PP38

BISPHOSPHONATES INFLUENCE ON BONE QUALITY AT MOLECULAR LEVEL: STUDY OF HUMAN JAW BONE SEQUESTERS BY RAMAN MICROSPECTROSCOPY

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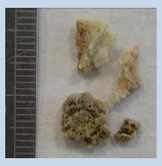
BACKGROUND

- **Bisphosphonates (BP) = anti-resorptive drugs with strong affinity for bone mineral, in particular for sites with high bone turnover**
- **Jaw bone = bone skeletal site with a high turnover**

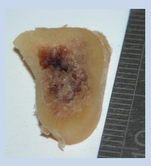
→ **Physicochemical changes of human jaw bone upon BP uptake?**

METHODS

Bone samples



BP group (n=24)
- BP-exposed bone sequesters
- 42 to 94 years old subjects
- underlying diseases requiring BP therapy: osteoporosis or malignancy (myeloma or bone metastasis)
- BP molecule: alendronate, ibandronate, zoledronate, pamidronate et clodronate
- therapy duration: 4 to 156 months



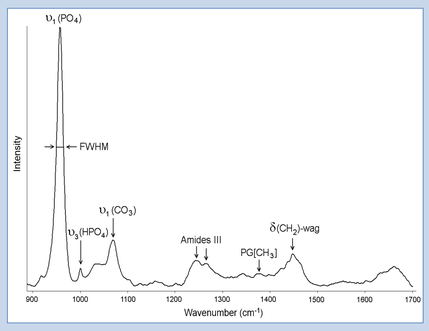
CTL group (n=24)
- healthy cadaveric cortical jaw bone sections (Anatomy laboratory of Lille medical school)
- 64 to 93 years old subjects

Raman microspectroscopy

Labram microspectrometer (HORIBA)
- λ = 632 nm
- x100 objective (ON = 0,8)
- spectral resolution: 2 cm^{-1}
- 900-1700 cm^{-1} range
- acquisition time: 10 x 60 s

Punctual analyses :
- cortical or lamellar bone tissue
- each 2 µm from a 50 µm analyze line
- 75 measurements per sample

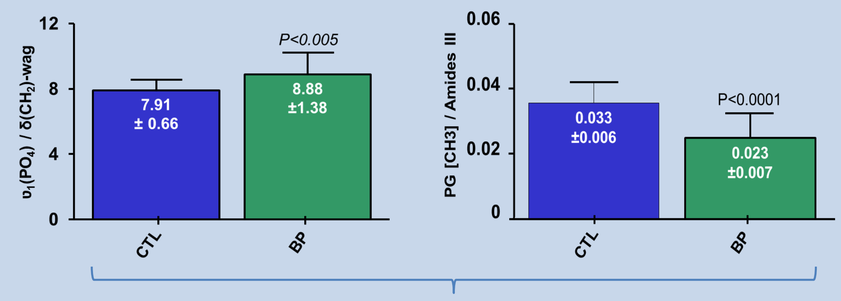
Physicochemical parameters related to bone quality
- Mineral to organic ratio: $\nu_1(PO_4)/\delta(CH_2)$-wag
- Relative proteoglycan (PG) content: $PG[CH_3]$/ Amides III
- Cristallinity: 1/FHWM $\nu_1(PO_4)$
- Monohydrogen phosphate content: $\nu_3(HPO_4)/\nu_1(PO_4)$
- Type-B carbonate substitution: $\nu_1(CO_3)/\nu_1(PO_4)$



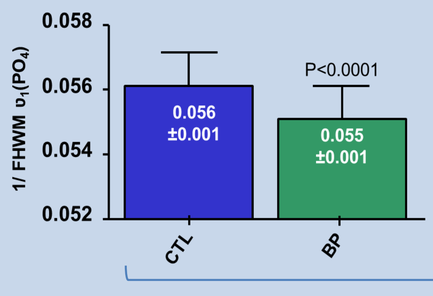
Partial-Least-Square Discriminant Analysis (PLS-DA, PLS Toolbox v6.7)
→ specific Raman spectral features of each group

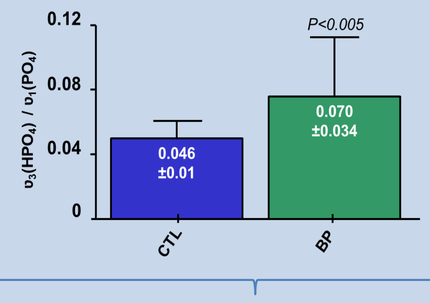
RESULTS

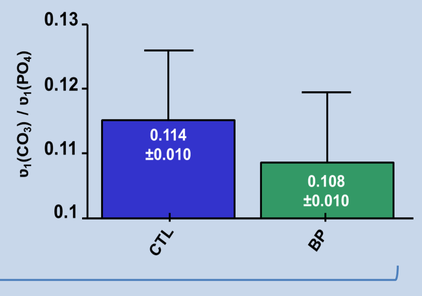
Raman physicochemical parameters (mean ± SD, t test)



Overmineralization in the BP group
- ↗ ratio mineral to organic (+ 12 %)
- ↘ PG content (- 30 %) i.e. promoting collagen matrix mineralization

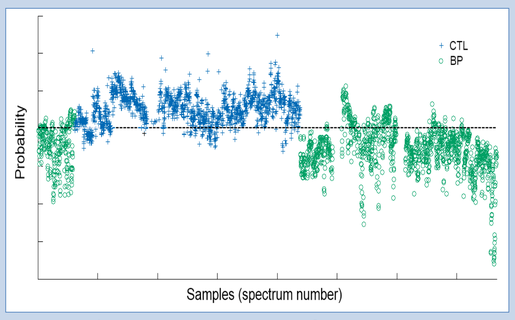




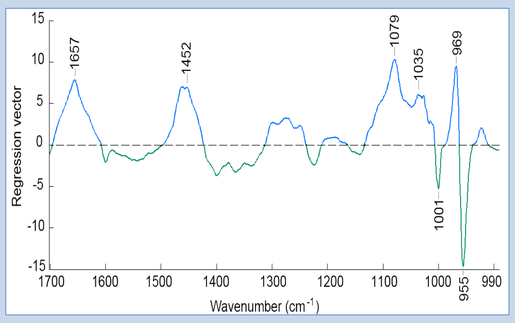


Structural changes on mineral components in the BP group
- ↘ cristallinity (- 2 %) and mineral maturity (- 52 %)
- carbonate substitution unchanged

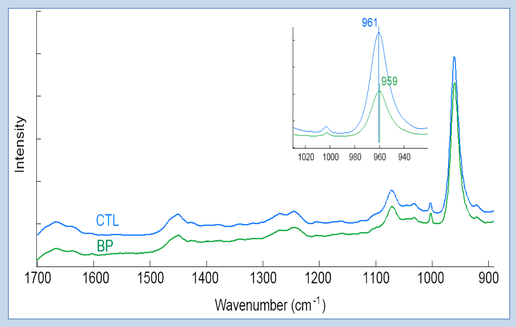
PLS-DA analysis



PLS-DA discriminate BP group from CTL group



Regression vector with Raman spectral features
(CTL: positive bands, BP: negative bands)



Average raw Raman spectra
→ inset shows the shift of the ν_1 PO_4 band

Spectral features to discriminate the 2 groups
- CTL group : organic components (collagen, amides I, lipids)
- BP group : mineral components (PO_4, HPO_4)

Structural changes on the apatite phosphate environment
- typical "S-shape" positive/negative = shift of the ν_1 PO_4

CONCLUSIONS

- **Cumulative BP uptake in human jaw bone causes bone quality alterations :**

  → **Overmineralization compared to healthy bone**

  → **Ultrastructural changes of mineral components, perturbations of the phosphate environment due to BP binding/interaction**

- **Additional studies are needed to evaluate the impact of these changes on pathophysiological behavior of bone**