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The Role of Carbonated Hydroxyapatite in Modulating Osteoblast Proliferation and Differentiation in Composite Bone Scaffolds: A Narrative Review

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ABSTRACT

Carbonated hydroxyapatite (CHA) is becoming widely used in composite bone scaffolds due to better similarity of its carbonate substitution to the mineral component of natural bone when compared with stoichiometric hydroxyapatite (HA). However, the role of CHA in osteoblast proliferation and differentiation within such systems is controversially described. This paper presents a narrative review on the physicochemical features of CHA regarding osteoblast behavior, CHA usage in composite bone scaffolds, and influence of CHA on proliferation and differentiation of osteoblasts. Generally, carbonate substitution decreases crystallinity and increases solubility and ion release when compared with HA, and there is a number of recent reports regarding stimulation of osteogenic differentiation of cells (alkaline phosphatase activity, RUNX2 and collagen I expression, and mineralization) by composite bone scaffolds in dependence of the amount of carbonate ions. The influence on proliferation is rather heterogeneous and sometimes, especially during direct CHA versus HA comparison, the effect is absent. There are two major problems for making conclusions on the effect of CHA: difficulty of separating the effect of CHA from co-substituents and design of composite scaffolds, and great heterogeneity of the methods used. Therefore, future approaches should include both CHA versus HA comparison and other approaches. This enhanced knowledge on the subject will help in creating better evidence-based designs of CHA-based composite scaffolds for bone regeneration.

Keywords: carbonated hydroxyapatite; osteoblast proliferation; osteoblast differentiation; composite bone scaffold; bone tissue engineering

INTRODUCTION

Bone tissue engineering represents one of the key approaches employed to restore the function of critical-size bone defects through the use of extrinsically applied biological stimuli [1]. The scaffold acts as a transient template for cell attachment and the subsequent formation of the new tissue, with the perfect degradation process that should occur in parallel with bone formation [2]. Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) constitutes one of the materials chosen to fabricate the scaffolds as the mineral phase of bone and thus represents an important component that mimics biological apatite [2,3].

The stoichiometric form of HA does not constitute a realistic apatite substitute because natural bone apatite possesses a poorly crystalline, deficient in calcium, and carbonate substituted structure where approximately 4-8 wt% of the carbonate groups occupy the phosphate positions in a B-type substitutional pattern [4,5]. In contrast with stoichiometric HA, carbonated hydroxyapatite demonstrates reduced crystallinity and increased solubility which are considered to be the biologically relevant properties since they affect the ion release and reactivity [4,6]. Thus, carbonated hydroxyapatite represents a promising material for bone tissue engineering applications due to greater similarity to natural bone apatite.

The ability to proliferate and differentiate osteoblasts is a well-established biomarker for the osteogenesis capacity of a scaffold, as these events occur prior to the deposition and mineralisation of the matrix [7]. Composites made of CHA and polymeric or other ceramic materials have been increasingly studied due to the scarcity of ceramic scaffolds with sufficient mechanical and biological properties as well as appropriate biodegradability for bone regeneration [3,8].

However, despite the growing number of publications, there is still controversy regarding the biological activity of CHA. While some papers show higher osteoblast proliferation and mineralisation in CHA scaffolds compared to conventional HA scaffolds [9], other studies demonstrate no differences in proliferation but changes in the surface dissolution process [6]. This may be attributed to the large variation in the amount of carbonate content, the particle size and the crystallinity, the preparation technique, and the type of the scaffold matrix that contains CHA. The purpose of this narrative review is to summarise available literature on CHA (and not HA in general) within composite bone scaffolds and to focus on the effect of CHA on osteoblast proliferation and differentiation, the underlying signal transduction pathways, the methods used in proving those results, and the future investigation.

CARBONATED HYDROXYAPATITE AS A BIOMIMETIC BONE MATERIAL

Carbonate ions replace HA in the crystal lattice mainly at two sites: phosphate (B-type substitution) and hydroxyl (A-type substitution). The former is predominant in natural bone minerals, with A/B ratio around 0.7 to 0.9 [4]. This substitution leads to reduction in size of a-axis of the unit cell. It forms the structural basis for reduced crystallinity found in CHA in comparison to stoichiometric HA [5,6].

Carbonate addition reduces crystallinity, increases solubility and causes decrease in crystallite size [4,5]. CHA ceramics dissolve significantly more in comparison to HA ceramics of the same microstructure and cause higher bioresorption in osteoclast cell cultures [9]. Increased solubility results in increased local supply of calcium and phosphate ions into the peri-scaffold microenvironment. It is believed though not experimentally proven to be one of the mechanisms by which CHA exerts its osteo-inductive properties [5,9].

Since carbonated substitution is the major chemical difference between apatite found in biology and stoichiometric HA ceramics, CHA is often called more biomimetic ceramic [4,5]. Indeed, from a chemical point of view, this statement is well justified, yet the successful transformation of the higher biomimicry in composition into a better biological response is not universally true and some authors report similar or even lower cell adhesion on CHA compared to HA [6].

ROLE OF CARBONATED HYDROXYAPATITE IN OSTEOBLAST PROLIFERATION AND DIFFERENTIATION

There is inconsistent evidence regarding the impact of CHA on the behavior of osteoblasts during the early stages. In the study carried out through a comparison of the pre-osteoblast MC3T3-E1, the CHA discs that had 4.9 wt% carbonate displayed lower cell attachment when compared to the HA at day 0, though no significant difference was seen between the two materials in terms of proliferation at days 7 and 28, with CHA displaying surface dissolution after 7 days as seen from SEM [6]. In contrast, a study carried out by comparing CHA/A-B type porous ceramics and HA with the same microstructure revealed that the CHA promoted higher pre-osteoblast proliferation as well as dissolution and bio-resorption, but without significant difference in osteoblastic differentiation markers RUNX2 and COLIA2 [9]. These conflicting results are probably attributable to variations in terms of carbonate composition, microscopic structure of particles and pores, as well as surface treatments performed on each of these materials. Indeed, in line with the fundamental idea that apatite-based grafts induce osteoblast proliferation, Indonesian research conducted *in vivo* has likewise found this relationship using non-carbonated HA as a control material. According to Setiawatie [10], the administration of hydroxyapatite in a rat alveolar bone model leads to an enhancement of osteoblasts compared to untransplanted animals, while its association with hyaluronic acid increases osteoblasts even more than hydroxyapatite treatment alone. This result shows that the biochemical microenvironment around an appetite graft, rather than the chemical nature of the apatite material, plays an important role in influencing osteoblast proliferation.

Particle Size and Osteoblastic Differentiation

The particle size is an independent factor affecting the osteogenic effect of CHA. It was shown that using the synthesis of nanoparticles based on the nanoemulsion method, which makes it possible to vary consistently the size of CHA particles from 200 to 900 nm, keeping their chemical structure unchanged, submicron-sized CHA particles with the size of 500 nm provide the fastest osteoblast differentiation with no effect on the viability of cells compared to particles of 200 nm or 900 nm sizes [11] This finding cautions against attributing differences between studies solely to carbonate content when particle size is a confounding variable.

Osteogenic Markers and Signalling Pathways

Of the osteogenic markers described in the reviewed literature, ALP activity, RUNX2, COL1, osteocalcin (OCN), and mineralised nodules formation are the most commonly observed osteogenic markers [7,9,12,13]. Other bone matrix-related proteins such as bone sialoprotein (BSP) and dentine matrix protein-1 (DMP1) have been used as *in vivo* markers related to osteoblast maturation and bone matrix mineralization.

In a study by Kamadjaja et al. [14], the expression of BSP and DMP1 were found to increase progressively, peaking in the treatment group on day 14 compared with the control group on day 7, using a hydroxyapatite scaffold made from crab shell (Portunus pelagicus) in extraction sockets, suggesting that apatite scaffolds can induce osteogenic markers that correspond to osteoblast differentiation and bone matrix mineralization *in vivo*. Although the results are quite similar to those observed with marine CHA scaffolds, as described in the next section, the upregulation of BSP and DMP1 alone, like the other osteogenic markers mentioned in the next section (Methodologies), cannot distinguish active osteoblast-induced matrix mineralization from passive protein adsorption on the graft.

The two signalling pathways shown to have a role in osteogenesis upon exposure to calcium phosphate materials are the Wnt/β-catenin pathway and BMP/Smad pathway, which are involved either independently or via cross-talk [15,16,17]. The canonical Wnt pathway activates cytosolic β-catenin, which moves to the nucleus and interacts with TCF/LEF transcription factors to induce expression of genes required for osteogenesis, as well as BMP2/4/7 expression, thereby highlighting the cross-talk between the two pathways [17]. BMP/Smad signalling pathway converges with Wnt signalling pathway by acting on RUNX2, a transcription factor for osteoblast differentiation [17]. Evidence linking this pathway specifically to carbonate-substituted apatite comes from an *in vivo* study in which carbonate apatite scaffolds seeded with stem cells from human exfoliated deciduous teeth enhanced BMP-2 and BMP-7 expression while attenuating MMP-8 during initial alveolar bone remodelling in Wistar rats [18].

Substrate physical parameters also have a link with the above pathways. Mechanistically, when polycaprolactone substrates were used with carbonated hydroxyapatite (CHA) particles at different densities, only a particular density of CHA particles (85 particles/cm^2) could increase osteoblast differentiation, which was due to focal adhesion maturation and nuclear translocation of β-catenin [15]. This shows that biological effects of CHA are not simply dependent on its chemistry alone.

CARBONATED HYDROXYAPATITE IN COMPOSITE BONE SCAFFOLDS

Natural Polymer/CHA Composites

Chitosan is one of the most commonly used natural polymers that has been paired with hydroxyapatite ceramic materials due to their structural similarity to glycosaminoglycan [8]. In a systematic review of *in vivo* research involving chitosan/ nanohydroxyapatite scaffold (20 studies included according to PRISMA guidelines), a favorable impact of this material on bone defect healing was observed; however, the presence of significant heterogeneity in scaffold preparation and defects among the included studies was mentioned [8].

Layered chitosan/HA scaffold containing therapeutic ions has also been proven to stimulate RUNX2, OCN, and COL1 while regulating osteoclasts via OPG/RANKL pathway [13]. A three-dimensional chitosan-hydroxyapatite scaffold seeded with amnion-derived mesenchymal stem cells similarly produced significantly greater RUNX-2, alkaline phosphatase, type I collagen, and osteocalcin expression than untreated controls in a rat calvarial defect model [25].

One example that directly ties into the discussion presented is that of the chitosan-gelatin/limestone-derived carbonate hydroxyapatite scaffold created by Rianti et al. [19], where scaffolds were prepared by freeze drying using three different chitosan-gelatin to CHA ratios (40:60, 30:70, and 20:80 w/w). This paper presents an example of the trade-off that will be described in greater detail later in this section between the strengthening capability of the polymer phase and the resorbability and bioactivity provided by the carbonate-containing apatite phase, while also presenting limestone-derived carbonate hydroxyapatite as one of the potential sources of the CHA along with marine animal-derived and synthetically precipitated. The biological counterpart to that material characterisation has since been reported for the same 30:70 chitosan-gelatin to CHA formulation, which significantly increased BMP-2, RUNX2, and RANK expression in Wistar rat extraction sockets at days 7, 14, and 21 relatives to untreated defects [20].

Synthetic Polymer/CHA Composites

Polymers like PCL are often blended with CHA for a trade-off between mechanical properties and biological activity. The incorporation of fish bone-derived CHA into 3D-printed PCL scaffold, with marine atelocollagen coating, proved that the increase in the CHA composition (from 2.5% to 10%) affected scaffold surface properties important in cell-matrix interactions [21]. Electrospun composite PCL/silk fibroin/carbonated hydroxyapatite scaffold was found to facilitate bone regeneration by controlling macrophage polarization towards a regenerative phenotype, suggesting the role of CHA in bone healing beyond osteoblasts [12].

Marine- and Mineral-Derived CHA Composites

The naturally aragonite cuttlefish bone (Sepia officinalis) can also be hydrothermally transformed into AB-type carbonated hydroxyapatite mimicking human bone composition and having a physiological range of 7.38 wt% of carbonates [22]. Cuttlefish bone-based nano-CHA can be introduced into PEO/chitosan fibrous networks using freeze-drying and tested for cytocompatibility *in vitro* [22]. The general review on biocompatibility of the cuttlefish bone-based biomaterials showed consistent results in terms of bioactivity and biocompatibility of these materials *in vitro* with respect to osteoblasts. In addition, the brittle nature of cuttlefish bone materials makes it necessary to combine them with polymer-based components for the load-bearing application [2].

Hydroxyapatite prepared from crustaceans can be considered as an example of naturally available raw material as well. As was mentioned above, crab shell-based hydroxyapatite scaffold materials were already used for extraction socket model for their influence on osteogenic markers [14], confirming the general trend for preparation of biogenic carbonate-containing calcium carbonates, such as cuttlefish bone, crab shell, sea urchin shell and limestone [2,19,22].

Functional Role of CHA Within Composites

CHA seems to play several non-exclusive roles in all of the reviewed composite structures. They are a bioactive mineral component delivering osteoconductive surface chemistry, a reinforcement material increasing stiffness of softer polymeric matrix [19], a source of ions activating osteogenesis [15], and, in certain scaffold types, an immunomodulator affecting macrophage polarization [12]. There is no way at the moment to rank these roles based on literature, although each scaffold system can be characterized in terms of its particular roles.

METHODOLOGIES USED TO EVALUATE OSTEOBLAST RESPONSE

Cell viability and proliferation tests (MTT, MTS, CCK-8) determine metabolic activity or cell numbers and can be used to rule out cytotoxicity, although no information is provided about the differentiation state [7]. This limitation is illustrated by a 24-hour MTT screening of an apatite–polymer composite at three ceramic-to-polymer ratios, which established non-cytotoxicity towards both osteoblasts and dental stem cells yet left the differentiation response entirely unaddressed at that time point [23]. ALP activity test is commonly used as an early osteogenic marker, usually reaching its peak at day 7 to 10 of *in vitro* incubation, but ALP activity cannot confirm differentiation itself [7,24]. Alizarin Red S (ARS) and von Kossa staining are the most common techniques for the identification of mineralisation nodules and serve as the late stage of differentiation markers; the results of systematic review of morphological assays conducted on 14 *in vitro* studies showed that ARS is the most frequently cited stain technique used in osteoblast differentiation studies, followed by von Kossa and ALP staining [7]. qPCR/RT-qPCR and Western blotting of osteogenic markers genes and proteins (RUNX2, ALP, OCN, OPN, COL1A1, OPG/RANKL) provide molecular confirmation of differentiation; a systematic review of gene expression profiling identified significant differences in gene and reference gene panels across the studies, which complicates the comparison between them [24]. The immunohistochemistry of matrix-bound proteins such as BSP and DMP1 was similarly applied as an *in vivo* differentiation and mineralisation marker for hydroxyapatite-based scaffolds [14], augmenting the previously described largely *in vitro* markers above; however, unlike ALP activity and mineralisation staining, BSP/DMP1 expression alone cannot discriminate between active osteogenesis-induced matrix development and passive protein binding. SEM is applied to evaluate cell attachment and cytoskeleton organisation and has been employed to correlate substrate CHA particle density with focal adhesion development and β-catenin relocation [15]. Animal model *in vivo* testing represents the most biologically relevant outcome measure but is quite resource demanding.

None of the essays presented above is alone enough to indicate differentiation on its own. Viability testing is incapable of identifying differentiation process altogether; ALP activity represents an indication of just a single and transient phase; and mineralisation/matrix protein staining indicates results that could potentially occur due to passive binding or deposition rather than successful osteogenic differentiation process. Thus, a conclusive proof of differentiation requires the combination of early marker, late functional marker, and molecular marker, an approach that is not followed by a significant part of the reviewed composite scaffolds papers.

COMPARATIVE METHODOLOGICAL ANALYSIS

A number of recurrent methodological differences prevent direct comparisons within the CHA literature. Cell responses to CHA are mainly evaluated using 2D CHA-coated discs or films [6], but such substrates do not reproduce the diffusion, mechanical, and geometrical limitations imposed by a 3D porous scaffold; 3D-printed or electrospun scaffolds containing CHA [12,19,21] better reflect the application in clinic but are rarer. Mechanistic studies on CHA rely mostly on immortalized MC3T3-E1 and MG-63 cell lines [6,9], whereas translational studies utilize human bone marrow-derived mesenchymal stem cells [13]; the animal alveolar-socket model is utilized *in vivo* osteogenic-marker studies [10,14]. *In vitro* tests focus on the mechanisms but do not account for angiogenesis or immunity, and *in vivo* studies give functional outcomes but rarely analyze the mechanisms in detail. Markers of differentiation change their pattern over the course of several weeks; short observation periods can be informative about proliferation or early ALP activity only, whereas *in vivo* tests such as the one conducted by Kamadjaja et al. [14] Only two markers have been studied on two different time points of days 7 and 14, which is enough to indicate the existence of any pattern but not enough to describe the complete time dynamics. The ALP and ARS analysis are functional and phenotypical indicators while qPCR and Western blots measure transcriptional processes. Very few papers in CHA provide both of these data points together with the same time points.

SYNTHESIS OF REVIEWED EVIDENCE

The scientific papers that are being synthesized in the current paper cover a wide variety of laboratory tests, from *in vitro* cellular models based mostly on MC3T3-E1 and MG-63 osteoblast-like cell cultures [6,9,11] to *in vivo* rodent and animal models with Wistar rat and Cavia cobaya extraction-socket or alveolar bone models [10,14] and non-cellular material characterization studies [19]. The vast majority of them were published within the timeframe of 2021-2026, while only some fundamental works from the

early 2010s and 2017 have been chosen to conduct comparisons between CHA and conventional HA [6,9]. Methods used to evaluate osteogenic properties vary from simple viability and proliferation tests [6,9] to ALP activity assessment, Alizarin Red S, von Kossa staining and mineralization [7] to molecular-level osteogenic marker expression analysis via qPCR and Western blotting of such markers as RUNX2, OCN and COL1A1 [12,13,24], and histological counting of osteoblasts in the case of *in vivo* models [10] as well as immunohistochemical staining of osteogenic matrix proteins such as BSP and DMP1 [14]. Sources of CHA that have been analyzed in this paper include synthetically precipitated apatite [6,9,11], marine-biogenic sources such as cuttlefish and fish bones [2,21,22], crustaceans such as crab shells [14] and mineral sources including limestone within chitosan-gelatin matrix [19]. This diversity of models, assays, and material sources underlies the methodological heterogeneity discussed above, and is the primary reason direct, study-to-study quantitative comparison of CHA's osteogenic performance remains difficult across the current literature.

MAJOR DEBATES AND INCONSISTENCIES

The idea that increasing carbonate content necessarily results in better osteogenic response through a straightforward dose-dependent relationship is not well-supported in the literature. For example, a study found that there was no difference between the two materials in terms of osteogenic activity between CHA with 4.9 wt% carbonate and HA [6], whereas a study comparing the AB-type CHA, which contained different carbonate arrangements, saw proliferation enhancement rather than differentiation enhancement [9]. Whether CHA is a better material than traditional HA across all conditions is also still an open question, considering the superiority of CHA appears to be highly conditional depending on carbonate content, crystallinity, and the type of assay conducted [6,9]. The particle size itself also influences osteogenesis in a non-linear way, where sub-micron-sized particles outperformed both smaller and larger particles in a certain study [11]. Although some composite systems achieved only a low level of *in vitro* differentiation signal, they managed to produce bone regeneration or matrix-marker upregulation *in vivo* [10,14], indicating that the *in vitro* assays might be unable to account for the bone-regenerative effects resulting from the release of ions, modulation of immunity, and vasculature growth *in vivo.*

TWO MAIN SCIENTIFIC CHALLENGES

Challenge 1: Lack of Standardisation in CHA Physicochemical Characterisation Across Studies

The carbonate content, A/B type ratio, crystallinity, particle size, and surface area parameters have been inconsistently determined and analysed using various methods in the reviewed literature [4,5,6,9,11]. It becomes very difficult to find out whether any biological inconsistency [6,9] is due to any biological differences or due to comparisons of materials which were different from each other in terms of factors other than the ones reported. Naturally and artificially obtained sources of CHA, such as cuttlefish bone, crab shell, sea urchin shell, and limestone, bring an additional factor of variability in composition for batches which are rarely characterised [14,19,22]. The solution to this problem lies in having a basic minimum requirement of CHA characterisation along with studies which vary just one parameter at a time while keeping other parameters unchanged, such as the one done for particle size [11].

Challenge 2: Disconnect Between *In Vitro* Differentiation Assays and Functional *In Vivo* Bone Regeneration

The *in vitro* experiments test certain partial aspects of the osteoblastic cell activity, and there is no such research presented in the current review that involves all these aspects together with an *in vivo* regeneration aspect for which the very same CHA composite would be used. In addition, there is also a problem related to the fact that the bone regeneration process *in vivo* depends on angiogenesis and immune regulation, the processes which cannot be measured through the current osteoblast *in vitro* assay [12], as well as because the *in vivo* marker studies are typically based on a few time points and only one marker set [10,14]. Resolving this challenge requires standardised multi-endpoint *in vitro*-to-*in vivo* correlation studies using the same CHA composite formulation across both testing regimes.

RESEARCH GAPS AND UNRESOLVED QUESTIONS

Material-based limitations: there is yet to be defined what the optimum carbonate concentration and ratio of A and B-type would be to enhance osteogenesis; the dependence of CHA particle size and morphology on differentiation, identified in one system [11], has not yet been repeated in other systems, including natural CHA [14,19,22]; the degradation rate and ion release from CHA incorporated into different polymers have rarely been quantified.

Scaffold-based limitations: the optimum loading percentage of CHA in scaffolds has not been yet defined for different polymer combinations. In even one well-studied system, a chitosan-gelatin/ Limestone-CHA composite scaffold, only three CHA-to-polymer ratios have been assessed [19]. The influence of pore architecture on the effect of CHA on osteogenesis is not yet investigated, along with the mechanical properties of CHA-containing scaffolds extracted from brittle marine minerals-derived scaffolds require further quantitative characterisation [2,19].

Biological gaps: the specific contribution of carbonate-dependent ion release on Wnt/β-catenin or BMP/Smad signaling activation remains experimentally unknown; the literature lacks long-term studies beyond 28 days with CHA composites; the number of comparisons between immortalized

cell lines and primary cells (or cells from patients) on identical CHA composites is low; the *in vivo* study on osteogenic markers in hydroxyapatite scaffold involve just one or two markers such as BSP and DMP1 [14].

Translational gaps: The reproducibility of CHA obtained from natural sources is affected by the variability of biological source and standard processing protocol is not yet defined [2,19,22]; there are not enough comparisons between CHA composites and bone graft substitutes clinically used in similar conditions; no long-term safety and efficacy studies in large animal models or clinical setting have been performed for CHA specific composites.

CONCLUSIONS

Carbonated hydroxyapatite constitutes a scientifically well-substantiated, biomimetic substitute for stoichiometric hydroxyapatite, which has been shown to be capable in a number of studies of increasing the proliferation of osteoblasts and, under certain physicochemical conditions, their differentiation [9,11]. But this is not the case across the board. Carbonate substitution alone is no guarantee of superior biological properties over conventional HA, and the results depend on an array of interrelated factors, such as the level of carbonation, size of the particles, packing density at the cell-biomaterial interface, degree of crystallization, and the matrix around it [6,9,11,15]. Scaffolds made of CHA combined with natural, synthetic polymers, as well as marine- and mineral-based matrices, including limestone-based CHA with chitosan-gelatin matrix [19] and crab shell-derived hydroxyapatite tested *in vivo* by BSP and DMP1 expression [14], These approaches have proven effective in individual studies, CHA having served in turn as a bioactive mineral phase, reinforcing agent, ion source, or immune modulator [2,8,12,13,19,21,22]. The osteogenic response is primarily mediated by the Wnt/β-catenin and BMP/Smad signalling cascades [15,16,17]. In terms of methodology, the current body of research utilizes a variety of assays, ranging from *in vivo* osteoblast counts and immunohistology for matrix proteins to the more familiar *in vitro* panel [7,10,14,24], neither of which alone can confirm osteogenic differentiation.

There are two fundamental flaws in the field at present, which hinder further development: the lack of an established standard for CHA's physicochemical characteristics between separate labs, and the lack of *in vivo* validation of *in vitro* differentiation results using the same material composition. The filling of these gaps can be achieved through experiments that focus on one chemical variable such as carbonate concentration or particle size while keeping others constant; combine molecular markers and functional endpoints at the latter stages of experimentation; and generalize naturally obtained CHA materials research that focuses on minerals and sea-based sources of CHA which have been gaining interest in the local scientific literature [14,19,22] to standardized and comparable characterization methods. Until that time, any claim about the superior nature of CHA relative to hydroxyapatite would not apply generally but conditionally to the experimental setting

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