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Application of Chitosan and Hydroxyapatite in Periodontal Tissue Regeneration: A Review

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Abstract

Chronic periodontitis is an infection caused by bacteria in the gum tissue that supports the teeth. The current periodontal therapy manages or removes periodontal infections and repairs the periodontium destroyed due to periodontal disease. Due to its biodegradability and biocompatibility, chitosan (CH) and hydroxyapatite (HAP) are employed for bone tissue healing. The purpose of this study was to compare the utilization of CH and HAP in the regeneration of periodontal tissue. The presented study is a systematic review prepared from a collection of recent relevant published articles. This research was conducted by reviewing articles from 2016 to August 2021. The analysis found that CH/HAP is a therapeutic strategy for chronic periodontitis patients that allow low-cost bone regeneration, mHA/CH scaffolds may inhibit the growth of periodontal pathogens, and CH or HAP has the potential to be developed bone tissue engineering.

Introduction

Periodontal tissue regeneration is a constant biological process. Wear and tear repair occur when new cells and tissues are regularly generated to replace those that have died under standard settings. According to the *National Institute of Dental and Craniofacial Research* of the National Institutes of Health in the United States, about 90% of adults over 70 have periodontal disease [1], [2].

Chitosan (CH) is a natural biopolymer derived from chitin, the main component of the outer crustacean skeleton, such as shrimp shell, and some studies state that CH is effective in accelerating wound healing, because it has specific properties, namely, the presence of bioactive, biocompatible, antibacterial, anti-fungal, and biodegradable properties [3]. CH is the result of chitin deacetylation using a solid base. Whereas chitin is a polymeric material found in natural materials such as shrimp shells, shellfish, crabs, yeast, insects, and fungi, the most chitin content is shellfish [4].

Chitin and CH have now received significant attention in applications medical and pharmaceutical. In addition, biopolymer CH is considered an antimicrobial agent significant for wound healing, concurrently with hemostatic function and its analgesic. Majority of traits biological activity of CH related to cationic and polymer chain size. These characteristics make CH ideal for use as a wound dressing [5].

Hydroxyapatite (HAP) is a bioceramic widely used in the biomedical field due to its similarity to the main mineral constituent of bones and teeth. Synthetic HAP has long attracted the interest of researchers to continue to be developed, because this material has excellent biocompatibility and has a high affinity with biopolymers [6]. HAP is proven to be biocompatible and very well tolerated by human oral tissues, has osteoconductive ability, and has been shown to stimulate osteoblast differentiation and bone formation [7].

Tissue engineering is a biomedical technology developed to assist the regeneration of body tissues in treating significant defects that the tissue itself cannot repair [8]. Tissue engineering is a multi-disciplinary knowledge based on clinical medicine, materials science, genetics, and other related sciences, life sciences, and engineering [9]. Bone tissue engineering (BTE) is based on an understanding of the bone structure, mechanics, and tissue formation that aim to support the formation of new bone functional tissue [10].

Research results in Boynuegri showed that CH gel was adequate and combined with demineralized bone matrix or collagenous membrane. All treatment modalities can be used in human intrabony defect treatment [11].

Study *et al*. showed that the HAP gel from the application of crab shells (Portunus pelagicus) showed a significant increase in the density of collagen fibers. The collagen fibers increased substantially up to 28 days after an extraction. The osteoconductive properties of crab shell HAP can induce and stimulate stem cells and osteoblasts to proliferate and differentiate the process of bone formation or new bone regeneration [12].

The research of Mukherjee *et al*. evaluates glutamate CH paste and HA as a synthetic bone graft material in mice. They concluded that pasta made from CH glutamate and HA could provide factors osteoinductive such as bone marrow aspiration or BMP-2 or osteoblasts cultured from bone marrow aspirates [13].

The research by Duan *et al*. demonstrated that NCMH microspheres could effectively heal bone defects *in vivo* without external preloading with cells or simultaneous application of bioactive growth factors. Therefore, NCMH microspheres can induce bone regeneration and provide sufficient channels for cell invasion and proliferation, as well as flexible adaptation to bone defects. This new exciting concept for the bone scaffold discovered in this study may serve as a design consideration for the next generation of bone grafts in tissue engineering [14].

The purpose of writing this systematic review is to compare how the use of CH and HAP in the regeneration of periodontal tissue.

Methods

Search strategy

This systematic review was conducted based on PRISMA guideline. Literature research was primarily performed using the PubMed, Wiley, and ProQuest to search for studies about application of CH and HAP in periodontal tissue regeneration, with the keywords: 1. “CH” AND/OR “HAP;” AND, 2. “Periodontal Regeneration” AND, and 3. “Graft,” published in the past 5 years, and ones written in English. After that, we combed through all articles cited and citing the articles so as not to miss any relevant articles.

Quality evaluation

First, all authors screened eligible studies through the titles and abstracts based on inclusion criteria. Then, all authors screened the full articles of all the collected studies. The authors had a meeting and agreed on highly relevant publications to be included in this study. All authors performed an appraisal of the study quality independently and any disagreement was resolved through discussion. All inherent aspects of the studies, including study quality, variables for which data were sought, and assessment of the risk of bias, were appraised by all authors independently by filling up forms. The forms were collected by the first author and the contents were scanned for any disagreement. The authors then gathered again for discussing any contradicting points.

Results

This resulted in a total of 398 journals identified through a database search ProQuest (n: 220) WILEY (n: 117) PubMed (n: 61); b). Selected articles were then screened, irrelevant study titles were excluded (n: 377), abstracts and titles identified (n = 21), study results and reviews that were not relevant were excluded (n = 14), because subject results were irrelevant (n = 10) and reviews (n = 4). Subsequent reviews of full-text articles were assessed for eligibility (n = 7). Full-text articles were retrieved and reviewed individually by all authors for additional screening. The remaining records were abstracted for analysis.

Further studies were included in the systematic review (n = 7) articles. The remaining records were abstracted for analysis. This selection process yielded

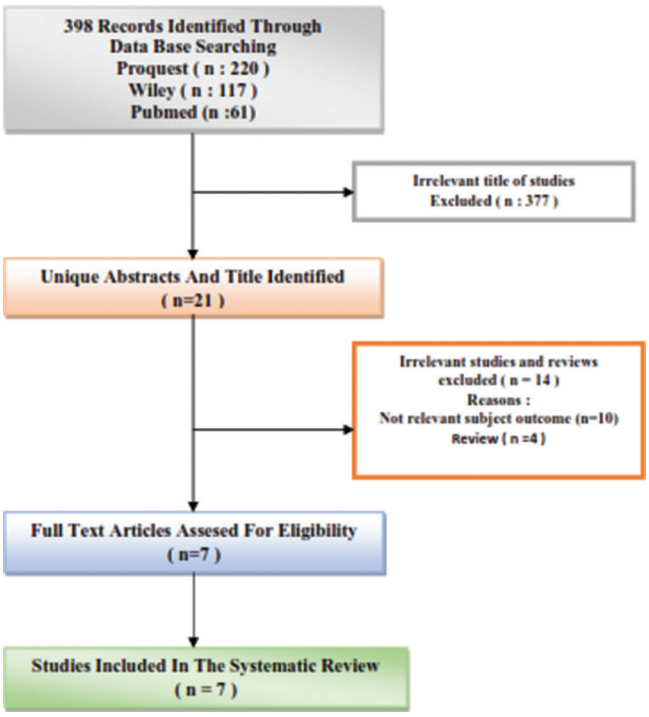

Figure 1: Flow diagram research procedure

seven final articles to be included in the systematic review and is depicted in Figure 1.

In this section, based on the review results, the use the method to classify selected articles based on the author, year, research methodology, and research results as in Table 1 as follows.

Discussion

HAP is chemically and physically the same mineral content as bone and teeth in humans. It also has calcium phosphate ceramics which are entirely biocompatible and non-toxic and are an integral part of existing bone and tooth tissue. Hence, it is essential that these materials are produced independently.

The results showed that HAP is considered one of the ideal scaffolds in periodontal tissue engineering. Its resemblance to bone mineral composition has good biocompatibility and bioactive properties [20], [21], [22].

Those findings agreed with Liao *et al*., which show that the mesoporous hydroxyapatite (mHA)/CS scaffold can inhibit the growth of periodontal pathogens. The cumulative amount of rhAm released by mHA and mHA/CS was statistically higher than that of CS at day 7, confirming that mHA and mHA/CS had a greater rhAm loading capacity than CS. Composite scaffolds loaded with rhAm significantly increased ALP activity and expression levels of RUNX-2, OPN, and DLX-5 genes and proteins *in vitro*. In addition, it successfully induces cementum-like tissue formation *in vivo*. This study provides a novel surrogate scaffold for periodontal regeneration that exhibits antibacterial activity and promotes osteogenic effect [17].

Therefore, the mHA scaffold retains the benefits of hydroxyapatite and mesoporous structure. Experimental studies have shown that nanostructured HAP is beneficial for osteogenic proliferation and differentiation of hPDLC [23]. When HAP and CH are combined as a composite scaffold, it can retain the benefits of good biocompatibility and mechanical strength. Level degradation is adjusted. Organic-inorganic nanocomposites have proven useful in many fields, such as tissue engineering and administration drugs [16].

Shavandi *et al*.'s research showed that irradiated squid-derived CH bio-scaffold had high water absorption and retention properties, showing stronger hydrophilic bonds than commercial crab CH derived from bio-scaffold. Another thing shows that CH-based bioscaffolds' physical and biological properties can be a promising biomaterial for bone tissue regeneration [16].

The results of Boynuegri research showed that no inflammatory reaction was observed after applying CH in the form of a good gel alone or in combination with a demineralized bone matrix. Furthermore, statistical analysis revealed no significant difference between the four treatment groups concerning clinical parameters. However, if compared with radiographic values, except for the group control, all groups showed significant differences at the end of the study ($p = 0.001$), indicating that the use of CH in infrabony increases bone filling [24].

Hoemann *et al*.'s study showed that CH hydrogel guides macrophages to wounds, thereby contributing to wound repair *in vivo*. Macrophages are also responsible for the *in vivo* degradation of CH by releasing cytokines as tumor necrosis factor and interleukin-1, which attract fibroblasts into the wound

Table 1: Article result of systematic review

S. No.	Authors, Year	Method	Outcomes
1.	Cornejo *et al*., 2017 [15]	A quantitative research design U-test Mann–Whitney	The CH/HAP application produces 5.77mm flat alveolar bone growth. The initial tooth mobility level was 2.44 mm, and at the end of the study, it was 0.8 mm, with a significant difference. Bone density in the area affected by periodontitis is similar to bone density adjacent to it. CA/HAP applications show alveolar bone growth in periodontitis patients
2.	Duan *et al*., 2017 [11]	A quantitative research design	NCMH microspheres can effectively cure bone defects *in vivo* without external preloading with cells or simultaneous application of bioactive growth factors. Therefore, NCMH microspheres can cause bone regeneration and provide sufficient channels for cell invasion and proliferation and flexible adaptations for bone defects. The new exciting concept for this bone scaffold found in this study can serve as a design consideration for next-generation bone grafts in tissue engineering
3.	Shavandi *et al*., 2016 [16]	A quantitative research design test Kruskal–Wallis and Mann–Whitney	Cell viability tests with osteoblasts show that bio-ceilings produced from irradiated CH are non-toxic and biocompatible, which have the potential for bone tissue engineering applications in the biomedical field
4.	Liao *et al*., 2020 [17]	A quantitative research design test ANOVA and t-Test	The mHA/CS scaffold can inhibit the growth of periodontal pathogens. The cumulative amount of rhAm released by mHA and mHA/CS is statistically higher than CS on day 7 (which confirms that mHA and mHA/CS have greater rhAm loading capacity than CS. Composite scaffolding loaded with rhAm significantly increases ALP activity and the level of expression of RUNX-2 genes and proteins, OPN, and DLX-5 *in vitro*. In addition, it succeeded in inducing tissue formation such as cementum *in vivo*. This study provides a new replacement scaffold for periodontal regeneration, showing antibacterial activity and promoting osteogenic effects
5.	Jayash *et al*., 2016 [18]	A quantitative research design analysis of Turkey HSD and parametric ANOVA test	The OPG CH gel can demonstrate enlarged protein release patterns, enhance cell development, and undergo particular lysozyme decomposition, making it useful in bone mending. It may be concluded that OPG CH gel has various valuable properties for network engineering
6.	Dahlan *et al*., 2020 [12]	A quantitative research design was analyzed using the Kolmogorov–Smirnov, Levene test, one-way ANOVA, and Turkey HSD test.	HAP gel from crab shell application (Portunus pelagicus) shows a significant increase in collagen fiber density. The collagen fiber increases substantially up to 28 days after an extraction. Osteoconductive properties of crab shell HAP can induce and stimulate stem cells and osteoblasts to multiply and distinguish new bone formation or bone regeneration processes
7.	Kamadjaja *et al*., 2019 [19]	A quantitative research design one-way ANOVA and *post hoc* Turkey HSD	HAP graft powder from Portunus pelagicus has biocompatible properties in human gingival fibroblast cell culture and at the lowest concentration of 25 ppm has optimal biocompatibility compared to the other two concentrations

initiate the reconstruction process. This study absorbed CH in approximately 3 months, with moderate alveolar bone growth. However, no macrophages were observed in the biomaterial analysis [21], [25].

Growth factors are an essential element in tissue engineering. Enamel matrix proteins have been widely used in periodontal regeneration and have shown the capacity to promote wound healing, limit epithelial growth, and support equipment regeneration complete periodontal attachment [26].

Of the seven studies, four existing studies conducted trials on CH/HAP materials and the final results of the three studies showed that the CH/HAP material was biocompatible and non-toxic, so it has the potential for BTE applications [16], [17], [18], [19]. Two studies conducted studies on experimental animals that showed healing of bone defects. Both of these studies showed the occurrence of cell invasion and proliferation, so it can be concluded that the CH/HAP material is considered as a bone graft in tissue engineering [11], [12]. Moreover, only research conducted by Cornejo directly conducted clinical trials on humans. Application of CH/HAP gearshift rate was initially 2.44 mm, and at the end of the study, it became 0.8 mm with a significant difference. In periodontitis patients, the application of CA/HAP to the socket preservation shows alveolar bone formation. Glutinous bone in the area affected by periodontitis after application of CH/HAP was similar to the density of healthy alveolar bone close to it [15].

Conclusions

Based on a review of the literature on the use of CH and HAP in the regeneration of periodontal tissue, the addition of CH/HAP seems a therapeutic strategy for chronic periodontitis patients that allow a feasible bone regeneration. The mHA/CS scaffold may inhibit the growth of periodontal pathogens. CH and HAP showed a remarkable potential for BTE to be developed.

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