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Novel Applications of Phyco-Oxyapatite – A Hydroxyapatite from a Marine Seaweed

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Abstract

Phyco-oxyapatite, a novel form of hydroxyapatite (HAp), is derived from marine seaweed and holds immense potential in the fields of regenerative medicine and dentistry. Traditionally, HAp, a major component of bone and teeth, has been sourced from bovine bones. However, ethical concerns and the risk of disease transmission have prompted the exploration of alternative sources. Seaweed, particularly from the Indian coastal region, provides a sustainable and safer source of HAp. In this study, Halimeda sp., a calcareous seaweed, collected, processed, and chemically treated to extract phyco-oxyapatite. The material was characterized using SEM, EDS, FTIR, and XRD techniques to confirm its morphology and elemental composition. In vivo studies on zebrafish (Danio rerio) demonstrated that phyco-oxyapatite derived from seaweed significantly enhanced caudal fin regeneration compared to control. The von Kossa staining further confirmed enhanced mineralization in treated zebrafish. These results suggest that phyco-oxyapatite is a promising biomaterial for bone tissue engineering, offering superior regenerative capabilities compared to conventional sources of HAp. Future research should focus on the material's application in clinical settings and its long-term performance in human bone regeneration. The study highlights the potential of seaweed-derived hydroxyapatite in transforming the landscape of biomaterials and regenerative medicine.

Keywords: *Bone Regeneration, Calcareous Seaweed, Phyco-Oxyapatite.*

Introduction

Hydroxyapatite (HAp), a naturally occurring calcium phosphate mineral, is a fundamental component of human bones and teeth. Due to its remarkable biocompatibility and osteoconductive properties, HAp has become an essential biomaterial in a wide range of medical and dental applications [1, 2]. It is particularly used in bone grafts, dental implants, and tissue regeneration due to its ability to support new bone formation and integrate seamlessly with surrounding tissues [3, 4].

Traditionally, hydroxyapatite is derived from bovine bone, a process that, while effective, raises significant concerns. These concerns include the risk of disease transmission, such as bovine spongiform encephalopathy (BSE), and ethical issues surrounding the use of animal-derived materials [5, 6]. Furthermore, the use of synthetic hydroxyapatite, although widely researched

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and applied, often falls short in terms of metabolic activity and bio-integration when compared to natural HAp sources [7]. This has fueled the search for alternative, sustainable, and ethically sound sources of hydroxyapatite, leading researchers to explore marine environments, specifically seaweed.

Seaweed, a renewable marine resource, has emerged as a viable alternative for hydroxyapatite extraction [8, 9]. The marine environment is rich in bioactive compounds and minerals that contribute to the structural complexity and functionality of seaweed-derived materials [10]. Among these compounds, calcium carbonate plays a critical role in the formation of hydroxyapatite. Calcareous seaweeds, such as *Halimeda sp.*, accumulate calcium carbonate in their cell walls, making them an ideal candidate for HAp extraction [11, 12].

Phyco-oxyapatite, a form of hydroxyapatite derived from marine seaweed, is attracting growing interest due to its unique properties [13]. Unlike synthetic variants, phyco-oxyapatite retains trace elements naturally present in seaweed, such as magnesium and strontium, which are known to enhance the osteogenic potential of hydroxyapatite [14, 15]. These elements support crucial biological processes, including osteoblast proliferation and differentiation, which are vital for bone regeneration [16].

Materials and Methods

This section details the procedures for extracting and characterizing phyco-oxyapatite, including the collection of marine seaweed, chemical treatment, and analysis techniques.

Collection of Marine Seaweed

Halimeda sp., a calcareous seaweed, was harvested from the Mandapam Coastal Region of Rameswaram, Tamil Nadu, India. The biomass was subjected to a multi-step cleaning process to remove salts and impurities. This was followed by drying and grinding the seaweed into a fine powder using a mortar and pestle.

Extraction of Phyco-Oxyapatite

Phyco-oxyapatite was extracted using a combination of chemical treatments. The powdered seaweed was treated with concentrated hydrochloric acid (HCl) and orthophosphoric acid to dissolve the calcium carbonate present in the biomass. The solution was neutralized with sodium hydroxide (NaOH), precipitating hydroxyapatite. The mixture was centrifuged, and the resulting precipitate was dried to obtain the final phyco-oxyapatite product (applied for Copyright).

Characterization

The structural and elemental properties of the extracted phyco-oxyapatite were analyzed using several advanced techniques. Scanning electron microscopy (SEM) was employed to observe the morphology of the particles, while energy-dispersive X-ray spectroscopy (EDS) was used to determine the elemental composition. Fourier-transform infrared spectroscopy (FTIR) helped confirm the chemical bonds, and X-ray diffraction (XRD) was used to identify the crystalline structure.

In *Vivo* Analysis: Caudal Fin Regeneration in Zebrafish

The biological efficacy of phyco-oxyapatite was evaluated through *in vivo* studies using zebrafish (*Danio rerio*). Zebrafish have regenerative capabilities, making them a suitable model for studying bone regeneration. The experiment involved two groups: control, and seaweed-derived hydroxyapatite-treated. The treatments were administered every two days (5 mg of phyco-oxyapatite), and caudal fin regeneration was measured over 14 days.

Results

The results section presents the outcomes of the extraction process, characterization analyses, and *in vivo* studies, providing a detailed assessment of phyco-oxyapatite's potential as a biomaterial.

Extraction Yield

The extraction process yielded 4.51g of phyco-oxyapatite from 5g of *Halimeda sp.* powder, representing a high extraction efficiency (≈ 90%) (**Fig. 1**).

SEM and EDS Analysis

SEM imaging revealed that the phyco-oxyapatite particles had a tetrahedral morphology, with densely packed particles and interstitial voids that promote cell infiltration—an essential feature for bone tissue engineering . EDS confirmed the presence of calcium and phosphate, with a Ca/P ratio of 2.2, which aligns with the composition of natural hydroxyapatite (**Fig. 2 & 3**).

Caudal Fin Regeneration in Zebrafish

The zebrafish treated with green seaweed-derived phyco-oxyapatite exhibited superior regenerative performance compared to the control. By day 20, the fin length in the green seaweed-derived HAp-treated group reached 4.1 mm, compared to 3.3 mm in the control group. This significant improvement highlights the osteoconductive properties of phyco-oxyapatite (**Fig. 4**).

Von Kossa Staining

von Kossa staining revealed extensive calcium deposition in zebrafish treated with green seaweed-derived phyco-oxyapatite. The mineralization was more uniform and integrated compared, suggesting better bone regeneration properties (**Fig. 5**).

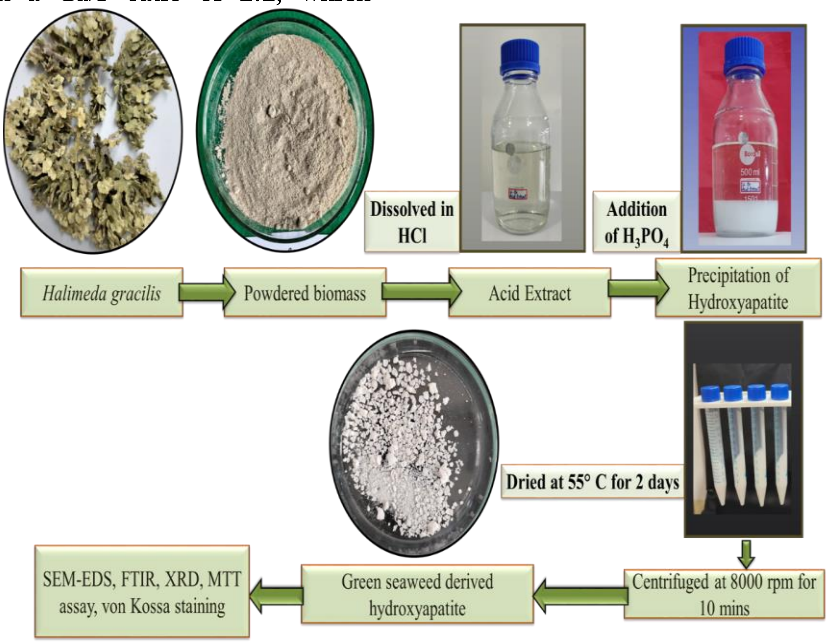

Fig. 1. Schematic Representation of the Extraction of Phyco-Oxyapatite from Marine Seaweed (Copyright Applied)

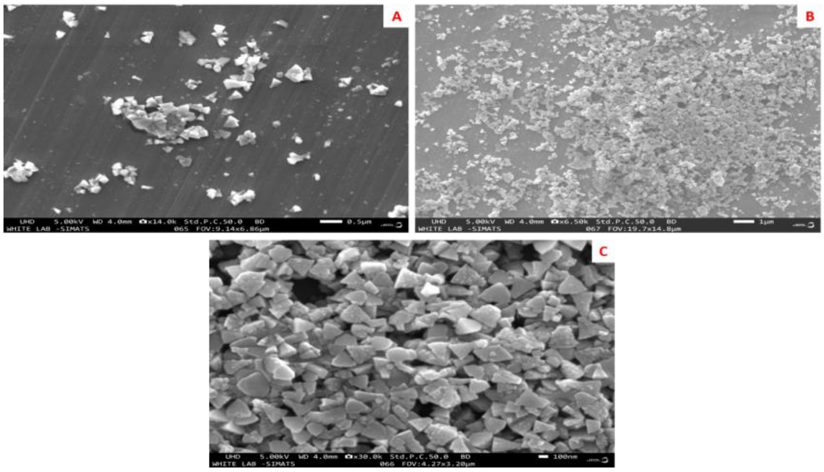

Fig. 2. SEM Images of Green Seaweed Derived HAp (A) Scale of 0.5 µm (B) Scale of 1 µm (C) Scale of 100 nm

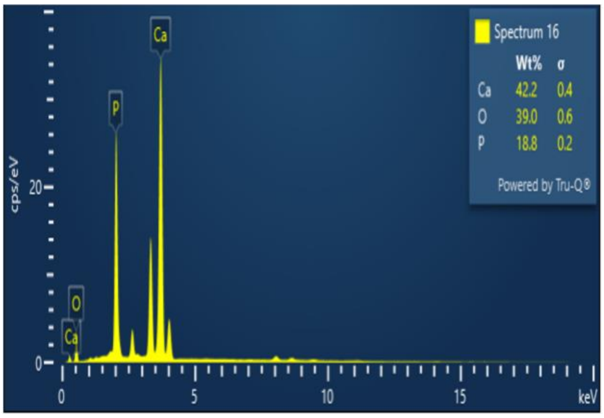

Table 1. Composition (wt.%) of element in green seaweed-derived hydroxyapatite

Spectrum 16		
Element	Ca/P ratio	Wt%
O	42.23/ 18.78 = 2.2	38.99
P		18.78
Ca		42.23
Total		100.00

Fig. 3. EDS Spectra of Precipitated Green Seaweed-Derived Hydroxyapatite

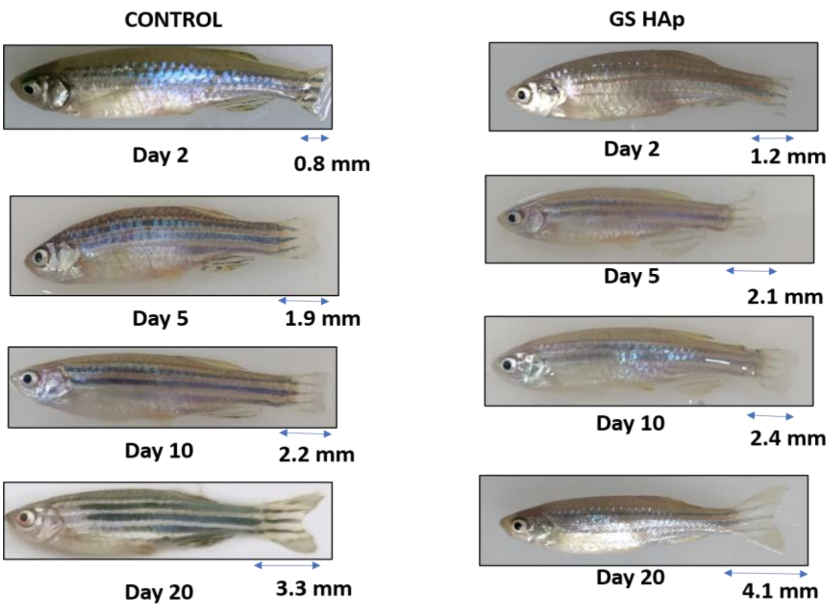

Fig. 4. Zebrafish Caudal Fin Development on the Phyco-Oxyapatite Derived from Marine Seaweed

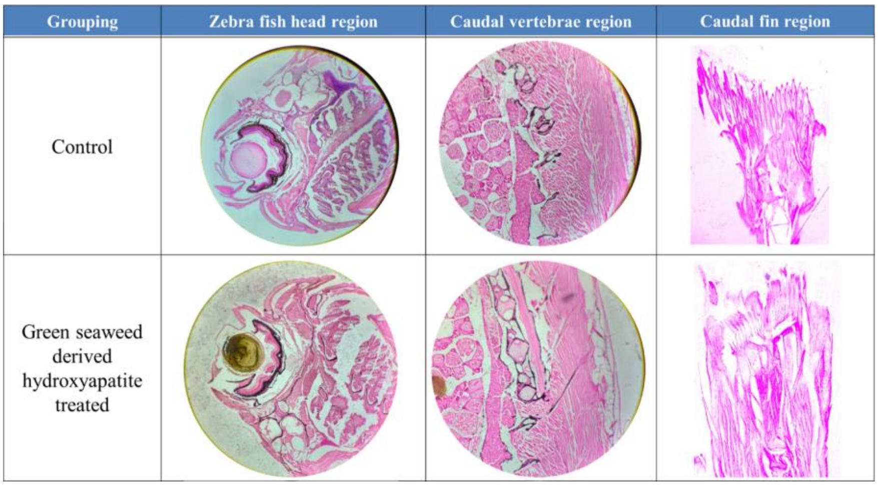

Fig. 5. Histopathological Sections of Zebrafish Showing Bio-Absorption of Calcium in Bones and Fins

Discussion

The results of this study provide compelling evidence that phyco-oxyapatite derived from *Halimeda sp.* offers distinct advantages over traditional hydroxyapatite sources in terms of biocompatibility, regenerative capacity, and sustainability [17, 18]. The in vivo experiments on zebrafish (*Danio rerio*) demonstrated that green seaweed-derived hydroxyapatite significantly improved caudal fin regeneration, indicating that this material possesses strong osteoconductive properties [19, 20]. By day 20, zebrafish treated with phyco-oxyapatite exhibited superior fin regeneration compared to control, underscoring the material's potential in bone tissue engineering [21].

The presence of bioactive trace elements, such as magnesium and strontium, within the phyco-oxyapatite may explain its enhanced regenerative performance [22, 23]. Magnesium plays a vital role in promoting osteoblast differentiation and mineralization, while strontium has been shown to improve bone density and strength by reducing osteoclast activity [24]. These trace elements, naturally found in seaweed, contribute to the superior bioactivity of phyco-oxyapatite compared to synthetic HAp, which often lacks these beneficial components [25, 26].

The structural analysis using SEM and EDS revealed that the phyco-oxyapatite particles had a tetrahedral shape with densely packed particles and interstitial voids [27]. This morphology is advantageous for bone tissue engineering, as the porosity of the material facilitates cell infiltration, vascularization, and nutrient transport, all of which are essential for effective bone regeneration [28]. Additionally, the Ca/P ratio of 2.2 observed in the extracted material aligns with the ideal ratio found in natural bone, further confirming the suitability of phyco-oxyapatite for biomedical applications [29].

One of the key advantages of phyco-oxyapatite is its origin from a renewable and sustainable source [30]. As concerns over the ethical use of animal-derived materials continue to grow, the development of biomaterials from marine sources offers a promising alternative. Seaweed, particularly *Halimeda sp.*, is abundant along the Indian coastline and can be harvested sustainably without harming marine ecosystems [8, 9].

However, while the findings of this study are promising, several limitations must be acknowledged. First, the study was conducted on zebrafish, a model organism that, although useful for preliminary research, may not fully replicate the complexities of human bone regeneration [19]. Further studies involving larger animal models, such as rodents or non-human primates, are necessary to confirm the efficacy of phyco-oxyapatite in more complex biological systems.

Additionally, the long-term stability and performance of phyco-oxyapatite in clinical settings remain to be explored [16, 17]. While the material demonstrated strong regenerative potential in short-term studies, its durability and integration over extended periods are critical factors that must be evaluated before it can be considered for widespread clinical use. Future research should focus on testing the material in more challenging environments, such as load-bearing bone grafts or dental implants, where mechanical stability is essential [28, 29].

The industrial scalability of phyco-oxyapatite production also warrants further investigation [21, 22]. While the extraction process described in this study is effective at a laboratory scale, scaling up the production to meet clinical demands will require optimization of the extraction and purification methods. Collaborations between academic institutions and industry could facilitate the development of cost-effective and efficient production processes, ensuring that phyco-oxyapatite becomes a viable option for large-scale biomedical applications [25, 26].

This study highlights the potential of phyco-oxyapatite as a sustainable, biocompatible, and osteoconductive biomaterial for bone tissue engineering [27, 28]. Its superior regenerative capabilities, combined with its ethical and environmental advantages, make it an attractive alternative to traditional bovine and synthetic hydroxyapatite [29, 30]. While further research is needed to confirm its clinical efficacy and industrial viability, the findings from this study represent a significant step forward in the development of seaweed-derived biomaterials for regenerative medicine.

Conclusion

The conclusion summarizes the key findings, emphasizing that hydroxyapatite derived from *Halimeda sp.* not only matches but surpasses traditional HAp in terms of biocompatibility and regenerative properties. The material's high calcium-to-phosphate ratio, combined with its trace element composition, makes it an ideal candidate for bone tissue engineering.

Future research should focus on scaling up the extraction process, testing the long-term stability of phyco-oxyapatite in clinical applications, and exploring its potential as a scaffold in tissue engineering.

Conflict of Interest

The authors declare that there is no conflict of interest.

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