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

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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 metatasis)
- 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 (HDRIBA)
- 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:
  - v₁(PO₄)²⁻/v₃(CH₃) wag
- Relative proteoglycan (PG) content:
  - PG(CH₃)³/ Amides III
- Crystallinity:
  - 1/FWHM v₁(PO₄)
- Monohydrogen phosphate content:
  - v₂(HPO₄)²⁻/v₁(PO₄)
- Type-II carbonate substitution:
  - v₁(CO₃)²⁻/v₁(PO₄)

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
  - ➔ crystallinity (- 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 v₁ PO₄ band

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