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## COMMENTARY

# What bridges mineral platelets of bone?

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**Commentary on:** Davies E, Müller KH, Wong WC, Pickard CJ, Reid DG, Skepper JN, Duer MH. Citrate bridges between mineral platelets in bone. *Proc Natl Acad Sci USA* 2014;**111**:E1354–E1363.

The interactions between constituents of the bone composite, at the molecular level, have long been investigated for their potential role in the cohesion and mechanical properties of the tissue. Despite recent efforts involving sophisticated techniques, the nature of interactions, at the chemical level, between the two main bone components—collagen matrix and calcium phosphate apatite nanocrystals—has not yet been clarified. Another question is that of interactions between the apatite nanocrystals, which is addressed in the paper of Davies *et al.*<sup>1</sup> As it is often the case, regarding bone mineral, the work of the authors relies strongly on the analyses of synthetic samples, which are considered to represent more or less bone mineral crystals, in this case octacalcium phosphate (OCP) incorporating citrate anions. Two main questions arise: is OCP a good model for bone crystals? Is the crystal structure of OCP-citrate a pertinent representation of an intercrystalline chemical bonding between bone crystals?

The hypothesis that OCP is a precursor of apatite bone crystals was formulated by Brown<sup>2</sup> and is supported by several common characteristics of OCP and bone mineral crystals: platelet morphology, close chemical composition at very early stage of bone formation, with a Ca/P ratio close to 1.33 and a high-HPO<sub>4</sub> content,<sup>3</sup> and ionic product of blood serum close to the solubility product of OCP.<sup>4</sup> However, OCP has never been reproducibly evidenced in bone as distinct crystalline entities. Several reasons have been given: the fast hydrolysis of OCP crystals into apatite, and the nanosize of the crystals, especially their very small thickness, which would prevent observing the most intense diffraction line of OCP at low angle (100). Sparse reports identified OCP in bone as crystals, but these most probably resulted, like dicalcium phosphate dihydrate crystals (DCPD), from the evolution of the very reactive bone mineral crystals.<sup>5</sup> Another difficulty with the OCP precursor theory is that, even at very early stages, bone mineral forms in a carbonate-containing media and contains carbonate ions, which is incompatible with an OCP structure. Brown's<sup>6</sup> conception of OCP apatite relationships was extended to surface characteristics of apatites, and he considered that the surface atomic structure in aqueous media could be well represented by half of the internal hydrated layer of OCP. Spectroscopic data

regarding this point seem to support Brown's view and synthetic nanocrystalline apatites, free of carbonate ions, exhibit FTIR spectra close, although not identical, to that of OCP.<sup>7</sup> This hydrated surface structure appears, however, very fragile and is altered by reversible surface ion exchange preserving the bulk characteristics of apatite crystals.<sup>8</sup> In the presence of foreign ions such as carbonate and magnesium, the surface hydrated layer may remain structured (carbonate), but with alterations, or become amorphous-like (Mg<sup>2+</sup>). The very fast ion exchange reactions justify the term 'labile' often used to characterise the hydrated layer. This does not mean that ionic species are moving in the hydrated layer, which appears often well structured: they are immobile, but they are loosely bound and can be easily exchanged. Therefore, the surface model of OCP for biomimetic nanocrystalline apatite surfaces, including bone mineral, has some relevance, but it needs to be adapted to take into account variable ion content with potential ionic substituents.

The representativity of OCP-citrate crystals as a model for intercrystalline interactions may appear in some ways as the continuation of Brown's hypothesis on the relationship between a crystalline OCP structure and a surface of nanocrystalline apatite. The proposed model is in fact one monolithic block containing apatite domains and OCP-citrate hydrated layer. In this case, there are no separate nanocrystals, and citrate cannot be depicted as a bridge between preformed nanocrystals. In the proposed model, citrate appears simply as an element of a single continuous structural unit. The authors refer to a publication, indicating that bone crystals form a continuum; however, this is rather misleading as the use of sodium hypochlorite to remove the organic matrix of bone, especially during excessive contact time, significantly alters biological apatite crystals.<sup>9</sup> Bone crystals can be isolated using gentler procedures,<sup>10</sup> and they appear as individualised entities. In addition, the effect of citrate ions on apatite crystals is not clear, and it has been reported in different papers<sup>11</sup> that these molecules do not act as agglomerating agents, but, on the contrary, as dispersing ones, maintaining a high negative zeta potential at the apatite crystals surface and preventing them from getting into close contact. Returning to the proposed

model, a further question is: do we need citrate to bind crystals? The OCP hydrated layer with its mineral ions is also, in fact, a binding layer between apatite domains. Citrate ions, because of their size, produce a 'swelling' of the hydrated layer, and it has to be demonstrated that a citrate-OCP layer gives more cohesion compared with a regular OCP hydrated layer with its calcium and  $\text{HPO}_4^{2-}$  ions. The hydrated layer at bone nanocrystal surface could well act as a bridging and structuring agent, as proposed in a recent paper.<sup>12</sup> Synthetic nanocrystalline apatites with a chemical composition, structure and morphology very similar to those of bone crystals can be easily obtained.<sup>3</sup> They show a plate-like habit, without citrate addition or formation of OCP transient phase. Monoliths of these biomimetic apatite nanocrystals can easily be obtained by simply drying water suspensions,<sup>13</sup> and once obtained they cannot be re-suspended as individual crystals. Application of pressure on similar nanocrystals also leads to solid monoliths,<sup>14</sup> and it is even possible to sinter these nanocrystals at very low temperature by preserving their nanometric dimensions, without decomposition of the apatite.<sup>15</sup> In all of these monoliths, which can exhibit interesting mechanical properties, strong intercrystalline bonds and crystal orientations are created without the use of citrate ions. Nevertheless, the adsorption of citrate ions on apatites is well documented,<sup>16</sup> and it is likely that these ions are on the surface of nanocrystals and could be in the intercrystalline space like other ions of the hydrated layer of bone nanocrystals. Thus, they can be part of the bridging. However, their specific role in this bridging action has to be clarified and implemented.

One of the difficulties in the study of interactions between bone constituents lies in taking into account the dynamics of the system and the heterogeneity of the tissue. Considering the mineralisation process, for example, it does not involve one step, as it is implicitly understood in the paper of Davies *et al.*<sup>1</sup> to produce a continuum. Two stages of mineralisation are distinguished: primary and secondary mineralisation.<sup>17</sup> The primary mineralisation is described as a rather fast precipitation process (a few days) corresponding to 60–70% of the mineral load and the secondary mineralisation as a slow one (several months), which ends when a full mineral load is achieved. It is probable that the change in formation conditions, associated with these processes, leads to crystals with different characteristics and creates a heterogeneous population.<sup>18</sup> Remodelling, furthermore, is an important source of heterogeneity. There are also other sources of heterogeneity such as bone type, bone site, bone age, or within the tissue, at the osteons level in lamellae<sup>19</sup> and at the cement line. An important

characteristic of bone tissue is a strong heterogeneity inter- and probably intra-crystalline. Although these heterogeneities are not well known, they seem to affect the apatite domains as well as the hydrated layer,<sup>20</sup> and intercrystalline interactions may consequently exhibit some heterogeneity. The publication of Davies *et al.*<sup>1</sup> has the merit of highlighting the question of intercrystalline interactions proposing a role for citrate ions based on sound results with an accurate, although schematic, model for the intercrystalline bridging.

### Conflict of Interest

The authors declare no conflict of interest.

### References

1. Davies E, Müller KH, Wong WC, Pickard CJ, Reid DG, Skepper JN *et al.* Citrate bridges between mineral platelets in bone. *Proc Natl Acad Sci USA* 2014;**111**:E1354–E1363.
2. Brown WE. Crystal growth of bone mineral. *Clin Orthop* 1966;**44**:205–220.
3. Rey C, Hina A, Tofighi A, Glimcher MJ. Maturation of poorly crystalline apatites: chemical and structural aspects *in vivo* and *in vitro*. *Cells Mater* 1995;**5**:345–356.
4. Eidelman N, Chow LC, Brown WE. Calcium phosphate saturation level in ultrafiltered serum. *Calcif Tissue Int* 1987;**40**:71–78.
5. Muenzenberg KJ, Gebhardt M. Brushite, octacalcium phosphate, and carbonate-containing apatite in bone. *Clin Orthop Relat Res* 1973;**90**:271–273.
6. Brown WE, Mathew M, Chow LC. Roles of octacalcium phosphate in surface chemistry of hydroxyapatite. In: Misra DN (ed.) *Adsorption and Surface Chemistry of Hydroxyapatite*. Plenum Press: New York, pp13–28, 1984.
7. Eichert D, Slihi H, Combes C, Rey C. Specific characteristics of wet nanocrystalline apatites. Consequences on biomaterials and bone tissue. *Key Eng Mater* 2004;**254–256**:927–930.
8. Eichert D, Combes C, Drouet C, Rey C. Formation and evolution of hydrated surface layers of apatites. *Key Eng Mater* 2005;**284–286**:3–6.
9. Tomazic BB, Brown WE, Eanes ED. A critical evaluation of the purification of biominerals by hypochlorite treatment. *J Biomed Mater Res* 1993;**27**:217–225.
10. Kim H-M, Rey C, Glimcher MJ. Isolation of calcium phosphate crystals of bone by non-aqueous methods at low temperature. *J Bone Miner Res* 1995;**10**:1589–1601.
11. Tan J, Chen M, Xia J. Water-dispersible hydroxyapatite nanorods synthesized by a facile method. *Applied Sur Sci* 2009;**255**:8774–8779.
12. Wang Y, Von Euw S, Fernandes FM, Cassaignon S, Selmane M, Laurent G *et al.* Water-mediated structuring of bone apatite. *Nat Mater* 2013;**12**:1144–1153.
13. Sarda S, Tofighi A, Hobatho M-C, Rey C. Associations of low temperature apatite ceramics and proteins. *Phosphorus Res Bull* 1999;**10**:208–213.
14. Tadic D, Epple M. Mechanically stable implants of synthetic bone mineral by cold isostatic pressing. *Biomaterials* 2003;**24**:4565–4571.
15. Grossin D, Rollin-Martinet S, Estournès C, Rossignol F, Champion E, Combes C *et al.* Biomimetic apatites sintered at very low temperature by spark plasma sintering: physico-chemistry and microstructural aspects. *Acta Biomater* 2010;**6**:577–585.
16. Skwarek E, Janusz W, Sternik D. Adsorption of citrate ions on hydroxyapatite synthesized by various methods. *J Radioanal Nucl Chem* 2014;**299**:2027–2036.
17. Boivin G, Meunier PJ. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. *Calcif Tissue Int* 2002;**70**:503–511.
18. Rengers GAP, Mulder L, Langenbach GEJ, van Ruijven LJ, van Eijden TMGJ. Biomechanical effect of mineral heterogeneity in trabecular bone. *J Biomech* 2008;**41**:2793–2798.
19. Paschalis EP, DiCarlo E, Betts F, Sherman P, Mendelsohn R, Boskey AL. FTIR micro-spectroscopic analysis of human osteonal bone. *Calcif Tissue Int* 1996;**59**:480–487.
20. Cazalbou S, Combes C, Eichert D, Rey C, Glimcher MJ. Poorly crystalline apatites: evolution and maturation *in vitro* and *in vivo*. *J Bone Miner Metab* 2004;**22**:310–317.