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Natural Biphasic Calcium Phosphate Derived from Fish Bones as Biomaterial in Vital Pulp Therapy – A Literature Review

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ABSTRACT

Vital pulp therapy (VPT) is performed to treat teeth affected by deep caries, trauma, or restoration-induced perforations. Synthetic bioactive materials (non-biologic materials), including Mineral Trioxide Aggregate (MTA), Biodentine, and Bioaggreates cement are commonly used in this procedure. The limitations of MTA were long setting times, difficult manipulation processes, and discoloration. Biodentine is characterized by low radiopacity, difficulty in obtaining optimal consistency, and high costs, while bioaggregate cement has an extended setting time, which can impact the success of the therapy. Therefore, this study aimed to explore the use of fishbone, an abundant biological waste and a natural biomaterial as a treatment alternative. Fishbone contains biphasic calcium phosphate (BCP), a key component in bioactive material synthesis for dentistry. The results showed that BCP had excellent properties such as calcium ions release to stimulate odontoblastic activity, osteointegrative, osteoinductive, and excellent bioactive. Additionally, the production of this material is cost-effective, safe, and less complicated. Considering these advantages, fish bone with BCP can be an excellent alternative for VPT.

Keywords: Vital Pulp Therapy, Marine Fish Bone, Biphasic Calcium Phosphate

ABSTRAK

Vital pulp therapy (VPT) merupakan suatu prosedur perawatan yang dilakukan pada gigi dengan karies dalam, trauma, atau kesalahan prosedur yang menyebabkan perforasi. Bahan bioaktif sintetik (bahan non biologis) seperti *Mineral Trioxide Aggreagate* (MTA), *Biodentine*, dan semen *Bioggregates* adalah biomaterial yang paling sering digunakan dalam perawatan pulpa vital. Namun, *MTA* memiliki kekurangan seperti waktu pengerasan yang panjang, sulit dimanipulasi dan menyebabkan diskolorasi. *Biodentine* dengan kekurangannya yaitu radiopasitas rendah, sulit mendapatkan konsistensi yang tepat, serta mahal. Semen *Bioaggregates* juga memiliki waktu pengerasan yang panjang sehingga seluruh kekurangan yang sudah disebutkan dapat memengaruhi tingkat kesuksesan VPT. Saat ini, telah dikembangkan biomaterial alami yang dapat mengatasi kekurangan tersebut. Tulang ikan merupakan limbah biologis yang melimpah dan mengandung kalsium fosfat bifasik atau *biphasic calcium phosphate* (BCP). BCP merupakan salah satu komponen penting dalam pembuatan bahan bioaktif dalam kedokteran gigi. BCP memiliki sifat yang unggul seperti pelepasan ion kalsium yang mampu menstimulasi aktifitas odontoblastik, bersifat osteointegratif, osteoinduktif, dan sangat bioaktif. Selain itu, produksi bahan ini lebih mudah, aman, dan membutuhkan biaya yang lebih sedikit sehingga kalsium fosfat bifasik yang berasal dari tulang ikan dapat menjadi alternatif yang baik digunaka sebagai bahan VPT.

Kata kunci: *Vital Pulp Therapy*, Tulang Ikan Laut, Kalsium Fosfat Bifasik

1. Introduction

The dentin pulp complex is crucial in maintaining both the integrity and vitality of the tooth. Furthermore, it is the primary tissue responsible for dentin formation, comprising fibroblast, odontoblast, immune cells, extracellular matrix (ECM), blood vessels, nerves, interstitial fluid, and other components. However, the complex is prone to injuries caused by external factors such as caries, trauma, and restorative procedures [1]. Caries and trauma remain significant challenges in dentistry because untreated cases can cause pulp injury, potentially leading to tooth loss [2]. In modern dentistry, the focus has changed towards maintaining and preserving pulp vitality. Pulp inflammation can induce dentin synthesis through the process of biomineralization [3]. To address concerns related to pulp vitality, treatment strategies have shifted toward conservative approaches, particularly vital pulp therapy (VPT) [4]. When properly applied, VPT not only allow the preservation of viable tissue in permanent teeth but also facilitate apical closure and root development in immature permanent teeth [5,6]. The procedure entails applying a protective biomaterial over the remaining thin layer of dentin [6].

VPT materials are bioactive agents that protect exposed pulp, facilitate tissue regeneration, and support normal function and viability. An ideal VPT material should be non-toxic to the pulp while possessing bioactive properties to promote cell migration, proliferation, and osteogenesis [7]. Bioceramics, widely used in endodontics, can be classified based on bioactivity, resorbability, and chemical composition. Common categories include calcium silicate-based, calcium hydroxide-based, and calcium phosphate-based materials [8].

Calcium phosphate-based materials are highly effective in VPT due to the ability to release calcium ions, which enhance cell differentiation, proliferation, and mineralization. When compared to calcium hydroxide, these materials do not cause tissue necrosis and pulp inflammation. Examples of calcium phosphate-based pulp capping materials include calcium phosphate cement (CPC), tricalcium phosphate (TCP), and hydroxyapatite (HA) [8]. Calcium phosphate cement (CPC) is part of a broad family of calcium phosphate (CaP) biomaterials widely used for bone repair due to their chemical similarity to natural bone mineral.

The main types of CaP include hydroxyapatite (HAP), α- and β-tricalcium phosphate (TCP), amorphous calcium phosphate (ACP), octacalcium phosphate (OCP), dicalcium phosphate anhydrous (DCPA), dicalcium phosphate dihydrate (DCPD), and tetracalcium phosphate (TTCP). Each type has distinct properties: HAP offers excellent osteoconductivity and osteoinductivity but has poor mechanical strength and slow biodegradability; α-TCP and β-TCP are more resorbable, with β-TCP being more stable; ACP provides rapid biodegradability and a large surface area, while OCP is known for initiating bone mineral deposition but is mechanically weak. DCPA/DCPD have good biocompatibility but can cause inflammatory responses due to their degradation products, and TTCP is highly biodegradable and stable but challenging to synthesize in aqueous environments. The choice of CaP type in CPC formulations significantly influences the cement's setting reaction, mechanical properties, resorption rate, and biological performance, allowing customization for specific clinical needs in bone regeneration [9].

Synthetic HA nanoparticles are the main component of calcium phosphate and have been proven to possess great properties due to their ability to release remineralizing ions and adhere to tooth surfaces, thereby restoring demineralized lesions. Calcium phosphate is also capable of forming mineralized barriers and closing the dentine tubules that have been exposed. Recently, there has been growing interest in replacing synthetic calcium phosphate with the natural counterparts [10]. Calcium phosphate from natural sources contains a biphasic mixture of β-tricalcium phosphate (β-TCP) and HA. Similarly, synthetic mixtures of HA and β-TCP, known as biphasic calcium phosphates (BCP) have shown properties comparable to natural calcium phosphate. BCP has been successfully applied in orthopaedics as bone substitution material due to its superior properties such as osteoconductive, osteo-integrative, and excellent bioactive.

Due to the hydrophobic properties of BCP, it has the ability to enhance adsorption of minerals compared to the single-phase components which only consist of HA. The additional of TCP in BCP will results in a better rate of degradation along with higher bioactivity. In the other hand, HA will stabilize the process, ensure the low absorption rate but still manage to have promising mechanical properties. In comparison with the single-fased ones, BCP will have a better result in the new bone formation. Fish bones are known for the rich calcium, carbonate, and phosphate contents. Thus making it, an excellent natural source of HA and CaP. The extraction of BCP from marine fish bones was also observed to be safe, cost-effective, and less complicated [11]. There is no specific species of fish to be sorted out as one of the aim is to utilize the environmental waste which happens to contain abundant amount of calcium phosphate. The material poses a great characteristic as a therapeutic agent for dental hard tissues due to the ability to remineralize and occlude dentine tubules, which needs to be explored deeper in the next study[10]. Other thing that can also be investigated further in the next research is to compare different species of fish or from different water such as fishes from fresh water and salt water. This study aimed to review the properties of BCP from marine fish bone in general along with the potential application as dental biomaterials, especially in VPT.

Vital pulp therapy (VPT) is primarily used in cases where an immature permanent tooth have reversible pulpitis with signs of minimal bacterial contamination such as pulp exposure during restorative procedure or trauma [12]. It consists of procedures such as indirect pulp capping, direct pulp capping, shallow pulpotomy, and deep pulpotomy. Indirect pulp capping is a dental technique that includes preserving the carious dentin closest to the pulp to prevent pulp exposure, and this preserved dentin is then capped with a biocompatible material. The purpose of the treatment is to preserve the primary odontoblasts and stimulate the formation of reactive dentin at the junction between the pulp and dentin. The primary role of the bioactive lining material is to induce the odontoblasts to generate reactionary and reparative dentin, as well as facilitate the remineralization of the pre-existing dentin. This process promotes the health and functionality of the dentin pulp complex [5,13].

Direct pulp capping is a procedure used for healing mechanical or traumatic injuries exposed to the vital pulp. This process includes sealing the wound on the pulp with a biomaterial, which helps stimulate the growth of reparative dentin and preserves the vitality of the pulp. In the event of pulp exposure, the destruction of primary odontoblasts and the initiation of inflammation necessitate the recruitment and differentiation of stem cells in the healthy underlying pulp. This process is important for the production of reparative dentin [5,13].

Pulpotomy refers to the whole extraction of the upper part of the dental pulp, followed by the application of an appropriate dressing or medication that will facilitate healing and maintain the tooth's vitality. The indications for pulpotomy include cases where the pulp is exposed during caries removal in primary teeth. Additionally, the process also includes pulp exposure resulting from trauma, absence of a history of spontaneous pain, easily controllable haemorrhage from the exposure site, the bright red colour of the haemorrhage, absence of bone loss within the root, lack of radiolucent areas within the root, absence of abscess or fistula, a young permanent tooth with vital exposed pulp and an incompletely formed root, as well as a history of spontaneous pain. Pulpotomy is classified into two distinct groups, namely partial and complete. Partial pulpotomy, also referred to as shallow or Cvek pulpotomy, entails the removal of a small portion of the coronal pulp while preserving the remaining coronal and radicular pulp tissues. Complete pulpotomy, known as deep pulpotomy, is a more invasive approach that removes the entire coronal pulp and maintain the vitality of the radicular pulp. The procedure can be performed as an emergency measure to temporarily relieve symptoms or a therapeutic measure, such as in the case of a Cvek pulpotomy, defined by the American Association of Endodontics. Complete coronal pulp is extracted, and the living pulp tissue is covered at the root canal orifice [14].

The success of VPT largely depends on the choice of pulp capping material. However, it should not be the only determining factor, as no material developed to date possesses all the ideal requirements as pulp capping materials. The ideal properties of such materials are the ability to maintain pulp vitality, stimulate the formation of reparative dentin, be bactericidal or bacteriostatic, adhere well, provide a tight seal, be radiopaque, and be able to resist forces [15].

In the past half-decade, bioceramics have been widely used in medical fields for joint replacement, heart valves, bone tissues, and cochlear replacement. In the dental field, these materials were used due to their odontogenic and osteogenic properties. Bioceramics is chemically stable, biocompatible, and inorganic material can be divided into several groups, including bioinert, bioactive, and biodegradable [15].

Calcium phosphate-based materials have been a major focus in recent years due to their exceptional biocompatibility and low toxicity. A key factor in the success of pulp capping treatment is the release of calcium ions, a fundamental property of calcium phosphate, which promotes cell differentiation, proliferation, and mineralization [10,16]. Additionally, calcium phosphate does not inflame the pulp and causes tissue necrosis, making it an excellent option compared to calcium hydroxide and calcium silicate. Several calcium phosphate-based materials, including calcium phosphate cement (CPC), tricalcium phosphate (TCP), and hydroxyapatite (HA), have been utilized in both medical and dental applications. TCP is resorbable due to its ability to degrade, while HA and CPC are non-resorbable. Despite the promising properties, these materials have not been widely adopted as pulp capping agents due to the limited availability of clinical trials [8].

CPC is a bioactive material that transforms into HA in the presence of moisture. It has excellent biocompatibility, bioactivity, osteoconductive, and osteointegration properties, as well as the ability to bind with both soft and hard tissues [10,11]. Considering these advantages, CPC can be an effective alternative for VPT. However, certain limitations, such as high biodegradability, low mechanical strength, and prolonged setting time, restrict its application. The advantages of CPC included biocompatibility, good compressive strength, and self-setting capability. Additionally, it is non-toxic, non-immunogenic, and has a lower risk of inducing genetic mutations or promoting cancer development. Therefore, CPC may be used as pulp capping material as it has the potential to initiate dentin regeneration [8].

HA has a composition similar to the bone mineral content and is among the most stable forms of synthetic calcium phosphate thermodynamically. It is biocompatible, osteoconductive, and capable of osseointegration. HA has been used widely in orthopaedics and dentistry due to the advantageous properties. However, the limitations include poor mechanical properties, less dental bridge formation, and low fracture toughness. These drawbacks pose challenges, particularly in areas subjected to high mechanical forces [8,11].

TCP has been extensively studied since the 1970s and exists in several polymorphic forms, with α- and β-tricalcium phosphate (β-TCP) being the ones commonly used as biomaterials. These materials are osteoconductive allowing bone growth to happen on the surface or to fill other spaces. It is also biocompatible, has good compressive strength, and will turn into HA over time. This superiority makes calcium phosphate a good option for pulp capping. However, there are some limitations with the use of these materials such as poor mechanical strength, fast and uncontrolled resorption rate, low crack resistance, and difficulty in sintering [8].

4. Discussion

The synthetic calcium phosphates are produced as stoichiometric HA nanoparticles. According to reports, synthetic calcium phosphates have some limitations that do not meet one of the ideal requirements as capping materials. In a study conducted by Esposti et al., synthetic calcium phosphate was obtained by wet precipitation to acquire nanosized HA. The process was carried out by adding 30 mL of an aqueous solution of phosphoric acid to 100 mL of calcium hydroxide ($Ca(OH)_2$ 10.00 g). Synthetic calcium phosphate is frequently produced in the form of HA nanoparticles which have been proven to have excellent properties such as remineralizing ions release, surface adhesion, repairing the demineralized lesions, and also responsible for exposed dentinal tubules closure thus forming a mineralized barrier [10]. However, the synthetic HA does not fully mimic the components of the bone mineral phase [11].

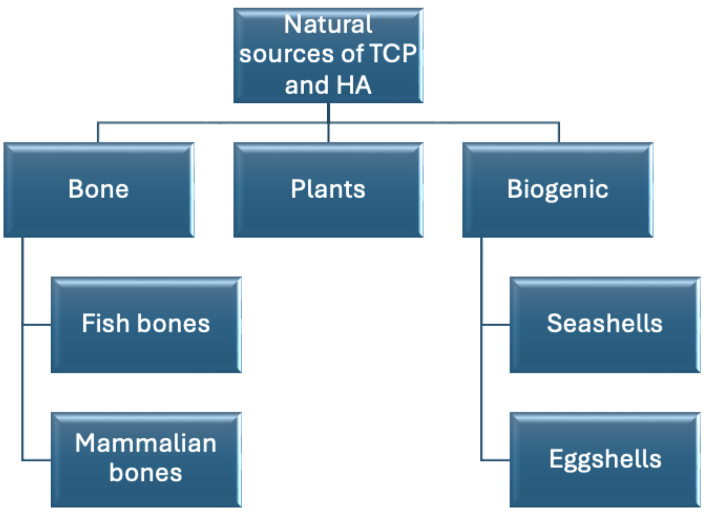

Figure 1. Biological sources of TCP and HA.

4.1. Biphasic Calcium Phosphate

Natural calcium phosphate contains a biphasic combination of β-TCP and HA. This combination makes the naturally derived calcium phosphate an excellent material to be used in remineralization and occlude dentinal tubules. Biphasic calcium phosphates (BCPs) belong to a category of bone-replacement materials, usually formed by combining two CaP components in different proportions: β-TCP, which degrades efficiently and is highly bioactive, and HA, which contributes structural stability, slower absorption, and robust mechanical strength [11].

β-TCP is found to be more soluble compared to HA both in neutral and acidic environments and this causes extensive release of ions needed for remineralization. The combination of BCP has been successfully applied as a bone substitution in orthopaedics [10,11,16]. Few studies were carried out regarding the use of HA derived from fish bone as fillers in resin composite and remineralization, but only pure HA minerals were used. However, no study has been carried out to explore the benefit of using BCP on dental hard tissues [10].

Scientists have proposed calcium phosphate substitution derived from natural resources such as marine supplies. Marine waste such as fish bones has produced tons of millions annually and has become a contributing factors to both environmental and economic problems. The fishbone by-products turned out to be the major source of calcium phosphate and were reported to be easily extracted [10,17]. The natural sources of calcium phosphate are mammals, fish, eggshells, or seashells. Fish bones contain calcium, carbonate, and phosphate which are essential in producing HA [11]. Thermal treatment is an effective method for extracting fish bones which leaves the BCP, and this composition has potential uses in remineralization and dentin tubules occlusion [10,18].

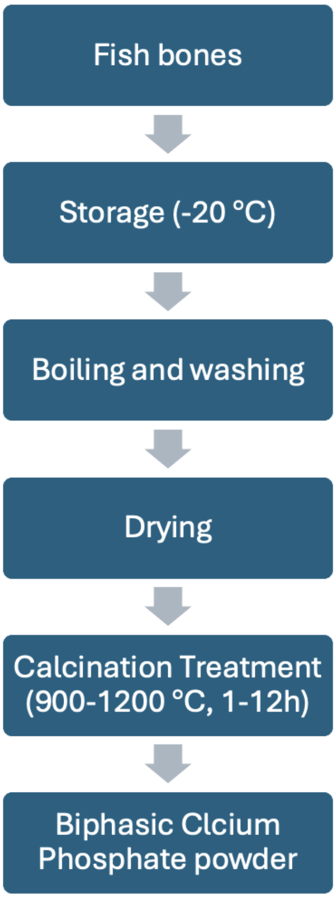

Figure 2. General flow diagram of BCP extraction from fish bones.

4.2. Role of BCP in VPT

HA derived from fish sources shows greater thermal stability, which can withstand temperatures around 1200 °C, compared to HA derived from bovine bones. Thermal treatment is also cost-effective and beneficial in keeping the original composition and qualities. Natural HA is found to have high compatibility due to its beneficial ion content that is important to properties such as solubility and morphology. These characteristics are very advantageous in bone formation, both in vitro and in vivo. It also have a low biodegradation rate which will adjust with other bone phases at a more optimal rate. Meanwhile, β-TCP has strong chemical stability, biological activity, and a suitable rate of biodegradation. Controlling the biodegradation rate could be a challenge and this will affect the bone formation rate [11].

BCP is very suitable to be used as bone substitution material due to its ability to act as a scaffold for new bone formation by osteoconduction, facilitated by HA [19]. Additionally, the reabsorption of β-TCP leads to an excess of calcium and phosphate ions in the surrounding area, which further promotes the acceleration of new bone formation. The mechanical characteristics, biodegradability, and overall stability of the final phases can be determined by achieving a suitable equilibrium between HA and β-TCP. BCP shows greater hydrophobicity compared to HA, but it is still lower than β-TCP. Therefore, the protein adsorption is increased in the case of BCP due to hydrophobic interactions, leading to a superior encouragement of bone growth compared to HA. The management of these qualities ensures a high level of stability while facilitating efficient bone ingrowth [11].

According to the study conducted by Ideia *et al*., HA and calcium phosphate derived from marine fish bones are more biocompatible with better metabolic activity compared to synthetic ones [20]. The report by Duta *et al*. showed that the crystallinity, rate of degradation, and biological performance in bone regeneration are more suitable in calcium phosphate obtained from marine fish bone [11].

Espoti *et al*. stated that the PXRD patterns of natural calcium phosphate contain a biphasic mixture of 33 wt% HA and 67 wt% β-TCP by Rietveld. Based on observation, natural BCP can induce mineral plugs formation which shows that the particles are small enough to penetrate and cause dentinal tubules occlusion. High magnification micrographs found that natural calcium phosphate produces two types of particles (rounded particles < 1 μm and small and irregular particles < 100 nm) which cover the surface of dentin evenly. Calcium phosphate also provides a more uniform increase in tissue hardness. This behaviour decreases the risk of fracture as external forces will be distributed evenly on the surface, thereby posing a higher fracture resistance [10]. Tan *et al*. conducted in vitro and in vivo study found that BCP can adhere easily to the dentin surface, occluding the dentinal tubules completely [21].

5. Conclusion

In conclusion, BCP derived from marine fish bone showed great potential as pulp-capping materials in VPT. BCP has the ability to enhace protein adsorption resulting in an excellent osteoconductive potential. Therefore, further investigation was necessary to evaluate the biphasic combination of β-TCP and HA for its ability to induce dentine bridge formation both in vitro and in vivo. Furthermore, the structural and compositional properties of different BCP material architectures can effectively support cell proliferation, which is a crucial step in accelerating bone tissue regeneration.

6. Future Directions

To this day, no material has met all the ideal requirements of a pulp capping material. Each of the bioceramics groups showed several limitations that cannot be an optimal option for VPT. However, calcium phosphate derived from fish bones is found to have the ability to overcome all the mentioned obstacles. Natural calcium phosphate has the superiority of being non-toxic, biodegradable, biocompatible, and able to neutralize acidic pH and high crystallinity. Furthermore, it produces both HA and β-TCP which in combination are found to have caused quicker and thicker reparative dentin formation compared to synthetics calcium phosphate which only consists of stoichiometric nanoparticles of HA.

7. Acknowledgements

Not applicable.

8. Conflict of Interest

The authors declare that there are no conflicts of interest to disclose concerning this study

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