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EXTRACTION OF FLUORAPATITE FROM BRACHIOPADS FOR USE AS A DENTAL MATERIAL

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ABSTRACT

Hydroxyapatite and carbonated-hyrdroxyapatite have been commonly used for orthopedic and dental applications because their composition is similar to bones and teeth. They have the ability to be partially dissolved, and so allow cells to proliferate in their place. However, such property is not suitable for dental applications as it promotes teeth dissolution even when tooth enamel cells do not regenerate. Furthermore, much of the commercially available apatite is currently produced through chemical synthesis, making the product expensive and technology-dependent.

Fluorapatite is known to be the harder and more insoluble alternative. It promotes re-mineralization, and is said to have anti-microbial properties. In addition, the mineral occurs in certain brachiopod shells found in the country. They are called Balay in Visayas. Fluorapatite was successfully extracted and characterized from these locally available shells. Protein analysis yielded an excellent degree of organic matter removal, while FTIR and XRD verified the extraction and purity of the obtained fluorapatite powder. Pellets made through pressing and sintering showed that it is also formable and compatible with current dental material fabrication techniques. The sintered pellets passed the ISO standards for dissolution of dental restoratives. In addition, the compression loading test and Vickers indentation showed that the milling time played a role in the resulting mechanical properties. Thus, to bring out these desired properties, one only needed to optimize the fabrication parameters. This study proves the viability of fluroapatite from brachiopod shells as an alternative biomaterial.

1. RATIONALE AND SIGNIFICANCE

Apatites are calcium (Ca) orthophosphate (PO_4^{3-}) minerals closely resembling bone and teeth. Investigations revealed that they promote bone re-growth and possess good biocompatibility, leading to their use as bioceramic materials for orthopedic and dental applications. Currently, hydroxyapatites (HAp) ($Ca_{10}(PO_4)_6(OH)_2$) and carbonated-hydroxyapatites (C-HAp) have been utilized for the said purposes. While naturally occurring, these materials are mostly artificially prepared .

Fluorapatite (FAP) ($Ca_{10}(PO_4)_6(F)_2$), also a naturally occurring form of apatite, is known to be more stable and resistant to tooth cavity (caries) formation, making it more appropriate for dental applications. However, little recognition has been given to this mineral.

Literature shows that certain brachiopods--marine invertebrates - have shells composed mainly of fluorapatite. The said organism is found in the country and is currently harvested for food. Its shells are discarded after the meat is consumed. Though considered trash and of no

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value by locals, these shells are actually good sources of fluorapatite. If utilized properly, the fluorpatite from brachiopod shells will provide a better alternative to HAp and C-HAp in dental applications. Moreover, developing a proper method to utilize these shells will be value-adding to a locally available resource and the community.

2. OBJECTIVES

This study aims to determine the viability of seashell-extracted fluorapatite for dental applications. Among its goals include extracting fluorapatite from the brachiopod lingula unguis, known locally as *Balay* (Photo 1), characterizing it and testing its ability to be formed into different dental restorative products. Responses to hardness indentation, compressive load, and dissolution will also be evaluated.

3. PROBLEM STATEMENT AND DESCRIPTION

HAp and C-Hap have gained recognition for their close resemblance to bones and teeth. They are biocompatible, able to bond to natural body tissues. The minerals are non-toxic and are easily accepted by the body's immune system as normal biological components. For dental applications, they are used as restorative materials, including but not limited to, fillers, crowns, and jackets. Among their desirable properties is the ability to partially dissolve and allow cells to grow in their place (bioresorption); with C-HAp being more resorbable. However, the same cannot be said for dental applications. Given the oral cavity environment, an insoluble material is needed to maintain the integrity of the teeth structure. Furthermore, tooth enamel cells terminate when matured and do not regenerate, thus no longer requiring bioresorptive capacity.

Fluorapatite has properties similar to that of the aforementioned minerals. The fluoride ion stabilizes the structure, making it the hardest and least soluble among the calcium orthophosphates. Thus, fluorapatite may be the more suitable apatite for dental applications. If it dissolves, the mineral releases fluoride ions which inhibit the metabolism of microbes, decrease acid production and possibly kill them all together. The free fluoride ions can also promote re-mineralization of the tooth by reacting with the calcium and phosphate in the saliva, thus, reforming fluorapatite.

Currently, studies on the three have been mostly prepared through chemical synthesis. This makes it expensive, not to mention technology-dependent. While HAp and C-HAp can be extracted from animal remains and eggs, there have been no studies on extraction of fluorapatite from renewable resources. However, fluorapatite has been found in shells of certain brachiopod species which are available in the country. A utilization method to extract or synthesize fluorapatite from the shells will introduce a sustainable, cost-effective and better performing material for dental purposes.

4. METHODOLOGY

The study proceeded in three phases: (1) extraction of the fluroapatite, (2) fabrication of ceramic into dental material, and (3) characterization of the ceramic powder and evaluation of its behavior under mechanical stress and dissolution. Because this is an exploratory study, no optimization of parameters was done. In addition, while there was no aim to reach a certain benchmark, the results were nonetheless compared to a set of values whenever possible to evaluate the material's performance.

Balay shells were shipped from Bacolod, Negros Occidental. They were cleaned with water and bleach, and then crushed. The shells were separated into two batches of 500g each for de-proteinization. De-proteinization was done through alkaline hydrolysis of the organic matter using sodium hydroxide (NaOH), pressure and heat. The shells were "cooked" in 4.0 L of 2M sodium hydroxide (NaOH) solution to remove organic matter. For this process, the researchers used a pressure vessel for 6 hours, replacing the solution once after 3 hours (Photo 2). The de-proteinized shells were washed with distilled water and were sun-dried. Prior to ball milling, the de-proteinized shells were dried in an oven at 70^oC for 24 hours. The two batches were milled for 135 minutes and 270 minutes, respectively, to check the effect of particle size on the mechanical and dissolution performance. The milled powders were then calcined at 900^oC for 1 hour for added measure of de-proteinization.

Proximate protein analysis via Kjeldahl Method, Fourier-Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) was used to examine the calcined powder. The protein analysis measured the success of organic matter removal, and the FTIR and XRD identified the composition and the phases while assessing the purity of the obtained powder.

The powder was shaped into pellets using a load of 15MPa, and sintered at 1000 ^oC for 90 minutes. The pellets were then subjected to Vickers indentation using a load of 2kg for hardness testing and compression load testing. Lastly, a dissolution test based on ISO 4049: 2000 (E) was conducted.

5. RESULTS AND RECOMMENDATION

Upon calcination, the powder changed from white to light blue. According to literature, this is due to the oxidation of Mn in the powder as well as the accumulation of Mn in brachiopod shells. However, sintering the product caused the surface to turn back into white while the bulk remained the same. This may have been caused by a possible diffusion of Mn during the cooling down of the specimen. Characterization done to the calcined powder showed promising results. Proximate protein analysis yielded 0.52% protein content, confirming the removal of significant amounts of organic component.

FTIR transmittance spectrogram (Fig. 1) showed relatively few peaks, implying an inorganic substance. Detected peaks corresponded to PO_4^{3-}, F, CO_3^{2-} and OH bonds, all of which are present in apatite. Detected OH bonds were attributed to adsorbed molecule as opposed to chemical incorporation. The same may be said of CO_3^{2-}.

The obtained diffraction pattern (Fig. 2) was compared to the American Mineralogist Crystal Structure Database (AMCSD). The database yielded 90% match to several entries from the research. All these involved the fluorapatite mineral, but with differences in stoichiometry. It was noteworthy that some of the entries were of Mn doped fluorapatite, further raising the suspicion of Mn contamination.

Results of the mechanical and dissolution tests are seen in Table 1. Both Vickers indentation and compression testing yielded a similar trend, with the longer milled powders performing better. While the values are far below requirements, the research showed that current dental restorative material fabrication techniques were compatible with the obtained batches of powder. The shorter milled powder fared better in the dissolution testing with a mass loss of less than $1ug/mm^3$. Nonetheless both batches did not go over the $7.5ug/mm^3$ benchmark set by ISO.

The researchers recommend that studies be done on the optimization of the design parameter to bring out the full potential of the product. Research on the effects of the detected Mn content on the product behavior and the body must be taken up as well. Further experimentation on the product's compatibility with other current dental restorative techniques is also worth pursuing.

Figures

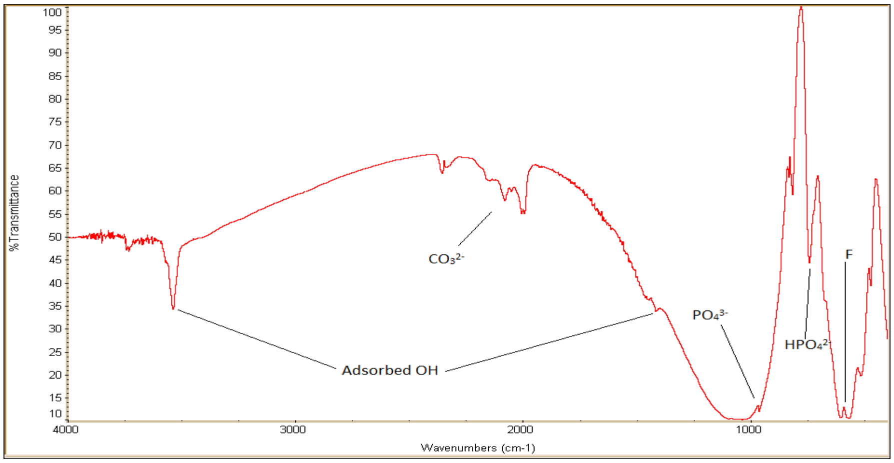

Figure 1: FTIR Spectogram

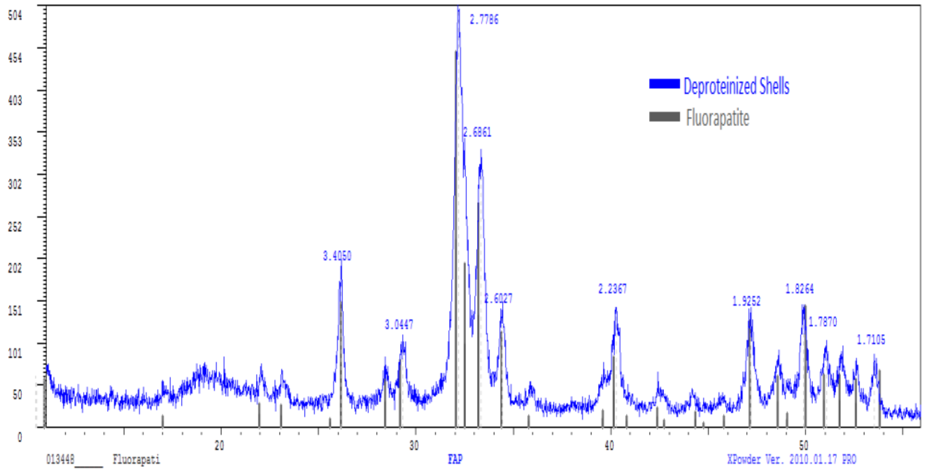

Figure 2: Deproteinized Shell vs Fluorapatite Diffraction Pattern

Tables

Table1: Mechanical Properties of Fabricated Fluorapatite Pellets

Mill Time (min)	Vickers Hardness (HV)	Hardness of Teeth (HV)	Max. Compressive Strength (MPa)	ISO Standard for Max Compressive Strength of Ceramic Enamel Restorative (MPa)	Mass Loss (ug/mm^3)	ISO Standard for dissolution (ug/mm^3)
135	33	300-400	0.013	50	0.80	7.5
270	42		0.017		3.04	

Photos

Photos 1a and 1b Brachiopod Lingula Unguis, known locally as Balay

Photo 2: Shells Deproteinized in Pressure Vessel for 6 hours

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