Main properties of nanocrystalline hydroxyapatite as a bone graft material in treatment of periodontal defects. A review of literature

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ABSTRACT

This study aims to provide a literature review on nanocrystalline hydroxyapatite (n-HA). n-HA constitutes the principle inorganic part of hard tissues. Therefore, preparation of commercial synthetic analogues, the so-called ‘biomimetic’, has gained a lot of attention since it can precisely mimic the physicochemical features of biological apatite compounds. Due to its improved osseointegrative properties, n-HA may represent a promising class of bone graft materials. n-HA binds to the bone and by stimulation of osteoblast activity and enhancing local growth factors it improves bone healing. Periodontitis is an inflammatory condition in response to microbial plaque that leads to periodontal tissue destruction and osseous defects in alveolar bone. A review of the extant literature reveals that n-HA has certain advantages in periodontal tissue regeneration including minimal patient morbidity, better biocompatibility, and lack of toxicity.

KEYWORDS

Nanocrystalline hydroxyapatite; bone graft; periodontal defects; periodontal regeneration

Introduction

Inflammation of tissues surrounding teeth and loss of periodontal ligament attachment with bony support is regarded as periodontitis [1]. Periodontal disease can cause various types of bone deficiency. Additionally, periodontal osseous defects are considered as major challenges for clinicians. The chance of bone regeneration is higher for cases with angular defects than horizontal bone loss, if the topography of remaining bone and the number of osseous walls are satisfactory [2].

One of the successful ways to accomplish reconstruction of lost attachment apparatus in deep intra-bony defects is grafting of bone substitute biomaterials [3]. Bone substitute grafts consist of autogenous grafts, allografts, xenografts, and alloplasts. Alloplastic materials are synthetic, inorganic, biocompatible, or bioactive bone graft substitutes. They have been extensively used due to their availability [4]. Besides, calcium phosphate ceramics, as bone graft substitutes, have been widely applied to treat periodontal intra-osseous defects. Their application has produced clinically significant results [5, 6].

Two of the available forms of calcium phosphate bone grafts are hydroxyapatite (HA) and tricalcium phosphate (TCP) [7]. HA biomaterials are complex calcium phosphates that resemble bone minerals in their chemical component \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \). They have calcium-to-phosphate ratio of 1.67 [8]. The available forms of biomaterials include HA cement, nonporous HA, porous HA, nano-sized HA, and bio-ceramics. Bio-ceramics show a more dense structure compared to the other mentioned forms. When used within connective and bone tissues, HA presents biocompatibility with little inflammatory reaction [2].

Nanotechnology is the conception and control of matter at dimensions of roughly 1–100 nm. Nanotechnology contributes to investigating unique and relatively new phenomena and providing novel applications. Engineered nanoparticles were considered as an important class of new materials with several properties which attracted clinicians to work on them in the last decade. Nanocrystalline calcium phosphate apatites are the key elements in bio-mineralization and biomaterials field. Biological nanocrystalline apatites (n-HA) constitute the main inorganic components of hard tissues in mammals (bone and tooth, with the exception of enamel which is closer to stoichiometric hydroxyapatite). They are used in some pathological calcifications such as dental calculus, salivary and pulp stones, and blood vessel calcifications [9].

Unlike HA (a stoichiometric apatitic phase) which is highly stable yet with low soluble calcium phosphate at...
ambient conditions, nanocrystallineapatites are nonstochiometric, calcium- (and OH-) deficient. They may incorporate substituted ions in their nano-sized particles [10]. Specifically, their higher solubility accounts for calcium and hydroxide deficiencies than hydroxyapatite. Moreover, they are capable of being mature when exposed to humid environment. This supports why ‘mature’ bone crystals for vertebrates are less soluble and reactive compared to embryonic bone mineral crystals [11]. A crucial biological function in bone depends on the small size and non-stoichiometry of apatite nanocrystals. These nanocrystals probably cause mineral phase with the solubility needed for resorption of the bone by osteoclasts. Therefore, they enable bone mineral to act as an ion ‘reservoir’ capable of either capturing or releasing ions (or small molecules) under the control of the body to ensure homeostasis. Given these unique features, bone is a living tissue, not an inert, which continuously undergoes remodelling and repairing processes.

Synthetic apatite demonstrates good biological properties including biocompatibility, bioactivity, lack of toxicity or inflammatory, immunity reactions, and a relatively high bioresorbability. The significant increase of these properties lies on the improvement of their biomimetic. Different synthetic ways have been utilized to prepare nano-sized apatite crystals. Yet, preparation of actual biomimetic nanocrystalline apatites might be considered as a scientific and technological challenge. It is worth noting that the most fundamental characteristics which confer special properties to nanocrystalline apatites are nano-sized particles. Thus, the large surfacetovolume ratio, the existence of a surface hydrated layer, and non-apatitic in nature are important in the formation procedure of a solution. This layer is bound to disappear progressively as the stable apatite domains (in the core of the crystals) improve with time. Possessing a great ionic mobility, ion exchange and adsorption capacities allows for participation of this hydrated layer in the interaction with macromolecules [12]. As such, it is assumed that this layer on bone mineral nanoparticles is actively involved in homeostasis and other regulation procedures [11]. In this review study we try to introduce n-HA in brief and mention some examples of n-HA clinical usage in periodontal regenerative surgery.

**Effects of n-HA on epithelial cells**

Kawai and colleagues [13] stated that n-HA might have a therapeutic effect on periodontal epithelium. Therefore, they conjectured that healing process of open wound by contraction effect could be increased through intravenous calcium-based nanoparticles.

**Role of n-HA in differentiation and proliferation of periodontal ligament (PDL) cells**

Kanaya and co-workers [14] observed n-HA could stimulate differentiation of PDL cells, mediated by mechanosensitive signalling pathway and expression of BMP-2. Besides, Yang et al. [15] conducted an animal study through which they found n-HA could be used as a coating on silk scaffolds. Thus, they pointed out that n-HA-coated silk scaffolds might be potentially good biomaterials for regenerating periodontal tissue. Along with the above-mentioned studies, there are several research articles in the extant literature that emphasize n-HA effects on different cells in the periodontium.

**Fibroblast**

Based on the results of a study by Saleh et al. [16], it was proven that silver n-HA could enhance fibroblast cell maturation and proliferation. This could eventually result in connective tissue regeneration. In contrast, n-HA was found to be much more biocompatible than silver nanomaterial in a study of evaluating the biocompatibility of silver and n-HA on fibroblast cells by Shahoo et al. [17]. An *in vitro* study by Sun and colleagues [18] revealed that n-HA could increase proliferation and differentiation of PDL fibroblast cells in comparison to dense hydroxyapatite. Additionally, it was pointed out that n-HA was more biocompatible than dense HA.

**Osteoblast**

Shnettler et al. [19] found that n-HA could bind to the bone and stimulate the osteoblasts in the early stage of periodontal defect repair. This can lead to bone formation. Similar results were found in a study by Thian and co-workers [20]. Moreover, Pilloni et al. [21] proved that n-HA can increase the proliferation and differentiation of osteoblasts. In a report by Webster et al. [22] greater protein adsorption and osteoblastic cells adhesion on n-HA were shown. Liu et al. [23] found that n-HA could stimulate binding and proliferation of osteoblast-like MG-63 cells. It was proven that n-HA exhibits biocompatibility and minimal toxic effect on osteoblast cells in studies by Motskin et al. [24], Hsieh et al. [25], and Zhao et al. [19].

**Osteoclast**

In a study by Detsch et al. [26], it was shown that n-HA with low or no carbonate content can enhance the differentiation of osteoclast-like cells. This can result in having a great number of osteoclast cells on the material compared to carbonate-rich group. Activated osteoclast recruited mesenchymal cells from the bone marrow to differentiate them into osteoblasts. Additionally, it was shown by Matesanz et al. [27] that osteoclastic cell
differentiation could be constrained by n-HA with silicon. Consequently, a small number of osseous resorption of these cells was found on their surface.

**Effects of n-HA on bone regeneration**

Jahangirnezhad et al. [28] reported that n-HA contains osteoconductive properties which make it capable of producing sufficient amount of bone as bone grafting material. In the same vein, Vullo et al. [29] indicated that n-HA possesses both osteoconductive and osteoinductive properties in periodontal defects in dogs. Gotz et al. [30] evaluated the immunohistochemical properties of hydroxyapatite nanocrystalline silica gel on biopsies obtained from jaw bone. The results revealed n-HA had osteoconductive and biomimetic properties. These properties were integrated into human physiological bone turnover at an early stage. By obtaining clinical results which were comparable to autogenous graft materials, Huber et al. [31] concluded that n-HA paste was appropriate for filling bone defects. In addition, Zuev et al. [32] indicated that n-HA paste was not suitable for treating periodontal osseous defects such as periodontal abscesses. Based on a study by Talal et al. [33] n-HA-poly-lactic acid composite may be a suitable graft material for guided tissue regeneration (GTR) membrane. Although this material acts as a barrier, it can enhance bone regeneration via delivery of biologically active molecules. These results were supported in a study by Busen et al. [34] where they found n-HA could compete with Bio-Oss in bone reconstruction surgeries. However, Bertobili et al. [35] found contradictory results through investigating the amount of bone formation in Bio-Oss and n-HA after 4 months. They found that bone formation in Bio-Oss group was greater than n-HA.

**Effects of n-HA on macrophage activity**

The cell toxicity of colloid and gel forms of n-HA on monocyte-derived macrophages was evaluated by Motskin et al. [24]. The results revealed that n-HA gel application might be highly toxic. Other prepared n-HAs were toxic at concentration up to 250 ppm. Based on the findings by Scheel and colleagues [36] n-HA at higher concentrations (>500 ppm) might be toxic for macrophages. It was also proven that cell viability and macrophage proliferation was not mainly impaired in concentrations less than 500 ppm.

**Effects of n-HA on angiogenesis**

Bing Du et al. [37] found that neovascularization within the early stage of bone healing procedure could be increased by n-HA coralline blocks coated with recombinant human vascular endothelial growth factor (VEGF). As such, it was proposed that VEGF/n-HA/coral blocks can be considered a potential scaffold for enhancing bone regeneration in implant dentistry.

**Effects of n-HA on growth factors release**

Lock and Liu [38] stated that n-HA loaded on a nano-composite scaffold might stimulate attachment and differentiation of undifferentiated mesenchymal cells in a similar way to shorten peptides of bone morphogenetic protein 7 (BMP-7). A large amount of proteins on n-HA films compared to hydroxyapatite particle films were found by Zhou et al. [39]. Furthermore, Jain and co-workers [40] indicated that degradation of calcium sulphate particles which decreases local pH can result in demineralization of defects walls and release of growth factors, such as BMP-2, BMP-7, TGF-β, and PDGF-BB. The results of a study by Pezzatini et al. [41] showed that n-HA increased upregulation of FGF-2, and stimulated endothelial cells proliferation. This study indicates that n-HA can stimulate cell responsiveness to VEGF.

**Resorbability of n-HA**

Since n-HA has a small particle size and an extensive surface area that leads to quick resorption and accelerated substitution by vital bone of this material, it has several unique and beneficial properties. As proven in prior studies, complete resorption of n-HA takes 12 weeks. Therefore, it is appropriate for post-extraction socket preservation and minimizing the very small amount of remnant graft material around dental implants [42]. The evaluation of the efficacy of two forms of calcium sulphate, crystalline and nanocrystalline, in fresh extraction socket by Jain et al. [40] revealed that both materials can be considered as an appropriate regenerative graft material with density compared to the surrounding bone. Canullo et al. [43] conducted an assessment of fresh post-extraction sockets grafted with Mg-enriched n-HA. They revealed that Mg-n-HA permits complete healing of hard tissue around the graft. Additionally, graft material demonstrated significant resorption within the studied time frame.

**Clinical outcomes**

Treatment of periodontal defects with n-HA and its comparison to other graft materials have the centre of attention by several studies in the literature on either animals or humans. Recently, Vullo et al. [29] confirmed the successful application of n-HA with crystals varying in size between 70 and 100 nm as a graft material in
regenerative periodontal therapy in dogs. A comparison was made by Chitsazi et al. [44] between the efficacy of n-HA and autogenous bone graft in the treatment of periodontal intrabony defects. The results were favourable for both treatments in two- and three-walled osseous defects. These results could be compared with each other. Furthermore, Jain and co-workers [40] compared n-HA and β-tricalcium phosphate (β-TCP) in the treatment of human periodontal defects. It was found that both materials were beneficial in the pocket reduction, CAL gain, and radiographic bone fill at 3 and 6 months follow-up. This was a slightly better result of n-HA in comparison to β-TCP in pocket reduction and CAL gain which were not statistically significant at 6 months. The efficacy of n-HA with enamel matrix derivatives in the treatment of intra-bony defects was compared by Al Machot et al. [45]. The results revealed similarity of both materials in clinical outcomes. Enamel matrix derivative has some advantages compared to nanohydroxyapatite regarding patient’s comfort.

Prathap et al. [46] used n-HA in the treatment of bilateral grade II furcation involvement in the mandibular first molars. Application of n-HA, both with and without collagen membrane, resulted in a significant horizontal and vertical probing depth reduction and clinical attachment level gain. Although it was not statistically significant, utilizing collagen membrane revealed superior results compared to sites treated with bone graft alone. The clinical and radiographic results of n-HA alone and in combination with bioabsorbable collagen membrane in the treatment of periodontal intra-bony defects were evaluated by Singh et al. [47]. It was concluded that combination therapy was significantly greater to open flap debridement alone.

**The usage of n-HA in socket preservation among orthodontic movement**

A major concern for clinicians is the amount of alveolar bone resorption after tooth extraction for orthodontic treatment purpose, according to the recent study by Seif et al. one of the clinical advantages of n-HA in socket preservation process after tooth extraction among orthodontic movement is induce neovascularization and osteogenesis, and it does not have an important effect on the amount of root resorption. Histological evaluation in their study showed significant osteoblastic activity and remodelling environment of n-HA [48].

**The usage of n-HA in human periodontal intrabony defects**

Regenerative treatment for periodontal intrabony defect is a best clinical approach, although selection procedure of appropriate bone substitute material is always difficult for each dental practitioner because a good clinician must determine advantages and disadvantages of every biomaterial before periodontal regenerative surgery. Kamboj et al. evaluated the efficacy of nanocrystalline hydroxyapatite bone graft in the treatment of human periodontal intrabony defects and they proposed n-HA has significant effect in pocket depth reduction, the depth of osseous lesion, and a statistically significant gain in attachment level [49].

Bansal et al. revealed the application of n-HA can improve clinical and radiographic parameters in the treatment of periodontal intrabony defect [50].

Kasaj et al. compared the clinical outcome of intrabony periodontal defects following treatment with n-HA paste and open flap debridement. They conclude treatment of intrabony periodontal defects with nano hydroxyapatite (NHA) paste significantly improved clinical outcomes compared to open flap debridement alone [51].

Elgendy et al. compared clinical and radiographic outcomes of nanocrystalline hydroxyapatite with or without PRF membranes in the treatment of periodontal intrabony defects and concluded n-HA is a suitable bone substitute in the periodontal treatment of intrabony defect. They proposed adjunctive use of PRF membrane in combination with n-HA bone graft resulted in clinically, radiographically, and statistically significant compared with n-HA bone graft alone [52].

Hena et al. used PRF in combination with n-HA with collagen for treatment of periapical lesion and they revealed this novel technique is an effective approach to induce faster periapical healing in cases with large periapical lesion [53].

Figliuzzi et al. tested clinical and radiograph outcomes of n-HA application in periodontal intrabony defect and they demonstrated with respect to accurate case selection procedure, the usage of n-HA in periodontal defect has a significant clinical and radiographical improvement [54].

Vullo et al. analysed the effect of n-HA application in periodontal intra osseous defects in dog for regenerative capacity assessment and they concluded the usage of n-HA has both osteoinductive and osteoconductive effects in dog periodontal defect regeneration [55].

**Conclusion**

Nanohydroxyapatite can be considered a suitable alternative for autogenous bone graft in periodontal tissue regeneration. The use of this class of materials has been increased recently since they possess several advantages including minimal patient morbidity, biocompatibility, lack of toxicity, and so on. Once nanohydroxyapatite is combined with other active particles or biologic
mediators, it can stimulate periodontal tissue regeneration more than its application alone. It is required to conduct more randomized controlled clinical studies on different applications of biomaterials in treating periodontal defects.

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