Labex MATISSE

Axe Biominéralisation

"In situ and ex situ study of apatite formation in bone tissue models"

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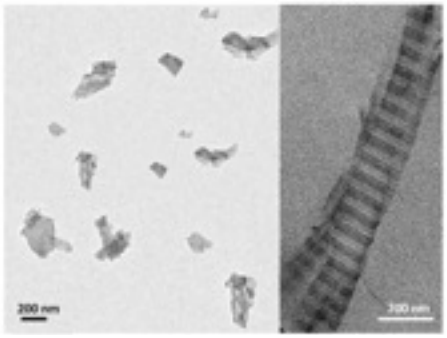

Figure 1 – Biomimetic hydroxyapatite crystals (left) and Type I collagen fribril (right) observed on TEM. The formation of apatite crystals is monitored by *in situ* Raman and *ex situ* ^{31}P solid state NMR.

Hosting laboratories, teams and and thesis supervisors names:

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Research project (10 lines)

Bone biomineralization is a complex time-related biological process that leads to the deposition of bone mineral in close association with Type I collagen to form a functional mineralized tissue. The comprehension of bone formation is a long-standing goal and a scientific as well as a technical challenge for researchers. Despite important *in vivo* and *in vitro* contributions aiming to clarify the implication of the main components and parameters involved in bone biomineralization (e.g. collagen, ions that form the bone mineral phase, collagen/ apatite interactions, matrix vesicles, cells and non-collagenous proteins) no consensus emerged to describe the whole scenario of bone mineral formation. In particular, the question of bone mineral precursors is still controversial and the key parameters that direct mineral formation are not established yet.
For this purpose, we developed a panel of *in vitro* bone models focusing on the reproduction of bone 3D collagen architecture found in native bone (density and organization) as well as on the synthesis of biomimetic hydroxyapatite (carbonate content, presence of an amorphous layer, oriented self-aggregation in water). The intrinsic biomimetic nature of these models makes relevant the kinetic study of their synthesis. Here we combined two spectroscopic techniques to assess mineralization as a function of time. Thus using *in situ* Raman spectroscopy we monitored various bone models formation following the calcium phosphate (CaP) precipitation and we identified the main events in the mineral formation in correlation with time. Based on these *in situ* Raman data we complementary performed *ex situ* ^{31}P solid state NMR (ssNMR) to clearly identify the different CaP phases and propose precipitation scenarios.

Summerize your scientific results & impacts

We evidenced that for our models the first formed CaP phase is an amorphous calcium phosphate (ACP) that is next partially dissolved and follow by the precipitation of brushite and octacalcium phosphate (OCP) which transformed into biomimetic hydroxyapatite. As OCP to hydroxyapatite transformation may have relevance *in vivo* ^{31}P ssNMR allowed us to follow the mechanism transformation through ^{31}P chemical shift analysis. The role of different organic additives described to influence the mineral precipitation (e.g. citrate and polyaspartate) has also been studied.
Finally, we observed a significant kinetic effect (slow down) and the stabilization of ACP due to collagen in consistence with very recent findings.

Main key facts

Poster and oral presentation at the « Journée MATISSE » the 03/07/14
Oral presentation at LCMCP laboratory meeting the 13/11/14
Oral presentation at the Collège de France for the visit of Melinda Duer the 10/03/15

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