CHAPTER 33

Marine Algae Possess Therapeutic Potential for Ca-Mineralization via Osteoblastic Differentiation

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Contents

Abstract

One of the important natural product investigations from marine algae is to focus on the pharmaceutically important compounds that can be applied in bone health. Osteoporosis is one of the bone diseases caused by an imbalance between bone formation and resorption. Promotion of osteoblast differentiation is one of the best therapeutic ways to combat osteoporosis. Osteoblasts are the cells responsible for bone formation by increasing the proliferation of the osteoblastic lineage or inducing differentiation of the

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osteoblasts. In this review, we describe the central effects of osteoblast differentiation by various bone therapy biomaterials from marine algae.

I. INTRODUCTION

Osteoblasts and osteoclasts are the bone cells, which are present and active in mature bones. These are effectively involved in interacting with the bone remodeling system and their balance activities remain the homeostasis of bone. Calcium phosphate compounds such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is present in substantial amounts in the mineralized tissue of the vertebrates, for example, 60–70% of the mineral phase of the human bones (Constantz *et al.*, 1995). As bones grow, osteoblasts lay down an organic matrix that is then mineralized by deposition of calcium (Ca) and phosphate to form hydroxyapatite. If this process is not properly regulated, the result can be of less mineralization or more, either of which can impair bone health. Osteoclasts are cells responsible for bone resorption that play a critical role in bone modeling and remodeling as they grow within the body. The disturbances in the relationship between these cell types can be found in many disease states. Bone diseases caused by abnormal bone homeostasis such as osteoporosis can be treated via the action of osteoclast and/or osteoblast. Therefore, identification of drugs that would affect the promotion of bone formation is the key tool for desirable therapies.

Osteoblast differentiation is regulated by various factors such as bone morphogenic proteins (BMPs), transforming growth factor β (TGF-β), Indian hedgehog (Ihh), Noggin, fibroblast growth factor (FGF2), insulin-like growth factor 1 (IGF-1), prostaglandins, parathyroid hormone (PTH)/parathyroid hormone-like peptide (PTHrP), and leptin via various signaling pathways lead by Smads, mitogen-activated protein kinases (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Osteoblast differentiation can be subdivided into three subsequent stages such as proliferation stage, extracellular matrix synthesis and maturation stage, and mineralization stage (Zamurovic *et al.*, 2004). The phenotypic markers of each stage are distinctively expressed. Active osteoblasts have high expression of alkaline phosphatase (ALP), collagen type I, early markers of osteoblast differentiation, while osteocalcin appears late, concomitantly with mineralization.

Marine natural products have attracted the attention of scientists in the world over for the past five decades. Marine organisms such as sponges, soft coral, marine algae, sea horses, tunicates, sea snakes, sea slugs, marine mollusks, and marine microorganisms are targeted nowadays for the investigation of new drug candidates. Many of the compounds

derived from marine organisms have shown very promising biological activity. The study of bioactive marine natural products has profoundly influenced the course of discovery in various fields ranging from pharmacology to cancer medicine.

According to Mayer's review in 2002, 2004, 2005, 2007, 2009, 2010, there has been a strong increase in studying marine compounds derived from a diverse group of marine animals, algae, fungi, and bacteria apply in pharmacology by years. In 1999, there have been 21 marine compounds demonstrating anthelmintic, antibacterial, anticoagulant, antifungal, antimalarial, antiplatelet, antituberculosis, or anti-viral activities; 23 compounds had significant effects on the cardiovascular, sympathomimetic, or the nervous system, as well as possessed anti-inflammatory, immunosuppressant, or fibrinolytic effects; and 22 marine compounds were reported to act on a variety of molecular targets. In 2003–2004, there have been highlights in the preclinical pharmacology of 166 marine chemicals: 67 marine chemicals demonstrated anthelmintic, antibacterial, anticoagulant, antifungal, antimalarial, antiplatelet, antiprotozoal, antituberculosis, or antiviral activities; 45 marine compounds were shown to have significant effects on the cardiovascular, immune, and nervous system as well as possessing anti-inflammatory effects; 54 marine compounds were reported to act on a variety of molecular targets. In 2007–2008, there have been 198 marine compounds which are part of the preclinical marine pharmaceuticals pipeline: antibacterial, anticoagulant, antifungal, antimalarial, antiprotozoal, antituberculosis, and antiviral activities were reported for 74 marine natural products; 59 marine compounds were reported to affect the cardiovascular, immune, and nervous systems as well as to possess anti-inflammatory effects; 65 marine metabolites were shown to bind to a variety of receptors and miscellaneous molecular targets. Thus, marine organisms that possess great potential of novel compounds and new drugs using for the treatment of treated numerous of disease categories are attracting attention of scientist. Each year, the increasing number of novel marine metabolites and marine natural compounds is reported in the literature indicating that the marine organisms will continue to be a prolific source of new natural products for many years to come.

Algae can be classified into two main groups as microalgae and macroalgae. Microalgae include blue green algae, dinoflagellates, bacillariophyta (diatoms), etc., whereas macroalgae (seaweeds) include green, brown, and red algae (Gamal and Ali, 2010). To the best of our knowledge, macroalgae, especially brown and red algae, are focused to show their effect on bone health. Although marine compounds derived from algae have exploited for a variety of purposes, the investigations for application in bone health are few and very recent.

II. THERAPEUTIC POTENTIAL OF MARINE ALGAE

Marine algae consist of numerous bioactive substances for known and unknown applications in medical and pharmacological fields. Natural products from marine algae can be used as pharmacological ingredients/materials for bone health or as functional foods for bone-strengthening applications. Marine algae have contributed numerous therapeutic compounds for the treatment of multiple disease categories such as antitumor (Fuller *et al.*, 1994, Guardia *et al.*, 1999), anticancer (Gerwick *et al.*, 1994), antibacterial (Ali *et al.*, 2002, Bennamara *et al.*, 1999, Smyrniotopoulos *et al.*, 2003), anti-inflammatory (Awad, 2000, Wiemer *et al.*, 1991), antiviral (Barbosa *et al.*, 2004, Wang *et al.*, 2007), antimicrobial (Barreto and Meyer, 2006), antimalarial (Lane *et al.*, 2007, Topcu *et al.*, 2003). In contrast, several investigations about compounds derived from marine algae effect on bone health as well as osteoblast differentiation processing were reported. Marine algae are also known as favorite food possessing enormous nutrient values. Thus, investigations to find out the medicinally important substances as drug candidates are very much needed to the development of marine materials from marine algae, which can be proposed for the bone health and/or to treat bone diseases.

A. Ca-mineralization via osteoblastic differentiation

Calcium level contributes to be crucial in the strengthening of bone and bone homeostasis. Investigations to find out natural products that promote Ca-mineralization are interesting nowadays. Marine algae have displayed a promising natural resource for this strategy, for example, unicellular coccolithophorid algae produce elaborate calcified scales called coccolith, which consist of fine pieces of $CaCO_3$ (calcite) crystals, known as one of the representative biominerals. A novel polysaccharide named coccolith matrix acidic polysaccharide (CMAP) was isolated from the coccolith of a coccolithophorid alga, *Pleurochrysis haptonemofera*, by Ozaki *et al.* (2007). By using chemical analysis and NMR spectroscopy including COSY, TOCSY, HMQC, and HMBC, the structure of CMAP was determined to be a polysaccharide composed of the following unit: [L-iduronic acid ($\alpha 1 \rightarrow 2$), mesotartaric acid ($3 \rightarrow 1$), glyoxylic acid ($1 \rightarrow$)]$_n$ (Fig. 33.1). The investigation showed that CMAP has a strong inhibitory activity on $CaCO_3$ precipitation and suggest that it serves as a regulator in the calcification of the coccolith. However, the effects of this compound on Ca-mineralization of osteoblasts were yet to be investigated.

Phlorotannins are the group of tannins and are found only in brown algae. Phlorotannins are oligomeric compounds using phloroglucinol (1,3,5-trihydroxybenzene) as a basic unit. Some phlorotannins have been

identified as the bioactive components in *Ecklonia* species such as *Ecklonia cava*, *Ecklonia kurome*, and *Ecklonia stolonifera* (Ham *et al.*, 2007). The compounds eckol, 2 dieckol, 6,6′-bieckol, and 1-(3′,5′-dihydroxyphenoxy)-7-(2″,4″,6″-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4,-dioxin (Fig. 33.2) were isolated from *E. cava* by Ryu *et al.* (2009), who investigated the potent effect of these compounds on a host of commonly

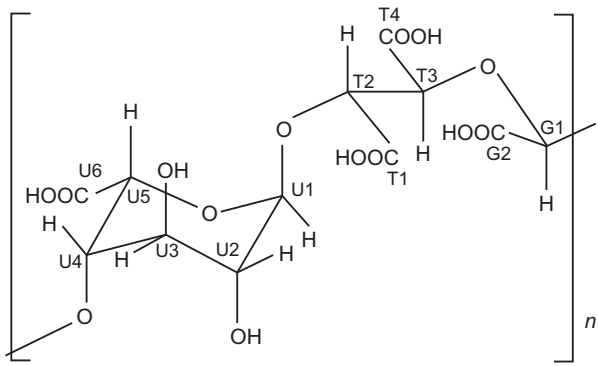


FIG. 33.1 Probable repeating structure of CMAP: [L-iduronic acid (α1 → 2), mesotartaric acid (3 → 1), glyoxylic acid (1 →)]$_n$.

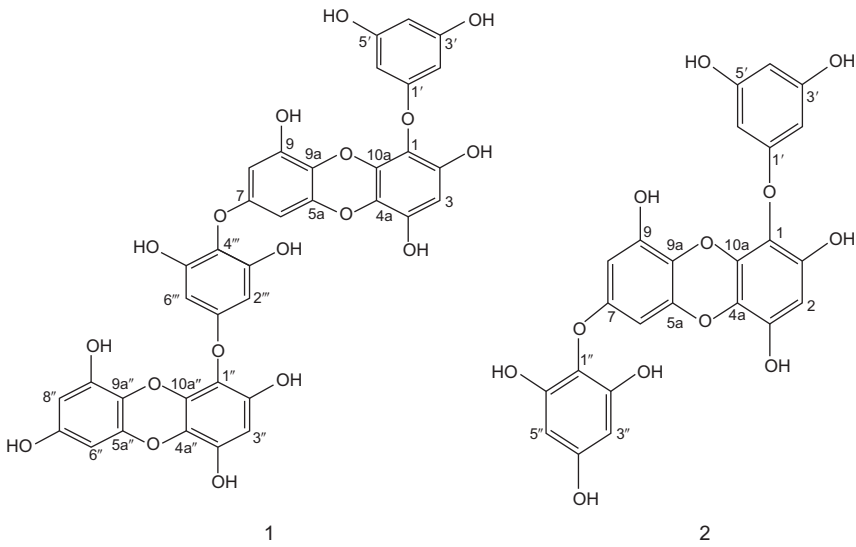
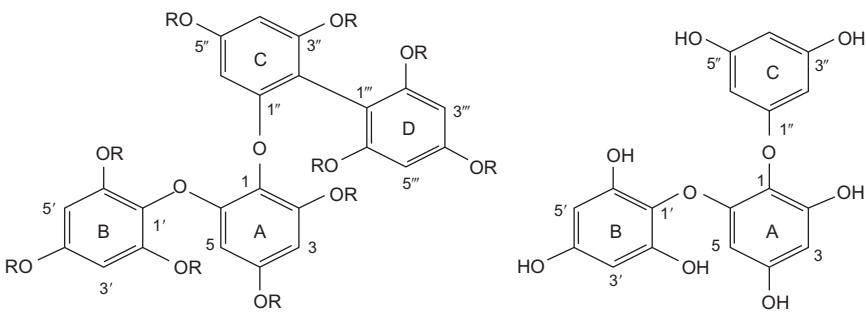


FIG. 33.2 Structures of phlorotannins from *Ecklonia cava* (1: dieckol; 2: 1-(3′,5′-dihydroxyphenoxy)-7-(2″,4″,6″-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin).

occurring diseases which possess an inflammatory component, including osteoarthritis, atherosclerosis, cancer, etc. These results suggested that the compounds help to stimulate the osteoblast differentiation at various stages and further confirmed that these compounds might have a therapeutic potential for the patients with osteoarthritis by stimulating production of proteoglycan. The phlorotannin derivatives showed the regulation of osteosarcoma differentiation by increasing ALP activity, mineralization, total protein, and collagen synthesis in human osteosarcoma cell (MG-63 cells). In addition, the phlorotannin derivatives could attenuate inflammatory response via MAPK pathway in chronic articular diseases.

Fucodiphlorethol G (Fig. 33.3), a new compound isolated from the methanol extract of *E. cava*, a brown alga, collected offshore in Jeju Island by Ham *et al*. (2007). By the examination of ^{1}H and ^{13}C NMR data, it was found that the structure of the compound is similar to that of trimeric phlorotannin triphlorethol-A (Fig. 33.3). Although these studies evidenced that these compounds can stimulate osteoblast differentiation at various stages, there is no clear demonstration of whether the phlorotannin compound, Fucodiphlorethol G, has direct effect on osteoblast differentiation.

The fucans of brown algae, often called fucoidans, have shown biological activities such as antioxidative, anticoagulant, antithrombotic, anti-inflammatory, antitumoral, and antiviral activities (Cho *et al*., 2009). Berteau *et al*. (2003) studied the fucoidan derived from brown algae and other common fucoidans (Fig. 33.4). Although fucoidan was extracted from the numerous species of brown algae (Table 33.1), to the best of our knowledge, there are a few reports on beneficial effects of fucoidan on bone health or formation. Cho *et al*. (2009) extracted fucoidan from brown algae *Undaria pinnatifida* and showed that fucoidan significantly effects



1, R = H
2, R = Ac

3, Triphloretol-A

FIG. 33.3 Structure of phlorotannin from *Ecklonia cava* (1: fucodiphlorethol G; 3: triphlorethol-A).

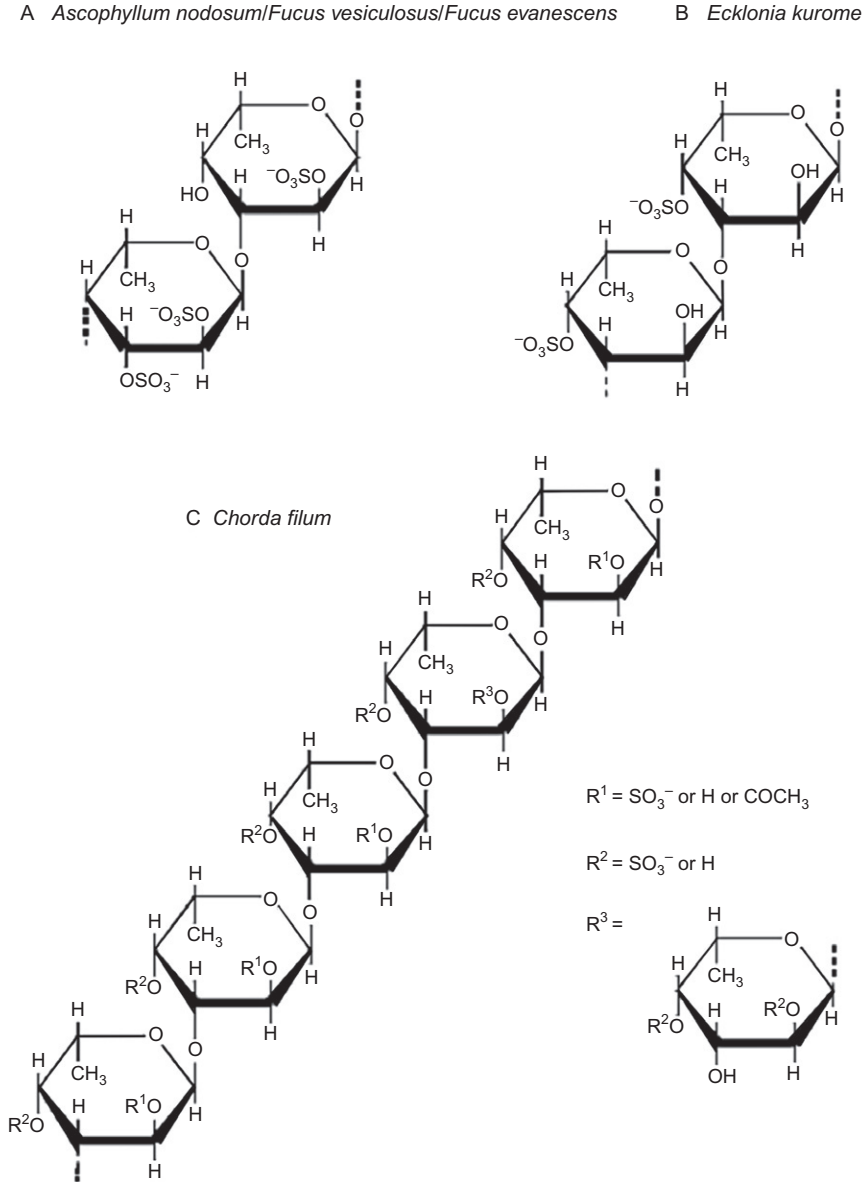


FIG. 33.4 Common structures in fucoidans from brown algae. (A) The disaccharide repeating unit [4)-α-L-Fuc*p*(2,3di-OSO_3^-)-(1 → 3)-α-L-Fuc*p*(2OSO_3^-)-(1] of a fraction of *A. nodosum* fucoidan representing the most abundant structural feature of fucoidans from both *A. nodosum* and *F. vesiculosus*. The same structure has been identified in the fucoidan of *F. evanescens*. (B) The 3-linked, preponderantly 4-sulfated fucoidan from *E. kurome*. (C) The quasirepeat unit identified in fucoidan from *C. filum*. Other substituents, such as *O*-acetyl, and branches are present in all these fucoidans and add considerably to their heterogeneity.

TABLE 33.1 Brown algae containing fucoidan

Species	Order
Cladosiphon okamuranus	Chordariales
Chordaria flagelliformis, *Ch. gracilis*	Chordariales
Saundersella simplex	Chordariales
Desmarestia intermedia	Desmarestiales
Dictyosiphon foeniculaceus	Dictyosiphonales
Dictyota dichotoma	Dictyotales
Padina pavonica	Dictyotales
Spatoglossum schroederi	Dictyotales
Adenocystis utricularis	Ectocarpales
Pylayella littoralis	Ectocarpales
Ascophyllum nodosum	Fucales
Bifurcaria bifurcata	Fucales
Fucus.vesiculosus, *F. spiralis*, *F. serratus*, *F. evanescens*	Fucales
Himanthalia lorea	Fucales
Hizikia fusiforme	Fucales
Pelvetia canaliculata, *P. wrightii*	Fucales
Sargassum stenophyllum, *S. horneri*, *S. Kjellmanium*, *S. muticum*	Fucales
Alaria fistulosa, *A. marginata*	Laminariales
Arthrothamnus bifidus	Laminariales
Chorda filum	Laminariales
Ecklonia kurome, *E. cava*	Laminariales
Eisenia bicyclis	Laminariales
Laminaria angustata, *L. brasiliensis*, *L. cloustoni*, *L. digitata*, *L. japonica*, *L. religiosa*, *L. saccharina*	Laminariales
Macrocystis integrifolia, *M. pyrifera*	Laminariales
Nereocystis luetkeana	Laminariales
Undaria pinnatifida	Laminariales
Petalonia fascia	Scytosiphonales
Scytosiphon lomentaria	Scytosiphonales

osteoblastic cell differentiation. The level of ALP, osteocalcin, and BMP-2 were increased in the presence of fucoidan. It is suggested that fucoidan could be an agent to promote osteoblast differentiation and has possibility for its application in bone health supplement. Synytsya *et al*. (2010) determined the structure of fucoidan extracted from brown algae *U. pinnatifida* and concluded that this fucoidan is sulfated galactofucan containing β-D-galactopyranose and α-L-fucopyranose at near equal amounts (44.6 and 50.9 mol%).

B. Marine algae-derived biomaterials for bone tissue engineering applications

The marine environment is rich with mineralizing organisms of porous structures, some of which are currently being used as bone graft materials and others are used in their early stages of development for bone repair. Some species of red algae (phylum Rhodophyta), specifically a group of coralline algae deposits calcium carbonate, have been used in bone tissue engineering (Clarke *et al*., 2011).

Felício-Fernandes *et al*. (2000) reported that calcium phosphate compounds such as hydroxyapatite were prepared by hydrothermal synthesis with phycogenic $CaCO_3$ as starting material. They showed that it may be suitable for use as a biomaterial. The biogenic material was obtained from algae of the Rodophycophyta. Calcium carbonate for the synthesis of calcium phosphates similar to bone can be obtained from several natural sources. Only the calcium carbonate originating from marine algae and corals shows characteristic porosity and interconnectivity that makes it like human bone.

Manufacture cell-seeded three-dimensional bone constructs based on hydroxyapatite ceramic granule calcified from red algae and mesenchymal cambial-layer precursor cells have been investigated by Turhani *et al*. (2005). The results showed that these 3D composites might possess suitable properties for bone reconstruction of the maxillofacial region *in vivo* and provide new insights into the development of novel strategies of bone tissue engineering. They conclude a positive effect of hydroxyapatite ceramic granules on mesenchymal cambial-layer precursor cell behavior in cell-seeded three-dimensional bone constructs.

Walsh *et al*. (2008) investigated a bioceramic from algal origin, which is suitable for bone tissue application. These reports confirmed the successful conversion of mineralized red alga to hydroxyapatite, via low-pressure hydrothermal process. Further, the synthesized hydroxyapatite maintained the unique microporous structure of the original algae, which is considered beneficial in bone repair applications. Investigations on hydrothermal transformation of mineralized red algae were performed by Walsh *et al*. (2010), which are shown to be a suitable candidate for conversion to calcium phosphate ceramics in terms of its physiochemical properties to hydroxyapatite that preserves the algae's unique structure. The optimum processing parameters for the thermal heat treatment were found to be in the range of 600–650 °C, with a ramp rate of 2 °C min^{-1}. These parameters are considered as a well-strategized approach for hydrothermal conversion of *Corallina officinalis* to hydroxyapatite.

Calcium is an essential mineral to support bone health and serves as a major therapeutic intervention to prevent and delay the incidence of

osteoporosis. Adluri *et al*. (2010) examined the effect of a novel plant-based calcium supplement from marine algae, which additionally contains high levels of Magnesium and other bone-supporting minerals [commercially known as AlgaeCal (AC)]. These supplements have potential effect on proliferation, mineralization, and oxidative stress in cultured human osteoblast cells and compared with inorganic calcium carbonate and calcium citrate salts. The results demonstrated that AC can serve as a superior and bioavailable calcium supplement than the inorganic calcium sources, and the effect of AC may be due to its content of other bone-supporting minerals and their influence on ALP, DNA synthesis, helping in the proliferation and mineralization of the osteoblast cells.

C. Marine algal diet with calcium supplement

The well-known correlation between diet and health demonstrates the great possibilities of food to maintain or even improve our health (Plaza *et al.*, 2008). Dietary factors are very important for osteoporosis, and Ca^{2+} is the most important cationic mineral in the bone (Aslam *et al*., 2010).

Different algae and their comparative composition to use as new sources of functional ingredients have been shown previously (Plaza *et al*., 2008). Algae have mainly been used in the Western countries as raw material to extract brown algae alginates and red algae agar and carrageenans. Thus, it is possible to conclude that these organisms have shown a high potential as natural sources of ingredients with many different biological activities.

Aslam *et al*. (2010) investigated whether a mineral-rich extract from the red marine algae *Lithothamnion calcareum* could be used as a dietary supplement for prevention of bone mineral loss. Their experiments showed that the mice receiving the mineral-rich supplement in the high-fat Western-style diet had better bone structure/function than the mice on the low-fat chow diet. The algae extract is already available as a food supplement under the name Aquamin (GRAS 000028), which is currently used in various products of human consumption in Europe, Asia, Australia, and North America.

III. CONCLUSION

Worldwide, there are about 1500–2000 species of brown algae, 5000–5500 species of red algae, 6000 species of green algae, approximately 4000 known species of microalgae. Among them, just small species groups have clarified the pharmacological potentiality. There are still more potential compounds derived from marine algae unidentified to be identified. Thus, marine algae are promising for investigations in future

toward their biomedical applications especially. Investigation of marine natural products from marine algae effect on osteoblast differentiation that can lead to regain bone homeostasis and possibility for its application in bone health supplement is beginning.

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