Surface properties of biomimetic nanocrystalline apatites; applications in biomaterials

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1. Introduction

Several types of nanocrystalline apatites have been described, obtained in various ways. Among these, biomimetic nanocrystalline apatites (BNA), whose characteristics are close to those of biological apatites, have been shown to exhibit specific properties mainly related to their surface structure and composition. The aim of this paper is to review current knowledge of these compounds.

2. Biomimetic nanocrystalline apatites

The term biomimetic is used with different meanings; applied to materials it is often intended to denote preparative techniques and/or properties mimicking those of biological materials. BNA are

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defined as apatites exhibiting the main characteristics of biologically produced apatites found, for example, in calcified tissues of vertebrates or ectopic calcifications. Four main characteristics can be identified:

- Non-stoichiometric composition.
- Presence of CO_3^{2-} and HPO_4^{2-} ions.
- Nanometric platelet crystals.
- Hydrated layer on the crystal surface.

2.1. Non-stoichiometry

Several types of non-stoichiometric apatites can be distinguished depending on the substituents and vacancies present in these widespread structures. Stoichiometric apatites are ideally represented by the following formulae:

$$Me_{10}(XO_4)_6Y_2 \tag{1.1}$$

where Me represents a bivalent ion (Ca, Sr, Ba, Pb, Cd, Mn, …), XO_4 a trivalent anion (PO_4, AsO_4, VO_4, …) and Y a monovalent anion (F, Cl, Br, OH, …). A model compound of biomimetic apatite is the calcium phosphate hydroxyapatite:

$$Ca_{10}(PO_4)_6(OH)_2 \tag{1.2}$$

Solid solutions often exist between apatites with different compositions [24]. Non-stoichiometry is related to vacancies in Me sites and Y sites. Significant number of vacancies in XO_4 sites have never been reported and theoretical calculations have shown that the creation of PO_4 vacancies in calcium phosphate hydroxyapatite would be associated with strong destabilization of the structure [17]. In other terms, the creation of large defects like XO_4 vacancies in the apatite structure would produce a collapse of the structure and the formation of other phases, whereas small defects like those corresponding to Me and Y vacancies allow preservation of the structure, although they are associated with a loss of cohesion and stability. Full characterization of non-stoichiometry in apatites thus requires, in principle, determination of the two atomic ratios, Me/X and Y/X, assuming that the number of vacancies in X sites is negligible. In a number of cases, however, only the Me/X ratio is considered, for several reasons; more specifically, concerning biomimetic apatites, the difficulty in determining the OH content.

2.2. HPO_4^{2-} and CO_3^{2-} in biomimetic apatites

Non-stoichiometry in BNA is mainly related to the substitution of PO_4^{3-} ions by bivalent ions like CO_3^{2-} (leading to type B carbonated apatites) or HPO_4^{2-}. All biological apatites contain variable amounts of carbonate and hydrogen phosphate ions [23,4]. In bone the level of HPO_4^{2-} ions has been found to decrease with age and to be associated with an increase in the CO_3^{2-} content. These incorporations of bivalent ions, which are related to a loss of negative charge, are mainly compensated for by a complex defect associating calcium and OH^- vacancies, as represented in the following formulae:

$$Ca_{10-x}(PO_4)_{6-x}(HPO_4, CO_3)_x(OH)_{2-x} \tag{1.3}$$

Rietveld analyses of non-stoichiometric apatites have partially confirmed this compensation mechanism [29,30], initially based on composition considerations only [9]. Other compensation mechanisms involving Ca^{2+} substitution by Na^+ have also been shown, in the case of carbonated apatites, although such substitutions remain very limited in bone mineral. The existence of vacancies clustering in biomimetic apatites, once proposed on the basis of Pauling's rule relative to crystal constitutions, is still debated. More accurate chemical formulas taking into account slight deviations

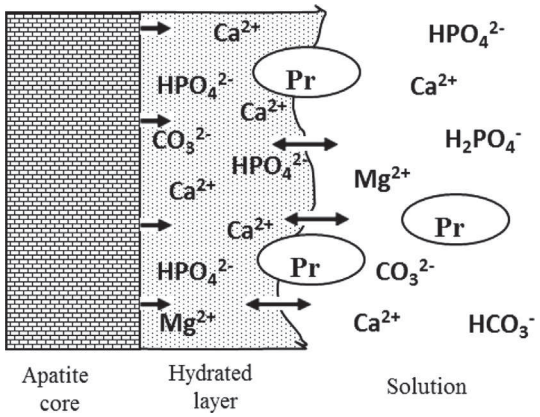
from Formula (1.3) have been proposed [24] that result in a disconnection of the content of vacancies from the proportion of bivalent ions in PO_4^{3-} sites. It should be noted that the quantity of OH^-, like that of HPO_4^{2-} ions, remains difficult to determine with accuracy, which introduces some imprecision in the chemical composition of biomimetic apatites.

2.3. Crystal morphologies

Crystal morphology is an important point as it has consequences for surface reactivity and interactions with biological molecules. Although controversies have been raised on the crystal morphology of bone apatites, it is accepted that the nanocrystals exhibit irregular plate-like shapes elongated along the *c* axis of the hexagonal apatite structure [15]. Generally, most geological or synthetic well-crystallized apatites show needle-like crystals, also elongated along the *c* axis; however, among biological apatites, only mature tooth enamel crystals, which are not really nanocrystals, adopt this morphology. The peculiar morphology of biological nanocrystals has been related to the existence of crystalline precursor phases, like dicalcium phosphate dihydrate (brushite) and octacalcium phosphate or amorphous calcium phosphate. This point, however, remains debated and no clear answer has been given that strictly excludes preparation or observation artifacts. The plate-like morphology is also observed for synthetic biomimetic nanocrystals, so cannot be related to biological conditions of crystal growth only.

2.4. The hydrated surface layer

Biological nanocrystals are characterized by the existence of non-apatitic domains, which have sometimes been interpreted as a sign of formation of precursor phases. In fact several consistent data, essentially obtained using spectroscopic methods, suggest that nanocrystals in bone are covered by a hydrated layer with a composition and structure different from that of crystalline apatite as schematized in Fig. 1. Fourier transform infrared spectroscopic (FTIR) investigations of bone crystals have revealed the existence of non-apatitic environments of CO_3^{2-} and HPO_4^{2-} with specific spectral features [4,5]. Solid state nuclear magnetic resonance (SS-NMR) studies of biomimetic apatites with similar FTIR characteristics as bone mineral have additionally established that the non-apatitic species were close to water molecules in domains distinct from apatite [26,28]. Finally, spectroscopic (FTIR and SS-NMR) analyses of wet samples revealed that the surface hydrated layers were structured, and that the structuralization was related to surface composition and easily altered by fast, reversible ionic exchange, leaving the apatite domains unchanged [7,8]. However, the



hydrated layer appears rather fragile and cannot be preserved in dried samples, leading to amorphous-like domains that can be observed by high resolution transmission electron spectroscopy (HR-TEM) [3].

The hydrated layer shall not be considered as a Stern double layer but a result of the precipitation process of biomimetic apatites. This layer is believed to decrease the water-crystal interfacial energy and to favour the formation of the nanocrystals in aqueous media.

From a thermodynamic point of view, however, the apatite domains are the most stable and with time they develop at the expense of the hydrated layer, incorporating some of the mineral ions present in this layer. Although the global composition of the nanocrystals can be determined by classical methods, the distribution of species between the hydrated layer and the apatite core seems more difficult to establish. Different data obtained by SS-NMR and FTIR suggest a predominance of bivalent ions in the hydrated layer [7,8]. Thus, the global composition of the nanocrystals does not represent that of the apatite domains and in the absence of adequate analytical techniques it seems, at this date, difficult to precisely assess the composition of the different domains of biomimetic nanocrystals. A consequence is that their global composition should not be discussed as related exclusively to apatite chemistry.

The structure of the hydrated layer is still unknown. FTIR data suggest an analogy with the hydrated layer of octacalcium phosphate, also containing bivalent species; however, some specific features of OCP are missing in spectra of the hydrated layer, and SS-NMR spectra do not confirm this analogy [7]. In addition, carbonate ions can be incorporated in this layer, although they are not incorporated in OCP.

3. Preparation and formation conditions of biomimetic nanocrystalline apatites

Several methods have been proposed for the formation of nanocrystalline apatite [9,16,24,25,27]; however, the “biomimetic” criteria are not always met. One of the most developed methods is probably precipitation from supersaturated solutions like simulated body fluid (SBF) [16]. However, this method does not allow the synthesis of large amounts of nanocrystals, and it does not facilitate control of their maturation rate and composition. One of the most convenient syntheses is double decomposition between a calcium salt solution and a phosphate (with or without carbonate) salt solution, with an excess of phosphate (and carbonate) for solution buffering [27] (Table 1). Usually, the cationic solution is rapidly poured into the anionic solution to confer the same ageing time to the nanocrystals. The choice of precipitation conditions allows the preparation of crystals with different characteristics corresponding, for example, to young or old bone mineral crystals [20]. Although drying changes the surface structure layer, freeze-drying preserves some surface characteristics and most of the reactivity. The nanocrystals' characteristics may vary depending on synthesis and post-synthesis parameters [27]. Analyses of the nanocrystals produced shall be done using different techniques in order to assess their main physico-chemical characteristics such as chemical composition, crystal shape and morphology, presence and extent of the hydrated layer. A stabilization of the hydrated layer has been observed with mineral ions like Mg^{2+} and CO_3^{2-}.

4. Properties of biomimetic nanocrystalline apatites

The surface reactivity of BNA is related to their hydrated layer. The mineral ions in this layer appear relatively mobile and may participate to different reaction such as ageing in solution, ion exchange,

molecular adsorption and dissolution properties. This layer also determines the interactions between crystals, crystals and surfaces, and crystals and macromolecules.

4.1. Ageing in solution (maturation)

Biomimetic apatite nanocrystals are unstable and upon ageing in solution the proportion of ions in the surface hydrated layer decreases and that of ions in the apatite domain increases (maturation). The driving force behind this process is the development of stable apatite domains. This phenomenon can be slowed down when inhibitors of apatite crystal growth like Mg^{2+} or CO_3^{2-} are present in the hydrated layer; however, it cannot be stopped.

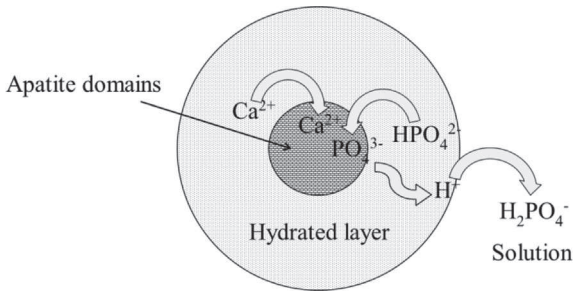
When biomimetic apatite nanocrystals are put in an aqueous solution, several phenomena are observed: an equilibration of the crystals corresponding to the dissolution of calcium and phosphate ions followed by a maturation process producing chemical changes and a loss of surface reactivity. The maturation process can vary depending on the solution, the nanocrystals' characteristics and the solid/solution ratio. For nanocrystalline apatites with low carbonate content, the phosphate concentration in solution increases and the pH and solution Ca/P ratio decreases, leading to an apparent incongruent dissolution [12]. These events can be related to the composition difference between the hydrated layer and the apatite domains: apatite is built mainly of PO_4^{3-} ions whereas the hydrated layer contains mainly HPO_4^{2-} ions. In the course of maturation, the development of stable apatite domains consumes ions from the hydrated layer and induces deprotonation of HPO_4^{2-} ions; the protons released in the hydrated layer react mainly with HPO_4^{2-} to give $H_2PO_4^-$ ions, which are not retained and are eventually rejected into the solution leading to its acidification (Fig. 2).

Over the long term, the increased acidity of the solution may lead to an increase in the proportion of the solid dissolved and the Ca/P ratio in solution may then increase, stabilizing the pH variation. When the solid/solution ratio is very high, the acidification can be so strong that brushite or monetite crystals can form.

Initially, even in a carbonate containing solution, the BNA contains very small amounts of carbonate, essentially in non-apatitic environments, in the hydrated layer. These carbonate ions are incorporated in the apatite domains during their growth. In this case, the release of $H_2PO_4^-$ ions related to the maturation process can be associated with that of bicarbonate ions, which can partly decompose:

$$HCO_3^- \rightarrow CO_2 + OH^- \tag{1.4}$$

attenuating the pH decrease and the evolution of the solution Ca/P ratio. These phenomena have to be considered when the biological activity of biomimetic nanocrystals is evaluated *in vitro* as well as *in vivo*.



4.2. Ion exchange

The ions in the hydrated layer can be rapidly and reversibly exchanged by ions in the solution. Several ion exchanges have been described, such as carbonate/hydrogen-phosphate, Mg/Ca or Sr/Ca. The exchange ratio depends on the ions involved and on the maturation stage, which determines the quantity of ions in the hydrated layer. It has been shown that the quantity exchanged decreased when the maturation time of the apatites increased [5,6]. Exchange experiments using ^{13}C-carbonate ions have revealed that only surface species were exchanged and that the apatite domains were not altered during the exchange experiments [21]. Although the ion uptake from the solution in exchange experiments can be well described as a Langmuir-type isotherm, this representation does not take into account the ion displacement from the solid. For example, the uptake of Sr ions on BNC can be expressed as:

$$Q = N\frac{K(\mathrm{Sr})}{1 - K(\mathrm{Sr})} \tag{1.5}$$

using a Langmuir-type representation, (with Q the amount adsorbed, N the amount adsorbed at saturation, K the Langmuir affinity constant and (Sr) the Sr ion activity in solution at equilibrium). Considering an exchange of Ca ions from the nanocrystals (nc) with Sr ions from the solution (sol) corresponding to the chemical reaction:

$$\mathrm{Sr}^{2+}_{(\mathrm{sol})} + \mathrm{Ca}^{2+}_{(\mathrm{nc})} \leftrightarrow \mathrm{Ca}^{2+}_{(\mathrm{sol})} + \mathrm{Sr}^{2+}_{(\mathrm{nc})} \tag{1.6}$$

gives:

$$Q = N\frac{K_{\mathrm{ex}}(\mathrm{Sr})/(\mathrm{Ca})}{1 - K_{\mathrm{ex}}(\mathrm{Sr})/(\mathrm{Ca})} \tag{1.7}$$

with K_{ex} the equilibrium constant of the ion exchange reaction (1.5), and (Ca) the calcium ion activity at equilibrium in solution.

The second expression is similar to that of a Langmuir adsorption equilibrium where (Sr) is replaced by the ratio (Sr)/(Ca). It allows us, however, to explain important characteristics of the exchange reaction such as its stability upon diluting the solution or washing the samples: although these treatments alter the Sr concentration in solution and should alter the amount of Sr taken up according to a Langmuir type representation, they preserve, in fact, the (Sr)/(Ca) ratio, which is all that matters. To remove the Sr ions from the surface, one must reduce this ratio, by adding calcium to the solution, for example, or other ions able to displace Sr.

The composition of nanocrystalline apatites can be easily modified using ion exchange and maturation, and heterogeneous compositions can be obtained within the nanocrystals. Several types of ions in the hydrated layer shall be distinguished:

- Ions that can be incorporated in the growing apatite domains like Sr^{2+}, CO_3^{2-} (and probably many others), which become non-exchangeable once they have entered the apatite domains (unless these apatite domains are destroyed)
- Ions that cannot enter the apatite domains, or that penetrate in very limited quantities into calcium phosphate apatites, like Mg^{2+}, which remain on the surface and can be exchanged at any time.

4.3. Adsorption of molecules

Adsorption is an important property of nanocrystalline apatites. Several types of interactions can be involved in the adsorption of molecules, as shown, for example, in theoretical modelling. However the strongest interactions seem to involve surface ion exchanges, which are not generally considered in models. Several publications have shown, for example, that the adsorption of molecules with negative functional groups is generally related to a phosphate (or carbonate) release [10,14,18]. Inversely, the separation of biological molecules by chromatography on apatite substrates involves the use of a phosphate solution with a concentration gradient [1]. Considering that the most important adsorption reactions are, in fact, related to a surface ion exchange, the maturation stage, composition and

development of the hydrated layer play a significant role in the adsorption process, although only a few data have been reported on this subject [20].

The molecules' adsorption is generally well represented by a Langmuir-type isotherm or some derivatives. However, if it is assumed that the adsorption is in fact a surface ion exchange, these representations, as explained in the previous paragraph, do not consider the implications of the ions displaced in the chemical equilibrium and more accurate equations can be used which can explain the apparent irreversibility of the adsorption phenomena upon dilution or washing [10]. It is generally observed that the amount adsorbed at saturation is higher for immature nanocrystals with a well-developed hydrated layer and decreases with the maturation time. The Langmuir affinity constant (or the exchange equilibrium constant) also varies according to the characteristics of the biomimetic nanocrystalline apatite substrate. The number of phosphate ions displaced per adsorbed molecules has been determined in a few cases and it depends on the molecules that are adsorbed [18] and on the characteristics of the nanocrystals. The number of displaced ions is not necessarily an integer and it has been suggested that adsorption could involve several steps [20].

4.4. Solubility behaviour

Several reports have revealed some variability in the dissolution properties of biological nanocrystalline apatites: preferential dissolution of carbonated domains, non-congruency (different mineral ion ratios in the solution and in the solid) or variable solubility products. This last phenomenon has been systematically studied [13] and it has been shown, in case of biological as well as synthetic samples, that the solubility products of nanocrystalline apatites could vary according to the amount dissolved, leading to the concept of metastable equilibrium solubility (MES). Although several explanations can be given for this observation, this behaviour could be related to existence of a hydrated layer. Currently, however, no indication of the evolution of the hydrated layer with the dissolution rate of the nanocrystals has been established.

5. Biomimetic nanocrystalline apatites in biomaterials

Biomimetic nanocrystalline apatites appear as very reactive and metastable compounds and they begin to decompose at about 200 °C, which does not favour their transformation into materials and their use as biomaterials. Among the different technical issues that must be solved are processing and shaping techniques, and the preservation of the physical—chemical and biological properties of biomimetic nanocrystalline apatites.

5.1. Processing of biomimetic nanocrystalline apatites for use as biomaterials

Several strategies have been used to produce bioactive orthopedic biomaterials using biomimetic nanocrystalline apatites, among which one can distinguish *in situ* formation, which allows the formation of nascent BNA with a high reactivity, and low temperature shaping.

5.1.1. In situ *formation*

The formation of a layer of nanocrystalline apatite from body fluids *in vivo* is being considered as a criterion for biological activity [16]. The ability of a material surface to nucleate and grow a nanocrystalline apatite layer *in vitro* from simulated body fluids (SBF) has even become a standard (ISO standard 14630). Several techniques can be used to promote the formation of this layer, although very often only the structure and nanometric dimensions of the crystals are considered and the chemical composition and development of the hydrated layer are very rarely mentioned. Two types of *in situ* formation of neo-formed nanocrystalline apatite and, correlatively, of biomaterial classes may be recognized:

- "Passive" biomaterials that favour the nucleation of nanocrystalline apatites without participating in the building up of the layer (in this group are apatites but also other nucleators of apatite, like titanium oxide and collagen).

- "Active" biomaterials that interact chemically with body fluids and increase their supersaturation locally with regard to apatite (biological glasses releasing Ca^{2+} ions, calcium carbonate, different calcium or phosphate salts).

In addition to these materials, another important class of biomaterials able to produce nanocrystalline apatites *in situ* is calcium phosphate cements. BNA are produced during the setting reactions of a number of cements and their characteristics vary, often considerably, depending on the composition of the cements and the setting conditions.

5.1.2. Low temperature shaping

Among other techniques that have been proposed to make materials from biomimetic apatite nanocrystals are gel setting, low temperature sintering, low temperature coatings and composite associations with polymers [22].

In all of these techniques the properties of the hydrated layer with its mobile mineral ions can be used to promote interactions:

- Intercrystalline interactions, for example, to build cohesive materials, as in spark plasma sintering (150 °C) of biomimetic nanocrystalline apatites at very low temperature [11].
- Interactions with a substrate to obtain an adhesive coating [2].
- Interactions with a polymer to obtain composite materials.

5.2. Biological properties and preservation of the BNA

The maturation of BNA can produce strong alterations in the aqueous media in contact with materials. In cell culture evaluations involving a very limited amount of solution in contact with materials exposing a large surface area, these phenomena can be unacceptable as they strongly change the solution pH and composition [12]. To avoid these alterations one can use low solid/solution ratios and pre-equilibrate the material with the cell culture media. The changes induced by BNA are, however, strongly dependent on their maturation stage and freshly prepared samples are the most reactive. Another complication for interpreting results is the progressive change in the material itself due to the maturation process [19].

The same maturation processes should occur *in vivo* but they have not really been studied. One can reasonably estimate that the buffering effect *in vivo* is much stronger than in cell culture wells and no adverse reactions have been reported concerning materials containing or inducing BNA formation. Implantation results in non-osseous sites have even shown an osteoinductive behaviour for BNA coatings on biphasic calcium phosphate ceramics [2]. However, the reason for this behaviour has not been clarified. Considering the correspondence between the solubility of a calcium phosphate and its bioabsorption capability, it can be inferred that mature BNA should resorb slower than non-mature BNA for similar types of materials, but there is presently no experimental support for this proposition.

The reactivity of BNA seems essentially related to the hydrated surface layer. Freeze-drying is probably the best way to preserve this layer, although there is an amorphization of the surface layer and particle aggregations occur. If freeze-dried samples are rehydrated the original structure seems partly reestablished, but there is a loss of reactivity and, for example, a reduced ion exchange capacity. As already mentioned, partial stabilization of the hydrated layer can occur when it incorporates apatite crystal growth inhibitors like carbonate or magnesium ions. Other crystal growth inhibitors (like pyrophosphate ions, protein adsorption) might well play an equivalent role but experimental evidence is scarce.

6. Conclusion

Although biomimetic nanocrystalline apatites have not yet received the industrial development of other traditional calcium phosphate ceramics, their reactivity and adaptable physical–chemical characteristics offer decisive advantages for biological applications. However the nanosize character of

these compounds should not be considered as their sole advantage. Behind the nano label there are a variety of chemical composition and reactivity issues that need to be explored and distinguished. The recent improvement in the characterization techniques for BNA and in understanding and controlling their reactivity in aqueous media should considerably favour the study and control of their biological effects and their biomedical applications.

References

[1] C. Andrews-Pfannkocj, D.W. Fadrosh, J. Thorpe, S.J. Williamson, Hydroxyapatite-mediated separation of double-stranded DNA, single-stranded DNA and RNA genomes from natural viral assemblages, Appl. Environ. Microbiol. 76 (2010) 5039–5045.

[2] H. Autefage, Rôle ostéoinducteur d'un revêtement d'hydroxyapatite carbonatée nanocristalline sur des céramiques de phosphate de calcium biphasique (Ph.D. thesis), Paul Sabatier University, Toulouse, 2009.

[3] L. Bertinetti, C. Drouet, C. Combes, C. Rey, A. Tampieri, S. Coluccia, G. Martra, Surface characteristics of nanocrystalline apatites: effect of Mg surface enrichment on morphology, surface hydration species, and cationic environments, Langmuir 25 (2009) 5647–5654.

[4] S. Cazalbou, C. Combes, D. Eichert, Rey, M. Glimcher, Poorly crystalline apatites: evolution and maturation in vitro and in vivo, J. Bone Miner. Metab. 22 (2004) 310–317.

[5] S. Cazalbou, D. Eichert, X. Ranz, C. Drouet, C. Combes, M.F. Harmand, C. Rey, Ion exchanges in apatites for biomedical applications, J. Mater. Sci. Mater. Med. 16 (2005) 405–409.

[6] C. Drouet, M.-T. Carayon, C. Combes, C. Rey, Surface enrichment of biomimetic apatites with biologically-active Mg^{2+} and Sr^{2+}: a preample to the activation of bone repair materials, Mater. Sci. Eng. C 28 (2008) 1544–1550.

[7] D. Eichert, H. Sfihi, C. Combes, C. Rey, Specific characteristics of wet nanocrystalline apatites. Consequences on biomaterials and bone tissue, Key Eng. Mater. 254–256 (2004) 927–930.

[8] D. Eichert, C. Combes, C. Drouet, C. Rey, Formation and evolution of hydrated surface layers of apatites, Key Eng. Mater. 284–286 (2005) 3–6.

[9] J.C. Elliott, Structure and Chemistry of the Apatites and Other Calcium Orthophosphates, Elsevier, Amserdam, 1994.

[10] F. Errassifi, A. Menbaoui, H. Autefage, L. Benaziz, S. Ouizat, V. Santran, S. Sarda, A. Lebugle, C. Combes, A. Barroug, H. Sfihi, C. Rey, Adsorption on apatitic calcium phosphates: applications to drug delivery, Ceram. Trans. (Adv. Bioceram. Biotechnol.) 218 (2010) 159–174.

[11] D. Grossin, S. Rollin-Martinet, C. Estournes, F. Rossignol, E. Champion, C. Combes, C. Rey, G. Chevallier, C. Drouet, Biomimetic apatite sintered at very low temperature by spark plasma sintering: physic-chemistry and microstructure aspects, Acta Biomater. 6 (2010) 577–585.

[12] J. Gustavsson, M.P. Ginebra, E. Engel, J. Planell, Ion reactivity of calcium-deficient hydroxyapatite in standard cell culture media, Acta Biomater. 7 (2011) 4242–4252.

[13] J. Hsu, J.L. Fox, W.I. Higuchi, G.L. Powell, M. Otsuka, A. Baig, R.Z. LeGeros, Metastable equilibrium solubility behavior of carbonated apatites, J. Colloid Interface Sci. 167 (1994) 414–423.

[14] S. Josse, C. Faucheux, A. Soueidan, G. Grimandi, D. Massiot, B. Alonso, P. Janvier, S. Laïb, P. Pilet, O. Gauthier, G. Daculsi, J. Guicheux, B. Bujoli, J.-M. Bouler, Novel biomaterials for bisphosphonate delivery, Biomaterials 26 (2005) 2073–2080.

[15] H.M. Kim, C. Rey, M.J. Glimcher, Isolation of calcium-phosphate crystals of bone by non-aqueous methods at low temperature, J. Bone Miner. Res. 10 (1995) 1589–1601.

[16] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 27 (2006) 2907–2915.

[17] K. Matsunaga, Theoretical defect energetics in calcium phosphate bioceramics, J. Am. Ceram. Soc. 93 (2010) 1–14.

[18] S. Mukherjee, C. Huang, F. Guerra, K. Wang, E. Oldfield, Thermodynamics of bisphosphonates binding to human bone: a two site model, J. Am. Chem. Soc. 131 (2009) 8374–8375.

[19] P. Pascaud, R. Bareille, C. Bourget, J. Amédée, C. Rey, S. Sarda, Interaction between a bisphosphonate, tiludronate and nanocrystalline apatite: in vitro viability and proliferation of HOP and HBMSC cells, Biomed. Mater. 7 (2012) 054108, http://dx.doi.org/10.1088/1748-6041/7/5/054108, 9pp.

[20] P. Pascaud, P. Gras, Y. Coppel, C. Rey, S. Sarda, Interaction between a bisphosphonate, tiludronate, and biomimetic nanocrystalline apatites, Langmuir 29 (2013) 2224–2232.

[21] C. Rey, C. Combes, C. Drouet, A. Lebugle, H. Sfihi, A. Barroug, Nanocrystalline apatites in biological systems: characterization, structure and properties, Materialwissenschaft und Werkstofftechnik 38 (2007) 996–1002.

[22] C. Rey, C. Combes, C. Drouet, H. Sfihi, A. Barroug, Physico-chemical properties of nanocrystalline apatites: implications for biomaterials and biominerals, Mater. Sci. Eng. C 27 (2007) 198–205.

[23] C. Rey, C. Combes, C. Drouet, M.J. Glimcher, Bone mineral: update on chemical composition and structure, Osteoporosis Int. 20 (2009) 1013–1021.

[24] C. Rey, C. Combes, C. Drouet, D. Grossin, Bioactive ceramics: physical chemistry, in: P. Ducheyne, K.E. Healy, D.W. Hutmacher, D.W. Grainger, C.J. Kirkpatrick (Eds.), Comprehensive Biomaterials, vol. 1, Elsevier, 2011, pp. 187–221 (Chapter 1.111).

[25] R.E. Riman, W.L. Suhanek, K. Byrappa, C.W. Chen, P. Shuk, C.S. Oakes, Solution synthesis of hydroxyapatite designer particulates, Solid State Ionics 151 (2002) 394–402.

[26] H. Sfihi, C. Rey, 1-D and 2-D double heteronuclear magnetic resonance study of the local structure of type B carbonate fluoroapatite, in: J. Fraissard, B. Lapina (Eds.), Magnetic Resonance in Colloid and Interface Science, Nato ASI Series II, vol. 76 , Kluwer Academic Publishers, 2002, pp. 409–418.

[27] N. Vandecandelaere, C. Rey, C. Drouet, Biomimetic apatite-based biomaterials: on the critical impact of synthesis and post-synthesis parameters, J. Mater. Sci. Mater. Med. 23 (2012) 2593–2606.

[28] Y. Wang, T. Azaïs, M. Robin, A. Vallée, C. Catania, P. Legriel, G. Pehau-Arnaudet, F. Babonneau, M.M. Giraud-Guille, N. Nassif, The predominant role of collagen in the nucleation, growth, structure and orientation of bone apatite, Nat. Mater. 11 (2012) 724–733.

[29] R.M. Wilson, S.E.P. Dowker, J.C. Elliott, Rietveld refinements and spectroscopic structural studies of a Na-free carbonate apatite made by hydrolysis of monetite, Biomaterials 27 (2005) 4682–4692.
[30] R.M. Wilson, J.C. Elliot, S.E.P. Dowker, L.M. Rodriguez-Lorenzo, Rietveld refinements and spectroscopic studies of the structure of Ca-deficient apatite, Biomaterials 26 (2005) 1317–1327.

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