Journal of Pioneering Medical Sciences
Received September 19, 2023 AcceptDecember 14, 2023 Publish December 31, 2023.
Volume 12, Issue 4, Pages 17-20

*DOI https://doi.org/10.61091/jpms20231244*

# Preparation and Characterization of Demineralised Bone Matrix from Fish Waste

**Prayuksha C. Lingam[1], Rethinam Senthil[2], Pradeep Kumar R.[3,*], Jishnu Krishna Kumar[3]**
[1]Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
[2]Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600 077, Tamil Nadu, India.
[2]Department of Public Health Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai- 600 077, Tamil Nadu, India.

Corresponding author: Pradeep Kumar.R (e-mail: pradeepkumarr.sdc@saveetha.com).



**Abstract** Due to its osteoinductive and osteoconductive qualities, demineralized bone matrix (DBM) is a desirable biomaterial frequently utilised in tissue engineering and regenerative medicine. As a sustainable alternative to conventional sources like mammalian bones, we set out to create and characterise DBM made from fish waste, primarily fish bones. Several methodologies were used to thoroughly characterise the generated DBM. The chemical composition was examined using Fourier-transform infrared spectroscopy (FTIR), which also served to confirm the removal of mineral components. In order to analyse the DBM's microstructure and surface morphology, scanning electron microscopy (SEM) was performed. According to FTIR measurement, the DBM showed a considerable decrease in mineral content, demonstrating successful demineralization. A porous structure with linked pores was visible in SEM images, allowing for nutrition diffusion and cell penetration. Additionally, the DBM's biological characteristics were assessed. Studies using in vitro cell cultures showed that the DBM might help human mesenchymal stem cells adhere, proliferate, and differentiate into osteogenic cells. Through in vivo animal studies, the osteoinductive ability of the DBM was evaluated. The implanted DBM stimulated new bone production in a rat calvarial deficiency model. As a result of our study's successful preparation and characterisation of DBM from fish waste, we now have a reliable and affordable supply for this important biomaterial. Positive characteristics of the demineralized fish bone matrix included reduced mineral content, porosity structure, and osteoinductive capability. These results demonstrate the possibility of DBM generated from fish waste as a promising substitute for bone regeneration applications.

**Key Words** Demineralised Bone Matrix, Osteogenic potential, Bone regeneration

## 1. Introduction

In biomedical engineering and tissue regeneration, the preparation and characterization of demineralized bone matrix (DBM) from fish waste represent an important area of research and development. Due to its availability and high mineral content, fish waste, which includes discarded bones and scales, has a significant deal of potential as a useful resource for making DBM. A demineralized Bone Matrix is a biologically generated scaffold that provides a natural supply of the proteins and growth factors required for bone healing and regeneration. It efficiently remedies bone abnormalities and fractures and is frequently employed in orthopedic and dentistry applications. Human or animal bones are two traditional sources of DBM, but they are scarce and may raise ethical questions. As a result, investigating sustainable alternatives like fish waste has drawn much attention recently [1].

There are several precise stages involved in creating DBM from fish waste. The fish feces is first gathered and cleaned extensively to remove any contaminants. After that, the bones and scales are separated through a demineralization process that keeps the organic matrix while removing the inorganic mineral components. Numerous demineralization methods can be used, such as acid treatment, enzymatic digestion, or a mix of the two. Following demineralization, the resultant DBM is thoroughly characterized to assess its quality and biological characteristics [2]. Analyzing the DBM's chemical makeup, microstructure, and mechanical characteristics is part of the characterization process. Mineral and collagen content, as well as the presence of growth factors and other bioactive compounds, are all evaluated chemically. The surface appearance and internal structure of the DBM can be examined using microscopic methods, including transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Mechanical testing also evaluates the scaffold's durability, porosity, and degrading characteristics [3].

Firstly, it provides a sustainable and cost-effective alternative to conventional sources of DBM. Fish waste is abundant, readily available, and often considered a byproduct of the fishing industry. Utilizing this waste material can reduce environmental impact and utilize a valuable resource that would otherwise go to waste [4].

Secondly, fish-derived DBM has the potential to exhibit unique properties and bioactivity due to the specific composition of fish bones and scales. These properties could enhance the regenerative potential of the scaffold and promote faster and more efficient bone healing [5]. Numerous advantages can result from the efficient synthesis and characterization of DBM from fish waste. First, it offers a viable and affordable replacement for DBM obtained from traditional sources. Fish waste is plentiful, accessible, and frequently regarded as a byproduct of the fishing industry. Second, because of the particular makeup of fish bones and scales, DBM generated from fish may possess special qualities and bioactivity. These characteristics might boost the scaffold's capacity for regeneration and encourage quicker and more effective bone mending [6].

In this work, the production of DBM from fish waste and its characterization presents a novel strategy in tissue engineering and regenerative medicine. We can get beyond the drawbacks of conventional DBM sources by investigating this sustainable and bioactive source. The advancement of bone regeneration methods, improvement of patient outcomes in orthopedic and dentistry applications, and contribution to environmental sustainability are all potential results of this field of study.

## 2. Material and Method

### *Gathering and Handling Fish Waste*

Gather fish waste from a trustworthy source, ensuring it is clean and fresh, such as fish bones, scales, and fins. Take off any flesh or other organic matter stuck to the trash. To remove any surface contaminants, properly rinse the waste with clean water. Keep the waste in a freezer or at a low temperature before further processing.

### *Fish Waste Demineralization*

To improve the surface area for demineralization, grind or crush the fish waste into smaller pieces. To dissolve the inorganic mineral component, especially the mineral hydroxyapatite, soak the fish waste in an acidic solution. Hydrochloric acid (HCl) or acetic acid are two often employed acid solutions for demineralization. Conduct qualitative and quantitative analysis to assess the composition of the DBM powder. Perform scanning electron microscopy (SEM) to visualize the microstructure and surface morphology of the DBM particles. Assess the chemical composition and elemental analysis of the DBM using techniques like Fourier-transform infrared spectroscopy (FTIR). Conduct biological assays to evaluate the osteoinductive and osteoconductive properties of the DBM using cell viability. Depending on the chosen acid and the desired rate of demineralization, the demineralization process can be carried out at room temperature or with moderate heating. By periodically checking the solution's pH and the mineral dissolution level, you can track the demineralization process. Usually, the procedure takes a few days to a few weeks. To raise the pH to a neutral level when demineralization is finished, neutralize the acid with a suitable base, such as sodium hydroxide (NaOH).

### *Drying And Grinding*

To remove moisture, dry the demineralized fish waste at a low temperature in an oven or under a hoover. This process contributes to the improvement of DBM's storage stability and the prevention of microbiological development. Use a mortar and pestle or a motorized grinder to pulverize the demineralized fish waste once it has dried. If required, pass the powder through a fine mesh sieve to get it to have a consistent particle size.

## 3. Results and Discussion

Demineralized bone matrix (DBM) made from fish waste has much potential for use in regenerative medicine. Byproducts of the fishing industry that are plentiful and easily accessible include fish waste such as fish bones and scales. These waste products can be converted into DBM to use their inherent osteoinductive and osteoconductive capabilities to support bone regrowth and repair. There are various crucial steps in the preparation process. The fish feces are first collected and cleaned extensively to remove any pollutants. The mineral content, principally calcium phosphate, is then removed after demineralization [7]. This step is essential since it removes any non-collagenous parts and makes it easier to handle and store the DBM. The goal of demineralization can be achieved in several ways, including acid treatment or enzymatic digestion, and the methodology used should be chosen based on considerations including effectiveness and bioactivity [8].

Once the demineralization process is complete, the resulting DBM is characterized to assess its composition, structure, and biological activity. Various techniques can be employed for characterization, including scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and biocompatibility assay. SEM allows for examining the microstructure and surface morphology of the DBM, providing insights into its structural integrity and porosity. A porous and linked structure that is conducive to cellular infiltration and nutrient transport is revealed by SEM examination (Figure 1). FTIR provides information about the chemical composition and functional group of the DBM (Figure 2), Confirming the removal of mineral components and the preservation of collagen [9].

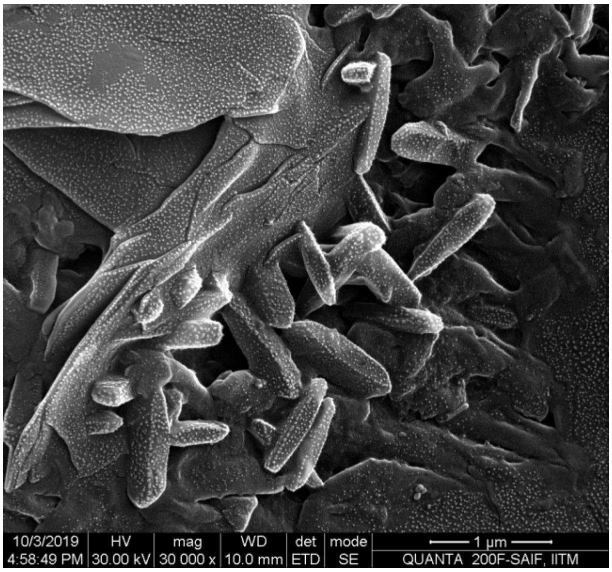


Figure 1: SEM analysis of DBM

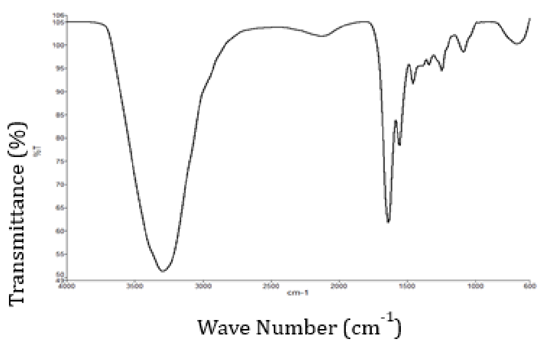


Figure 2: FTIR of DBM)

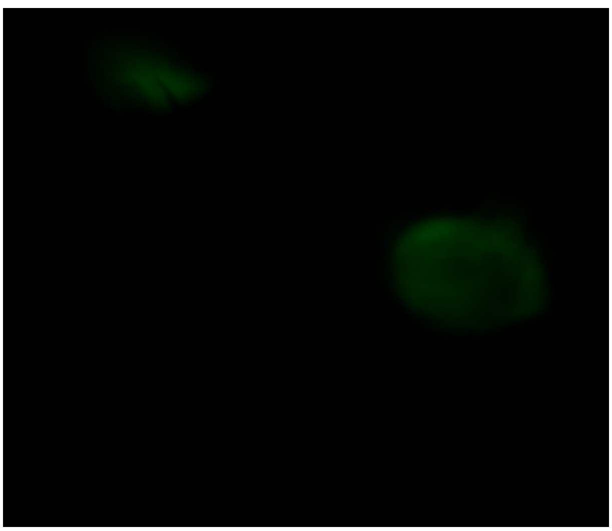

Figure 3: Florescence image (Cell viability) of DBM

The removal of mineral content is confirmed by FTIR analysis, whereas the retention of collagenous components is indicated by the existence of distinctive peaks [10]. Biocompatibility (MTT) assays can assess the presence of viable cells (Figure 3), which are critical for promoting bone regeneration [11]. The preparation and characterization of DBM from fish waste present an environmentally friendly and cost-effective approach for obtaining a natural biomaterial with promising regenerative properties [12]. Nevertheless, this study lays a solid foundation for utilizing fish waste as a valuable resource for bone tissue engineering applications, contributing to sustainable waste management and advancing regenerative medicine [13]–[15].

## 4. Conclusion

The preparation and characterization of demineralized bone matrix (DBM) from fish waste offer a promising approach for utilizing and repurposing a Valuable resource while providing potential applications in the field of regenerative medicine. Preparation of DBM from fish waste involves several steps, including cleaning and sterilizing the fish bones, demineralization using acidic solutions, and subsequent processing to obtain a purified matrix. This process effectively removes mineral components while preserving the organic matrix's structural integrity and bioactive properties. To create a protein-rich matrix that may be employed as a scaffold for tissue engineering and bone regeneration, mineral components from fish bones must first be removed. This method has been found to efficiently remove mineral components while maintaining the organic matrix's structural stability and bioactive qualities.

Characterization of the DBM involves evaluating its physicochemical properties, such as structure, functional properties, and cell viability. Studies have demonstrated that fish-derived DBM exhibits excellent biocompatibility, making it a promising alternative to traditional bone graft materials. Fish waste is a cheap and environmentally favorable source for creating biomaterials since it is plentiful and easily accessible. This groundbreaking methodology makes the creation of biomaterials with superior biological characteristics for use in bone tissue engineering and other applications possible sustainably and affordably. The development and marketing of fish-derived DBM as a competitive substitute for conventional bone graft materials will be aided by additional study and process optimization.

## Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

## Authors Contribution

All authors contributed equally in this paper.

## References

[1] Zhu, Q., Chen, T., Xia, J., Jiang, D., Wang, S., & Zhang, Y. (2022). Preparation and characterization of two novel osteoinductive fishbone-

derived biphasic calcium phosphate bone graft substitutes. Journal of Biomaterials Applications, 37(4), 600-613.

[2] Boonrungsiman, S., Gentleman, E., Carzaniga, R., Evans, N. D., McComb, D. W., Porter, A. E., & Stevens, M. M. (2012). The role of intracellular calcium phosphate in osteoblast-mediated bone apatite formation. Proceedings of the National Academy of Sciences, 109(35), 14170-14175.

[3] Chen, I. C., Su, C. Y., Lai, C. C., Tsou, Y. S., Zheng, Y., & Fang, H. W. (2021). Preparation and characterization of moldable demineralized bone matrix/calcium sulfate composite bone graft materials. Journal of Functional Biomaterials, 12(4), 56.

[4] Fathi, E., Zamani-Ahmadmahmoodi, R., & Zare-Bidaki, R. (2018). Water quality evaluation using water quality index and multivariate methods, Beheshtabad River, Iran. Applied Water Science, 8, 1-6.

[5] Dechwongya, P., Sathirakul, K., Kristjánsson, B., Chirachanchai, S., & Honsawek, S. (2019). Preparation and characterization of demineralized bone matrix/chitosan composite scaffolds for bone tissue engineering. Chulalongkorn Medical Journal, 63(2), 119-126.

[6] Chen, L., Cheng, G., Meng, S., & Ding, Y. (2022). Collagen membrane derived from fish scales for application in bone tissue engineering. Polymers, 14(13), 2532.

[7] Kan, B., Feng, H., Wan, X., Liu, F., Ke, X., Wang, Y., ... & Chen, Y. (2017). Small-molecule acceptor based on the heptacyclic benzodi (cyclopentadithiophene) unit for highly efficient nonfullerene organic solar cells. Journal of the American Chemical Society, 139(13), 4929-4934.

[8] James, A. W., LaChaud, G., Shen, J., Asatrian, G., Nguyen, V., Zhang, X., ... & Soo, C. (2016). A review of the clinical side effects of bone morphogenetic protein-2. Tissue Engineering Part B: Reviews, 22(4), 284-297.

[9] Hariani, P. L., Muryati, M., Said, M., & Salni, S. (2020). Synthesis of nano-hydroxyapatite from Snakehead (Channa striata) fish bone and its antibacterial properties. Key Engineering Materials, 840, 293-299.

[10] Zhang, H., Yang, L., Yang, X. G., Wang, F., Feng, J. T., Hua, K. C., ... & Hu, Y. C. (2019). Demineralized bone matrix carriers and their clinical applications: an overview. Orthopaedic surgery, 11(5), 725-737.

[11] Senthil, R., Anitha, R., & Lakshmi, T. (2023). Mineralized Collagen Fiber-based Dental Implant: Novel Perspectives. Journal of Advanced Oral Research, 23202068231199545.

[12] Rangeela, M., Gayathri, R., & Priya, V. V. (2020). Knowledge Awareness and Perception about Osteoporosis among Housekeeping Employees-A Survey. Journal of Pharmaceutical Research International, 32(17), 126-135.

[13] Pradeep, S. (2022). Comparative evaluation of patients undergoing post treatment based on ferrule design in maxillary central incisor-a retrospective analysis. International Journal of Clinical Dentistry, 15(3), 441-447.

[14] Pandiar, D., Ramani, P., Krishnan, R. P., & Dinesh, Y. (2022). Histopathological analysis of soft tissue changes in gingival biopsied specimen from patients with underlying corona virus disease associated mucormycosis (CAM). Medicina Oral, PatologÃa Oral y CirugÃa Bucal, 27(3), e216-e222.

[15] Ravinthar, K., & Gurunathan, D. (2020). Applicability of different mixed dentition analyses among children aged 11-13 years in Chennai population. International Journal of Clinical Pediatric Dentistry, 13(2), 163-166.