Journal of Biomedical Materials Research Part B: Applied Biomaterials

WILEY

Society For Biomaterials

TECHNICAL NOTE OPEN ACCESS

Workshop on the Latest Advances in Biomedical Applications of Octacalcium Phosphate

Nicola Döbelin[1] | Osamu Suzuki[2] | Christophe Drouet[3] | Jānis Ločs[4] | Gerard Insley[5] | Philip Procter[6]

[1]RMS Foundation, Bettlach, Switzerland | [2]Division of Craniofacial Function Engineering (Division of Biomaterials Science and Engineering), Tohoku University Graduate School of Dentistry, Sendai, Japan | [3]CIRIMAT, Université de Toulouse, Toulouse, France | [4]Riga Technical University, Faculty of Natural Sciences and Technology, Institute of Biomaterials and Bioengineering, Riga, Latvia | [5]PBC Biomed, R&D, Limerick, Ireland | [6]CPP SAS, Divonne-Les-Bains, France

Correspondence: Nicola Döbelin (nicola.doebelin@rms-foundation.ch)

Received: 11 July 2024 | **Revised:** 7 October 2024 | **Accepted:** 20 October 2024

Funding: The authors received no specific funding for this work.

Keywords: biomineralization | characterization | commercialization | crystal structure | functionalization | octacalcium phosphate | synthesis

ABSTRACT

The first workshop on the "latest advances in biomedical applications of octacalcium phosphate (OCP)" was organized as a satellite symposium to the Bioceramics33 conference in Solothurn, Switzerland, in October 2023. The event brought together leading researchers and industry representatives to present and discuss their latest groundbreaking research aimed at developing and commercializing advanced OCP-based biomaterials for bone regeneration. The topics presented by the six invited speakers ranged from a fundamental understanding of the OCP crystal chemistry to advanced processing and characterization methods, functionalization, biomineralization, and commercialization. With this summary report, we are laying the foundation for a continuation of a series of workshops on the subject of OCP biomaterials in order to promote the exchange between researchers and industry representatives and to drive forward the development and commercialization of new improved synthetic bone substitute materials.

1 | Introduction

Octacalcium phosphate (OCP) has received relatively little attention in the development history of synthetic bone graft substitutes compared to the better-known hydroxyapatite (HA), tricalcium phosphate (TCP), and biphasic materials (BCP). This is despite the fact that it has long been confirmed that OCP forms in the early phases of bone mineralization as a precursor of apatitic bone mineral [1]. Thanks to an increasing number of studies demonstrating a positive effect of OCP on bone formation [2] or showing interesting properties in combination with polymers or as a carrier of foreign ions [3–5], growing interest in OCP as a basis for commercial synthetic bone graft substitute products that promise faster bone formation compared to established materials has emerged in recent years.

In October 2023, we organized a workshop on the topic of "latest advances in biomedical applications of octacalcium phosphate" as a satellite symposium to the international conference "Bioceramics33", which took place from October 17 to 20, 2023, in Solothurn, Switzerland. Six invited speakers reported on their recent advances ranging from a fundamental understanding of the OCP crystal chemistry to the commercialization of OCP-based medical devices (Figure 1). The workshop provided a forum in which leading scientists, industry representatives, and all interested parties could enter into a dialogue and direct their focus toward a common objective, the improvement of synthetic bone graft materials.

The goal of this summary is to record the first global gathering of the leading OCP researchers and prepare the path for further such meetings for the sharing and dissemination of emerging

FIGURE 1 | The speakers of the first workshop on the "latest advances in biomedical applications of octacalcium phosphate (OCP)" at the Bioceramics33 conference in Solothurn, Switzerland, 2023. From left to right: C. Drouet, G. Insley, O. Suzuki, N. Döbelin, P. Procter, J. Ločs.

knowledge concerning this exciting new biomaterial. The translation of a new biomaterial to clinical application is always challenging and this high-level summary represents a first structured approach to characterizing OCP and, it is hoped that this will serve to encourage other workers in this field.

It is structured by topic each author presented as follows:

- Crystal structure and unique properties (C. Drouet)
- Synthesis and processing (J. Ločs)
- Characterization: Distinction from apatite (N. Döbelin)
- Modification and functionalization (G. Insley)
- Bioactivity, resorption, and chemistry in biomineralization (O. Suzuki)
- Commercialization and regulatory aspects (P. Procter)
- Discussion (all authors)

It is hoped that this opening meeting will be the first in a series of further meetings that will lead to the dissemination of the research by researchers who lead the OCP field.

2 | Crystal Structure and Unique Properties

OCP generally refers to the pentahydrate salt $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$ or "octacalcium bis(hydrogenphosphate) tetrakis(phosphate) pentahydrate" crystallizing in a triclinic structure. Other phases with the Ca-P composition of OCP have also been described, such as apatitic OCP (ap-OCP) and amorphous OCP (am-OCP) although these compounds received less attention, allowing for a generalized "OCP" terminology for the triclinic variety [6].

The crystal structure of OCP may be seen as an alternate stacking of "apatite-like" layers and "hydrated layers" (Figure 2). The hydrated domains share some characteristics with the hydrated ionic layer observed on the surface of bone (or biomimetic) nanocrystalline apatites [7]. To what extent the stacked hydrated layers in the OCP structure share substructural/compositional features with the hydrated layer on apatite nanocrystals is the subject of ongoing research. A variety of chemical modifications to the OCP structure have been reported over the years, including cationic substitutions, incorporation of linear carboxylate molecules, a variable H_2O content, and non-stoichiometric Ca^{2+} and HPO_4^{2-} contents. Such modifications may be seen as a way to convey additional properties to the material, for example, aiming at enhanced bone formation.

Regular OCP and its variants are all metastable and are progressively converted into calcium-deficient apatite when in aqueous medium. In the case of ion substituted-OCP, this may trigger cell activity typically to promote bone reconstruction. Several questions remain regarding OCP and its structural or compositional modifications depending on the conditions of formation/processing. For example, structural and compositional features appear to be strongly modified whether the OCP crystal formation is undergone in the presence or in the absence of sodium ions. Na^+ inclusion in the triclinic OCP structure remains under question. Recently, we have undergone the exploration of the possible preparation of nanosized OCP crystals (internal communication by C. Rey and C. Drouet et al., CIRIMAT—Toulouse)—expectedly more reactive than their micronic counterparts—again stressing the impact of the medium for formation; and the metastability/transformation path into apatite in humid conditions is still being explored.

3 | Synthesis and Processing

Many efforts have been placed into deciphering the OCP formation, but many background processes, which are influenced by supersaturation, molarity, pH, temperature, and mixing order/rate,

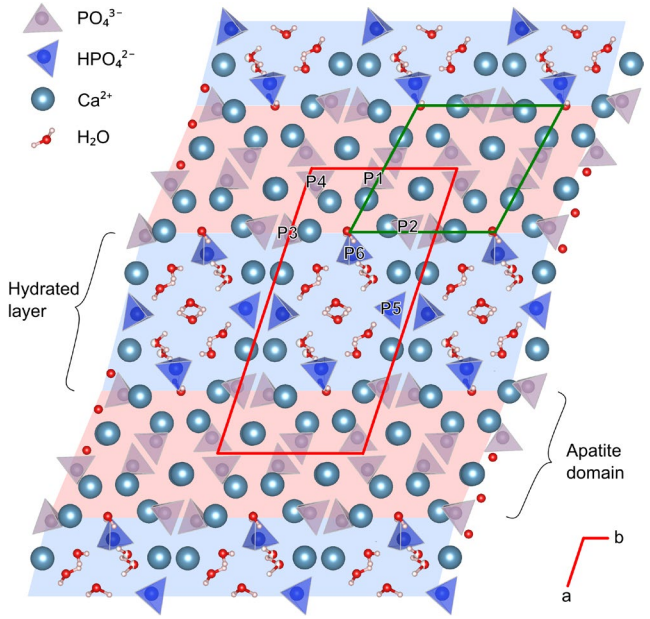

FIGURE 2 | The structure of triclinic OCP, evidencing the six phosphate ions per unit cell (P1 to P6). The OCP (red) and hydroxyapatite (green) unit cells highlight the inter-relation between the two structures.

have made it that much harder. Furthermore, even though the scientific community has been witnessing great progress in the utilization and processing of OCP in different systems, the core characteristics of the unique OCP crystal structure prevent it from undergoing any high-temperature treatments.

Recently, we developed two synthesis routes based on the hydrolysis of α-TCP (α-$Ca_3(PO_4)_2$) and on co-precipitation of OCP, respectively [8]. The hydrolysis route revealed that the gradual transformation from α-TCP to OCP phase transpired through brushite (DCPD, up to ~36%) as an intermediary phase. The co-precipitation route proved to be a novel, reproducible, and ultra-fast co-precipitation synthesis process that allowed us to produce tailor-made OCP with a specific surface area (SSA) ranging from 16 to 91 m^2/g.

Once both methodologies were mastered, the question of processing the obtained OCPs in ceramics, with an appropriate density and mechanical strength, was addressed. As OCP is a metastable phase and transforms spontaneously and irreversibly to apatite (> 80°C), it is impossible to sinter it by using conventional high-temperature sintering techniques. Instead, we reported for the first time that OCP was densified at room temperature by using a cold sintering process (CSP) and applying a uniaxial pressure of 1500 MPa, without altering its phase composition (manuscript in preparation).

4 | Characterization: Distinction From Apatite

The presence of calcium deficient hydroxyapatite (CDHA, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$) building blocks in the OCP crystal structure and the general similarity of the two structures present a challenge to many analytical techniques, including XRD, FTIR, Raman spectroscopy, and chemical analysis (e.g., XRF, ICP-MS), because the signals largely overlap and barely show any distinctive features. The few unique features, such as the iconic (100) peak at d = 18.637 Å (4.738°2θ for Cu$K\alpha_1$) of OCP in XRD signals and the OH^- stretch band at 3572 cm^{-1} of CDHA in FTIR spectra, clearly indicate the presence of OCP and CDHA, respectively. However since the opposite phase is devoid of a similar unique identifier in both scenarios, reliable phase quantification proves difficult nonetheless.

We found the molar Ca:P ratio to be a relatively reliable indicator for a more robust quantification of OCP and CDHA, assuming that pure OCP exhibits a ratio of 1.3333, and pure CDHA of 1.5000, respectively. A highly precise determination of the Ca:P ratio thus allows for derivation of the sample's OCP and CDHA content. We implemented this approach in a pilot study by heat-treating OCP samples at 1000°C for 30 min, thus converting OCP and CDHA to an equivalent mixture of the high-temperature phases β-CPP (β-$Ca_2P_2O_7$) and β-TCP (β-$Ca_3(PO_4)_2$). The converted phases were much easier to quantify by Rietveld refinement of XRD data and allowed the calculation of the molar Ca:P ratio from the quantities of the high-temperature phases. Assuming that the Ca:P ratio remained unaffected by the thermal treatment, the initial OCP and CDHA quantities could be calculated from the Ca and P content. To prevent the evaporation of phosphate during the thermal treatment [9], some sacrificial $CaHPO_4$ was added to the furnace to saturate the atmosphere with P gas species. This procedure allowed us to quantify the OCP phase purity with a 95% measurement uncertainty of ±3.0 wt-%. It requires standard laboratory equipment and is simple to implement, but the accuracy of the final result is directly linked to the accuracy of β-TCP and β-CPP quantification from XRD data, which requires relatively advanced data processing skills.

5 | Modification and Functionalization

Despite many years of research focusing on the clinical application of OCP for bone regeneration, there are still no products available to clinicians to date that will benefit patients. The material is known for its potential applications in bone tissue engineering, drug delivery systems, and dental materials. However, its inherent properties often require fine-tuning to meet specific requirements needed for diverse clinical applications. Functionalization and modification of OCP therefore represent the next frontier in materials science and biotechnology.

Functionalization refers to the process of introducing new chemical groups or functionalities onto the surface or within the structure of OCP crystals. These functional groups can impart a wide range of properties, such as improved biocompatibility, enhanced drug loading and release capabilities, or the ability to bind specific molecules. This tailored approach allows researchers to customize OCP to suit the needs of various biomedical and materials applications.

One example of an advanced functionality obtained by modification and functionalization of OCP was the development of a scaffold for the treatment of avascular necrosis (AVN) [10].

AVN is a debilitating condition causing a sub-condylar bone defect resulting in instability of the joint and eventual replacement by a medical implant. A scaffold has been developed that encompasses all the biological requirements for revascularization and regrowth of the AVN bone defect thus delaying the need for an invasive joint replacement procedure. A vascularity affinity precursor scaffold (VAPS) was fabricated such that a tri-layer collagen scaffold was produced. The two outer layers are collagen only with one layer designed to capture cells and the other layer designed to entrap signals and proteins added intra-operatively as autologous cells harvested from the patient. The central layer is designed to add structural support through the addition of α-TCP combined with an addition of a functionalized and modified OCP to enhance the bioactivity of the scaffold (Figure 3). The scaffold was implanted in a critical preclinical murine bone model and the bone growth was evaluated and reported. The addition of OCP to the scaffold resulted in enhanced bone healing when compared to α-TCP alone. This work has shown that OCP has a big role to play in the advancement of these scaffolds for compromised bone treatment.

6 | Bioactivity, Resorption, and Chemistry in Biomineralization

Positive effects of OCP implants on osteoblast and osteoclast activity in bone defects have been reported multiple times in the past [11–13]. We confirmed these observations by incubating mouse bone marrow stromal ST-2 cells on OCP or HA particles. As a result, osteoblast differentiation markers were significantly increased in an OCP dose-dependent manner while this tendency was not observed with HA [13]. Similarly, when mouse mesenchymal stem cells (MSCs) were cultured with OCP, β-TCP, and HA [13], OCP enhanced osteoblastic differentiation toward mature osteocytes with SOST/sclerostin expression, compared to β-TCP and HA.

The effect of OCP on osteoclast formation was examined using co-cultures of mouse bone marrow macrophages and osteoblasts [13]. OCP promoted the formation of tartrate-resistant acid phosphatase (TRAP)-positive cells without adding osteoclast differentiation factors, with increasing expression of osteoclast differentiation factor in osteoblasts. This indicated that OCP can induce osteoclast formation in itself. The migration of macrophages toward OCP can be enhanced through the inorganic ion exchangeable function of OCP, such as Ca^{2+} ions [13].

OCP also stimulates cells that are not involved in direct bone formation. The implantation of OCP/gelatin had effects on increasing collagenous Sharpey fiber formation at the tendon-to-bone insertion in the rabbit rotator cuff repair model and inducing angiogenesis in rat calvaria critical-sized defect model [11]. It was apparent that OCP can directly stimulate osteogenic differentiation of tendon stem/progenitor cells (TSPCs) [14] and human umbilical vein endothelial cells (HUVECs) [15] in the presence of OCP. The chemical environment induced by OCP dissolution should be involved in the stimulatory capacity of OCP in enhancing angiogenesis and bone regeneration [15].

We have found that bone regeneration by OCP and its biodegradation advance in association with a phase conversion from OCP into non-stoichiometric HA (CDHA) [16, 17]. The hydrolysis from OCP to CDHA taking place under physiological conditions is accompanied by the incorporation of Ca^{2+} and the release of inorganic phosphate (Pi) ions in OCP over time until it is completed (Figure 4). Solubility analysis of OCP in the simulated body fluid (SBF) verified that the supernatant became equilibrated toward a slightly saturated state with respect to OCP [18]. OCP is in fact converted to an apatitic phase and biodegraded in vivo conditions [13, 16, 17]. The analysis suggests that OCP is resorbed through the cellular biodegradation not only through simple dissolution [18]. The advancement of OCP hydrolysis into CDHA would be involved in increasing biodegradation to enhance bone regeneration by OCP implantation in bone defects.

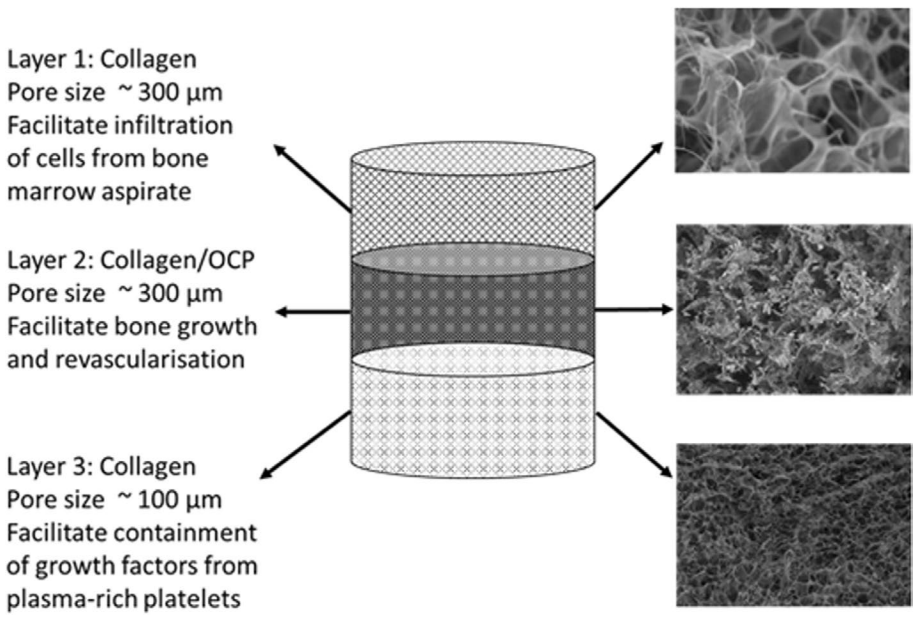

FIGURE 3 | Schematic of the tri-layered collagen OCP scaffold for bone regeneration of avascular necrosis defects.

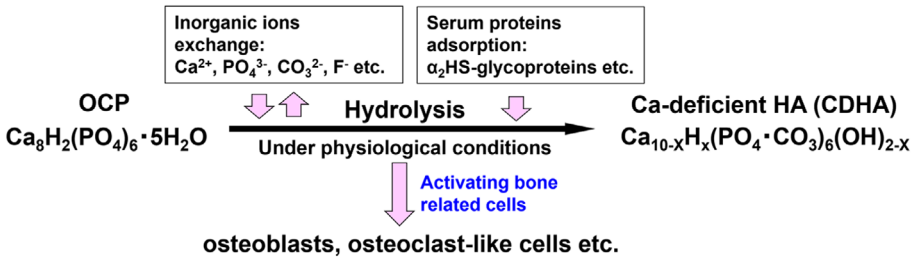

FIGURE 4 | Conceptual scheme for material and chemical properties of OCP and its cell activation function under physiological conditions based on the experimental evidence [2, 5, 11–18].

Thus biomineralization-like chemical property may be involved in the stimulatory capacity of OCP biomaterials.

7 | Commercialization and Regulatory Aspects

Unquestionably OCP is a fascinating biological material known to be a precursor to HA in the human biomineralization process that forms bone and tooth enamel. Research studies that have emerged in recent years suggest OCP's tremendous potential as a bioactive material. However, to successfully access this potential we have to agree "to what unmet clinical need is this biomaterial the best answer". If OCP enhances bone formation and has better biodegradability how will this translate into product claims that are both clinically and commercially attractive versus current biomaterials? What pre-clinical and clinical data will need to be collected to gain both clinical acceptance and the least burdensome regulatory pathway? Is there a feasible reimbursement strategy? The first OCP-containing products are now becoming visible and these appear to use "me too" regulatory pathways for existing bone void-filling biomaterials.

An implicit assumption in biomaterials projects is that the output will benefit patients and there will be better clinical outcomes ahead. Few researchers, however, are adequately prepared for the arduous translation journey from the laboratory setting to a commercially available product. All too often researchers identify an attractive new technology without first looking into the unmet clinical needs. An evidence-based approach will be mandatory to establish claims and indications in which OCP offers quantifiable benefits over the current standard-of-care treatments. The most promising pathway so far established is the regeneration of bone in dental indications.

8 | Discussion

As shown in the present paper, the OCP family of compounds, including the triclinic form and its many variants, represent promising bioinspired materials for biomedical applications. Gaining a deeper understanding of their conditions of formation, thermodynamic properties, evolution trends in solution and intimate structuration (including ion doping), as well as molecular sorption capabilities, should open new opportunities for research and innovation in the future. While a few OCP-based biomaterials are already considered for clinical use, their application has been limited to small volumes primarily in dental applications. Implantation of larger volumes is limited by the mechanically weak nature of the material and by a lack of larger scale production processes.

Due to its chemical and structural similarity to bone mineral in the early stages of mineralization, OCP offers the potential for improved bone regeneration compared to other bone filling materials. It therefore goes beyond the function of a pure filling material. However, clinical data will be required to support such a tissue regenerative claim, which in turn increases the hurdles for regulatory approval. The structural variability of OCP and the demanding manufacturing process both pose major challenges for would be manufacturers of OCP implants. Looking ahead, applied research will be needed to identify OCP compounds that overcome these manufacturing challenges. This should be accompanied by the development of advanced, multi-technique characterization strategies, as well as scale-up methodologies that will facilitate large scale industrial adoption and transfer to clinical applications. In parallel, standardization committees, regulatory agencies and medical device policy makers should consider the inclusion of the OCP family of bioactive compounds in their future guidelines and regulations.

9 | Conclusion

This first meeting has seen the presentation of groundbreaking research in the OCP field as summarized in the sections above. There can be no doubt that the translation into clinical applications is just on the very edge of happening and it is in translation to clinical application that the hoped-for promise from preclinical work will be fully realized. Bridging academic research and industry commercialization is a major challenge and the researchers will have to develop standardized forms of OCP that can be clinically evaluated, and the resulting outcomes used to fine-tune the formulation of these materials.

Conflicts of Interest

G. Insley is the founder of a company commercializing OCP-based products for bone repair (PBC Biomed Ltd., Shannon, Ireland). All other authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new original data were created or analyzed in this report.

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