Property Test of Phosphate and Hydroxyl Groups from Lates Carcarifer Fish Scale as a Candidate for Synthetic Hydroxyapatite using the FTIR Method

Dian Agustin Wahjuningrum[1]*, Setyabudi[1], Anuj Bhardwaj[2,3], Syania Edinda Febriyanti[4], Nadia Liliani Soetjipta[5], Latief Mooduto[1]

1. Departement of Conservative Dentistry, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.
2. Department of Conservative Dentistry and Endodontics, College of Dental Science and hospital, Indore, India.
3. Adjunct Professor at Departement of Conservative Dentistry, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.
4. Undergraduate Student of Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.
5. Postgraduate Student of Departement of Conservative Dentistry, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia.

Abstract

In Indonesia, the market price of Synthetic hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ reaches 1.5 million per 5 milligrams and its availability still depends on imported products .To solve this problem, alternative materials can be used, such as Lates calcarifer fish scales. Phosphate $(PO_4)_3$- and hydroxyl (-OH) groups are functional groups of synthetic hydroxyapatites. The presence of phosphate $(PO_4)_3$- and hydroxyl (-OH) groups in the sample can indicate the presence of hydroxyapatite. Therefore, phosphate and hydroxyl groups can be used as test parameters for the property test. In this study, the phosphate and hydroxyl characteristics of the Lates calcarifer fish scales group were compared with the "ALDRICH" chemical synthetic hydroxyapatite group using the FTIR analysis method.

Objectives to explain whether the scales of Lates calcarifer fish can be used as a synthetic hydroxyapatite candidate and to analyze the characteristics of the phosphate and hydroxyl groups produced from Lates calcarifer fish scales.

FTIR or Fourier-transform Infrared Spectroscopy analysis method was used by comparing the peak image of the phosphate and hydroxyl groups between the Lates calcarifer fish scales group and the "ALDRICH" chemical synthetic hydroxyapatite control group on the wave ranges of 1350–1250, 1050–990, 1240–1190/995–850, 1100-1000 for phosphate groups and on the wave ranges of 3650-3600, 3570-3200, 3400-2400 for hydroxyl groups.

The Lates calcarifer fish scales group showed that the peaks of the phosphate $(PO_4)_3$- and hydroxyl (-OH) groups had the same wave range as the synthetic hydroxyapatite control group "ALDRICH", namely in the 850-995 cm-, 990-1050 cm- / 1100-1000 cm- , and 3200-3570 cm- wave ranges.

Lates calcarifer fish scales can be used as a candidate for synthetic hydroxyapatite.

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Introduction

Background

Synthetic hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ is one of the materials that is often used in biomedical applications as a bone substitution therapy or bone graft. Synthetic hydroxyapatite is biocompatible or has the ability to adapt to the body. Hydroxyapatite structure has regular porosity that is similar to natural structure of the bone tissue. The pores have an open structure and biocompatible surface which has ideal condition for cell growth and tissue differentiation. Synthetic hydroxyapatite also has bioactive properties that can bind to bone tissue and provide a specific biological response. these abilities can stimulate osteoblasts to form new bone tissue so that it can help in the process of bone regeneration.[1,2]

The use of HA as a bone substitute or replacement includes complete or partial bone augmentation, filling bones and teeth or coating in orthopedics and dental implants. [3] The use of

***Corresponding author:**
Dian Agustin Wahjuningrum,
Dental Medicine Faculty of Airlangga University
St. Mayjen Prof. Dr. Moestopo No. 47, Surabaya, Indonesia.
E-mail: dian-agustin-w@fkg.unair.ac.id

synthetic chemicals in the hydroxyapatite synthesis process has an impact on the price of hydroxyapatite. In Indonesia, the market price of hydroxyapatite reaches 1.5 million per 5 milligrams and its availability still depends on imported products.[4] To solve this problem, alternative materials can be used using natural materials by utilizing the richness of Indonesia's marine resources such as the scales of *Lates calcarifer* fish. *Lates calcarifer* fish is a leading marine cultivation commodity in Indonesia, whose market demand continues to increase every year, causing an increase in the residual fish waste such as *Lates calcarifer* scales. This can cause problems with the environmental balance. [5, 6] The utilization of *Lates calcarifer* fish scales is needed because in addition to reducing *Lates calcarifer* scales waste and as an effort to maintain environmental balance, it can also be utilized in the medical field, namely as a synthetic hydroxyapatite candidate. Phosphate $(PO_4)^{3-}$ and hydroxyl (-OH) groups are functional groups of synthetic hydroxyapatites. The presence of phosphate $(PO_4)^{3-}$ and hydroxyl (-OH) groups in the sample can indicate the presence of hydroxyapatite in the sample. Therefore, the phosphate and hydroxyl groups can be used as test parameters for the property test. [7] In this study, the phosphate and hydroxyl characteristics of the *Lates calcarifer* fish scales group were compared with the “ALDRICH” chemical synthetic hydroxyapatite group using the FTIR analysis method.

Objectives to explain whether the scales of *Lates calcarifer* fish can be used as a synthetic hydroxyapatite candidate and to analyze the characteristics of the phosphate and hydroxyl groups produced from *Lates calcarifer* fish scales.

Materials and methods

Research Samples

The research sample used was the scales of 6 months old *Lates Calcarifer*, obtained from the Muara Karang Fish Auction, Jakarta.

Research Methods

FTIR or Fourier-transform Infrared Spectroscopy analysis method was used by comparing the peak image of the phosphate and hydroxyl groups between the *Lates calcarifer* fish scales group and the "ALDRICH" chemical synthetic hydroxyapatite (Merck KGaA, Darmstadt, Germany) control group. There were three stages.

1. Sample preparation stage

The fish scales were washed with distilled water for debridement and then soaked in a surfactant solution. Re-washing was carried out using a shaker for 4-6 hours to clean the surface and dry grease, and then the fish scales were soaked in 1N NaOH solution and heated at 120°C for about 30 minutes. The HA sediment was separated from the degraded protein, and the sediment was purified in distilled water. HA was dried in an oven at 60°C to remove water and moisture from the powder and HA was sterilized by irradiation. After that, the powder sample was mixed with powdered potassium bromide (KBr), then pounded until smooth and mixed in a mortar agate. Then, the mixture was subjected to a high pressure of about 12,000 psi for 1-2 minutes. The ratio between sample powder and KBr is about 1: 100 to form a very homogeneous mixture of KBr. The resulting KBr mixture could be inserted into the FTIR spectroscopy holder.

2. Sample Handling Stage

Infrared light was directed at the sample or solid crystal with a high refractive index, at a certain angle. Then, the infrared rays contacted the ATR crystal and produced some internal reflection, which then created an evanescent wave that extended beyond the surface of the crystal. The sample that contacted the evanescent wave would absorb the wave energy and as a result, the evanescent wave would be attenuated. The attenuated light would be reflected on the crystal, and then went out to the opposite end of the crystal and was directed to the detector in the infrared spectrometer. The detector recorded the attenuated Infrared light as an interferogram signal, which could then be used to generate the Infrared spectrum.

3. FTIR spectrum analysis

FTIR (Fourier Transform Infrared Spectroscopy) is an instrument technique used to identify functional groups present in organic and inorganic compounds by measuring their absorption of infrared radiation in a certain wavelength range.[8] FTIR analysis is generally used to determine the characterization of the desired compound which is characterized by distinctive pattern bands. [9] The pattern of the absorption spectrum is like a fingerprint that identifies the molecule, which lends itself to both quantitative and qualitative analysis. [10]

FTIR analysis was used to determine the phosphate and hydroxyl functional groups based on the peaks which were displayed on the monitor screen originating from the light captured by the FTIR detector. The peaks were observed for the presence of phosphate groups, which can be analyzed from the sharp peaks in the FTIR spectroscopy pattern in 1350–1250 (Organic phosphates), 1050–990 (Aliphatic phosphates), 1240–1190/995–850 (Aromatic phosphates), and 1100-1000 (phosphate ions) wave ranges. The presence of hydroxyl groups can be analyzed from the sharp peaks in the FTIR spectrum pattern on 3650-3600 (free alcohols, phenols), 3570-3200 (H-bonded alcohols, H-bonded OH, phenols), and 3400-2400 (carboxylic acid) wave ranges. [11, 12]

Statistical methods

The Mann-Whitney comparison test was used to see the significance of the two groups, *Lates Calcarifer* group and the ALDRICH group. Based on the statistical test of the two groups, it was stated that the phosphate group test resulted in P = 0.27, and the hydroxyl group test resulted in P = 1.000 so that P> 0.05. Thus, it can be stated that there was an insignificant difference data in the phosphate and the hydroxyl group between the *Lates Calcarifer* scales and the ALDRICH group.

Results

In the *Lates calcarifer* scales group, there were peak images of the phosphate $(PO_4)^{3-}$ and hydroxyl (-OH) groups with the same wavelength ranges as the synthetic hydroxyapatite control group "ALDRICH", namely in 850-995 cm^{-}, 990-1050 cm^{-} / 1100-1000 cm^{-}, and 3200-3570 cm^{-} wavelength ranges. At the wavelength of λ = ~873 cm^{-}, the transmittance number of *Lates calcarifer* fish scales group was ~ 66% and in the "ALDRICH" group was ~50%. At the wavelength of λ =~ 990 cm, the transmittance number of *Lates calcarifer* fish scales group was ~63% and the "ALDRICH" group was ~ 42%. At the wavelength of λ =~ 1050 cm^{-}, the transmittance number of *Lates calcarifer* fish scales group was~ 39% and the "ALDRICH" group was ~ 23%. On the hydroxyl groups, the *Lates calcarifer* fish scales group had a peak image on the wavelength of λ =~ 3407 cm^{-}, and its transmittance number was ~66%. Meanwhile, the "ALDRICH" group had two peaks at the wavelength of λ = ~ 3352 cm^{-} with its transmittance number was ~ 45% and at the wavelength of λ = ~ 3570 cm- with its transmittance number was ~50%.

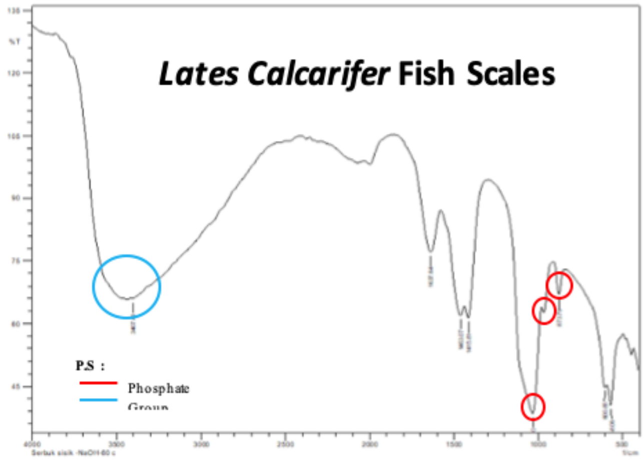

Figure 1. FTIR Spectrum of *Lates Calcarifer* Fish Scales Group.

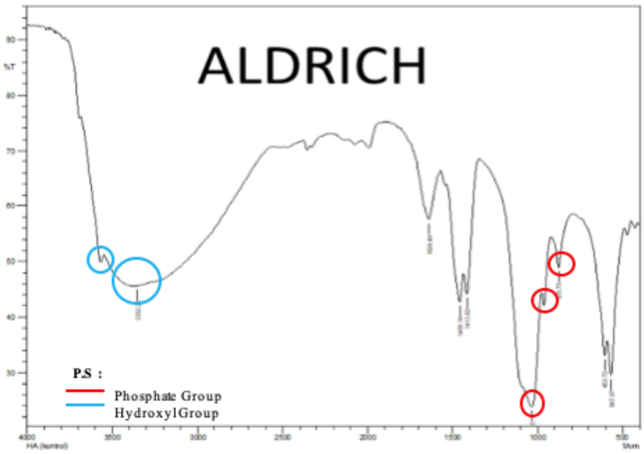

Figure 2. FTIR Spectrum of the synthetic hydroxyapatite control group "ALDRICH"(Merck KGaA, Darmstadt, Germany).

GROUP	PHOSPHATE		HYDROXYL	
	λ (cm^{-1})	Transmittance Number (%)	λ (cm^{-1})	Transmittance Number (%)
Lates Calcarifer Fish Scales	~873	~66	~3407	~66
	~990	~63		
	~1050	~39		
"Sigma ALDRICH" Synthetic Hydroxyapatite (Merck KGaA, Darmstadt, Germany)	~873	~50	~3352	~45
	~990	~42	~3570	~50
	~1050	~23		

Table 1. Comparison table of phosphate and hydroxyl peaks between the *Lates Calcarifer* fish scales group and the “ALDRICH” group.

Discussion

Synthetic hydroxyapatite is a mineral that can be derived from chemicals or natural materials commonly used for bone therapy or bone graft. Synthetic hydroxyapatite has the properties of osseointegration, osteoconduction, osteoinduction, and osteogenesis. Thus, it is said to meet the criteria as an ideal bone graft candidate. Synthetic hydroxyapatite has the chemical structure of $Ca_{10}(PO_4)_6(OH)_2$. The phosphate $(PO_4)^{3-}$ and hydroxyl (-OH) contained in synthetic hydroxyapatite have important roles in the process of osteogenesis or bone repair. [13] Phosphate $(PO_4)^{3-}$ and hydroxyl (-OH) groups are functional groups of synthetic hydroxyapatites. Therefore, the presence of phosphate $(PO_4)^{3-}$ and hydroxyl (-OH) groups in the sample can indicate the presence of hydroxyapatite in the sample. [7]

Based on the results of the FTIR spectrum, it is known that the *Lates calcarifer* scales contain phosphate and hydroxyl groups from the wave peaks in the resulting spectrum. The *Lates calcarifer* scales group had peaks present in the wavelength range of phosphate and hydroxyl groups which were the same as the "ALDRICH" control group, namely in the 850-995 cm- and 990-1050 cm-/ 1100-1000 cm- wavelength ranges, which indicated the presence of phosphate groups, and in the range of 3200-3570 cm-, which indicated the presence of a hydroxyl group. In the 850-995 cm- wavelength range, both the *Lates calcarifer* scales group and the "ALDRICH" control group had peak figures at λ =~ 873 cm- and λ =~ 990 cm-. In the *Lates calcarifer* group, the wavelength peak image was at λ =~873 cm- with its transmittance number = ~ 66% and wavelength peak image at λ =~990 cm- with transmittance number =~ 63%. Meanwhile, the control group "ALDRICH" had a slightly sharper peak image and lower transmittance number, in which at wavelength peak image λ =~ 873 cm- with its transmittance number =~ 50% and at λ =~990 cm- with its transmittance number =~ 42%. In the 990-1050 cm-/ 1100-1000 cm- wavelength range, both the fish scales group and the "ALDRICH" control group had a peak appearance at λ =~ 1050 cm. In the *Lates calcarifer* group, The wavelength peak image was at λ =~ 1050 cm- with its transmittance number =~ 39%, while the "ALDRICH" control group had a slightly sharper peak image and lower transmittance number of ~ 23% compared to the *Lates calcarifer* fish scale group.

The difference in the peak image of the phosphate groups between the two groups was slightly different with insignificant transmittance numbers. The frequency that passes through an organic compound or sample (which is not absorbed) will be measured as a percent of transmittance number. The higher the transmittance number means that the fewer infrared waves are absorbed by the compound or sample. Meanwhile, the lower the transmittance number or the sharper the wavelength peak, the higher the absorption intensity of infrared waves. [14] Thus, indicating that the content of chemical group constituents in a certain wavelength range will increase. Based on the results of the phosphate groups spectrum in the *Lates calcarifer* fish scales, they contained less phosphate than the "ALDRICH" group. The "ALDRICH" group contained ± 15% more phosphate content than the *Lates calcarifer* fish scales. According to the *Stanciu*, et al theory, the sharper the peak of the phosphate group $(PO_4)^{3-}$, the better the crystallinity growth of hydroxyapatite, which means that the better the hydroxyapatite is obtained. [15] So that based on the image of the phosphate group peaks in the "ALDRICH" group, it can be said to have a slightly better hydroxyapatite content than the *Lates calcarifer* fish scales.

The *Lates calcarifer* fish scales group had peaks present in the wavelength range of the hydroxyl group, which was the same as the control group "ALDRICH", namely in the wavelength range of 3200-3570 cm-. The fish scales group had a peak image at λ =~3407 cm- with a transmittance number =~ 66%, while the control group "ALDRICH" had two peak images at λ =~3352 cm- with a transmittance number =~ 45% and at λ =~ 3570 cm- with a transmittance number =~ 50%. Based on the results of the hydroxyl groups spectrum between the two groups, the peak image in the "ALDRICH" control group had a slightly sharper picture and a lower transmittance number compared to the *Lates calcarifer* fish scales. The "ALDRICH" group had two peaks in appearance while the *Lates calcarifer* fish scales group has only one peak. The sharper the peak of the hydroxyl group (-OH) shows a good level of crystallinity, which means that the more and better hydroxyapatite is obtained. [16] So that based on the content and the peak images of the hydroxyl group, the "ALDRICH" group had a better hydroxyapatite content because of more hydroxyl content that the absorption intensity of infrared waves was more than the *Lates calcarifer* fish scales group.

Conclusions

In conclusion, Based on the results of the analysis of the content of phosphate and hydroxyl groups using FTIR, it can be concluded that the *Lates calcarifer* scales can be used as a synthetic hydroxyapatite candidate because it contains hydroxyapatite as indicated by the presence of phosphate groups $(PO_4)^{3-}$ and hydroxyl (-OH) which had the same wave range with synthetic hydroxyapatite "ALDRICH" with a similar amount or less than the control group "ALDRICH", albeit not significant. However, the hydroxyapatite content produced from the "ALDRICH" group was better than that of *Lates calcarifer* scales.

Limitations

This study has a limitation, namely the property test carried out on *Lates calcarifer* scales was only the FTIR method. Therefore, it is advisable to do further research on: The property test of *Lates calcarifer* scales as synthetic hydroxyapatite candidates tested using other methods, such as XRD, TEM, and others, The effectiveness of *Lates calcarifer* scales on the bone or as synthetic hydroxyapatite, The toxicity test of *Lates calcarifer* in the body.

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Declaration of Interest

The authors declare that there are no conflicts of interest.

Ethical policy and institutional review board statement

Ethical clearance had been obtained from the Ethics Commission of the Faculty of Dental Medicine, Airlangga University, Surabaya (No.279/ HRECC.FODM / VI / 2020) on 11 June, 2020.

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