescent divalent cross-links**

- Non-crosslink type AGEs
  - Carboxyl methyl lysine (CML)
- Intermolecular cross-links
  - (pentosidine, vespertinoline)

**Advanced glycation end products (AGEs)**
3. Bio Organization, Biochemistry HPLC

- **Enzymatic cross-links**
- **The immature divalent cross-links**
  - deH-DHLNL
  - Hydroxylysine
  - + Helical Hyl
  - + Helical Lys
  - deH-HLNL
- **The mature trivalent cross-links**
  - Pyridinoline (PYD)
  - Pyroline (PYL)
  - Deoxy-pyridinoline (DPD)
  - Deoxy-pyroline (DPL)

- **Non-enzymatic cross-links**
  - Lys or Hyl in helical domain
  - + glucose, pentose
  - Glycation
  - Glycoxidation
  - Oxidative stress
  - Schiff base hexosyl-Lys
  - + Lys or Arg
  - Amadori product
  - Advanced glycation end products (AGEs)

**Process Details**:
- 1 process for DHLNL/HLNL
- 1 process for PYD/DPD
3. Bio Organization, Biochemistry HPLC

- 1 process for mature
- 1 process for immature
- 1 process for collagen amount
Current Paradigm: Adult ≠ Child
Results

Adult = Child

- Acoustics
  \[ V_f = 3000 - 3500 \text{ m/s} \]
- Elasticity
  \[ E_m \& E_a = 8 - 15 \text{ GPa} \]
- Organic
  \[ \% \text{ collagen} = 22 \]
- Mineral
  \[ \text{density} = 2 \text{ g/cm}^3 \]
Results

Adult = Child

- Acoustics
  \[ V_1 \text{ (m/s)} = 3000 - 3500 \text{ m/s} \]
- Elasticity
  \[ E_m \& E_a = 8 - 15 \text{ GPa} \]
- Organic
  \% collagen = 22
- Mineral
  density = 2 g/cm³

Adult \( \neq \) Child

- \( \omega_p \) and ratio cross links
  ***
Results, Plastic Energy $\omega_p$ and Ratio Cross Links

Parametric correlation $\omega_p$ and Ratio

$R^2 = 0.69$
**Question 1**: What is the mechanical difference between adult and children bone? *the green stick fracture model* a link between biological and mechanical values

Theoretical hypothesis *(Irwin et Mertz, 1997)*

Green stick = cross links explanation
Prothesis

Osteoporosis

Osteoarthritis

Green stick

Scoliosis

Pathologies of growth
Question 2: What is the impact of orthopedic treatment on bone material properties?
Multimodal Protocol 2: Ribs

1. Organization **Histology**
2. Mineralization and Geometry **CT-scan**
3. Elasticity **US**

AIS of 15-17 YO
6 pieces of ribs
(gibbectomy, 4th to 8th ribs)
1. Histology

organization and porosity
2. Image CT-scan BMD and Geometry

CT-scan : BMD
quantifying the geometrical modification of the AIS ribs
3. US
Porosity lower than 2.18%, average value: 1.35% ± 0.52
Results AIS Ribs

<table>
<thead>
<tr>
<th></th>
<th>$V_l$ (m/s)</th>
<th>cortical shell (mm)</th>
<th>Elasticity</th>
<th>BMD (mgHA/cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIS ribs</td>
<td>$V_l = 3200$</td>
<td>2.85 ($+/−0.32$)</td>
<td>$E = 15$ GPa and $\nu = 0.26$</td>
<td>2200-2500</td>
</tr>
<tr>
<td>Literature</td>
<td>$V_l = 3000 − 3500$</td>
<td>0.6 to 1.4</td>
<td>$E = 11.5$ GPa and $\nu = 0.3$</td>
<td>1200</td>
</tr>
</tbody>
</table>

(Berteau et al., 2014; Li et al., 2010; Chen et al., 2010, Boivin et al., 2002)

Increase geometry and BMD not elasticity
Question 2: What is the impact of orthopedic treatment on bone material properties?

- Brace impacts BMD and geometry, but not elasticity
- Load increase BMD » Julius Wolff Law
- Physical Therapy can impact bone biomechanics
- Find a good balance
Prothesis

Osteoporosis

Osteoarthritis

Green stick

Scoliosis

Pathologies of growth
**Question 3:** What is the change in the elastic properties and the permeability of cartilage during growth?
Experimental Set Up

What are the material properties of murine cartilage?

- n=7, C57BL/6 mice (2-3-5-7-9-12-17 week old)
- sacrifice
- storage -20°C, PBS + PI
- P indentation load
  - R radius of the indenter
  - h penetration depth
  - 2a 2*contact radius

Elasticity
Permeability K

(Extracted from Richard et al., 2013)
Results
Time/Load curve

- Lateral condyle (FLC), 2 week old mouse
- Displacement control, peak force 30 $\mu$N, loading rate 600 nm/s, t=5s-20s-5s, maximum depth 700 nm
Results

K and E Age

K depends on age, not E
Results

Location

- statistical difference for Elasticity between LFC and MFC
- no difference for K
Question 3: What is the change in the elastic properties and the permeability of cartilage during growth?

Permeability decreases with age, location changes the elasticity.
General Conclusion

- **Bone Elasticity**:  
  Adults and children: no difference  
  (Berteau et al., Ultrasonics, 2013)

- **Bone Plasticity**:  
  Explanation in the subnanostructure  
  (Berteau et al., review JBMR, 2014)

- **Brace Impact**:  
  Mechanical action increases the BMD and the size  
  (Berteau et al., JOB, 2012)

- **Cartilage properties**:  
  Permeability decreases with age, location changes the elasticity  
  (Berteau et al., in progress JMBBM, 2014)
Future

- **Non invasive detection osteoarthritis in juvenile population**: US characterization combined with histology » transfert to young population
Non invasive detection osteoarthritis in mice:
detect a default of 10 micron on a soft tissue layer of 50 micron

coll. S.J. Shefelbine NEU
Future

Role of nanostructure on the mechanical behavior of bone tissue: influence of mineral density and crosslinks

Os (cm)  Ostéon (Ø 100µm)  Lamelles (5 µm)  Fibrilles + minéral (100 nm)  Modèle moléculaire (10 nm)

coll. B.Depalle MIT
Future

- **Improve the PT action**:
  already develop for brace treatment (Berteau et al., TSJ, 2011)
  multimodal approach for the SMZ in the SpineFX project
Thank you for your attention, questions are welcome 😊