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Case Report

The Impact of Raman Spectroscopy on Concentration and Purity DNA Amelogenin from Dental Samples

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Abstract

Background: Analysis Laser by Raman spectroscopy is widely used to sex estimation of dental samples. Generally, sex estimation of dental samples can be identified by DNA amelogenin examination, but there is a lack of research on the impact of laser administration on concentration results and DNA purity in sex identification from dental samples. **Aim**: To determine the impact of laser application from Raman spectroscopy on the concentration and purity of DNA amelogenin for sex estimation. **Methods:** The tooth samples came from the same individual, with 1 right tooth and 1 left tooth. Samples were obtained from tooth extraction for orthodontic pretreatment. The tooth sample was cut horizontally on the occlusal surface to the tooth apex, then laser light was applied with a wavelength of 100-3400 cm^{-1}, on the enamel, dentin, and pulp chamber layers. The layers of teeth that had been shot with a laser were powder on each layer and DNA amelogenin analysis was performed. The total is 6 samples. **Results**: Examination of the concentration and purity of amelogenin DNA showed values between 1.57 – 1.87 µn, but the results of PCR analysis showed 1 of 6 did not have the same match in gender identification. **Conclusion**: This preliminary study showed that there was had impact of laser of Raman spectroscopy on the concentration and purity of DNA amelogenin from dental samples, and also there was an impact on gender determination.

1. Introduction

Laser spectroscopy studies provide the essential insight needed to gain insight into the atomic and molecular dimensional world. In particular, Raman spectroscopy is a powerful analytical method that provides detailed and specific information at the molecular level. In terms of its versatility, this method can provide information that maybe below the capabilities of other spectroscopic methods.[1] Raman spectroscopy has useful properties for forensic applications that

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identify body fluids (BFs) that non-invasively characterize a sample using light irradiation and highly selective spectral patterns based on the molecules that make up the sample. Detailed peak assignments show that the BF spectrum has a characteristic spectral pattern that can be interpreted with knowledge of the physiological components.[2] The use of Raman spectroscopy for biomedical applications, including dentistry, has increased significantly .[3] The research of Gamulin (2021) in dental aspect, concluded that sex examination of teeth with Raman spectrometric can be seen on the apex, dentin and enamel using a support vector machine (SVM) and artificial neural network (ANN) with a wavelength of 200-3500 cm−1.[4]

Amelogenin is involved in tooth development early in life. Previously known only from tooth enamel, amelogenin was also found in long bone cells.[5] Band readings in gender identification use amelogenin, according to Pfeiffer and Brenig (2005) if one band identifies the female gender and if two bands identify the male gender.[6] This is also affected based on the base length of bitter melon, male sex has two bands at ~ 1.3 kb and 1.5 kb, while for female gender there is only one band at 1.5 kb.[7] Based on this background, the uses of Laser from Raman Spectrocopy in dentistry can be in the form of sex examination of tooth samples[4], examination of the biological elements of ancient teeth[8], estimation of age from tooth samples[9], Spectrophotometric analysis of burnt tooth color can predict the feasibility of human DNA extraction for identification purposes.[10]

However, there is a lack of evaluation of the laser effect of Raman spectra on the purity of amelogenin DNA from dental samples. The purpose of this study was to determine the impact of laser application from Raman spectroscopy on the concentration and purity of DNA amelogenin for gender identification.

2. Material and Methods

Sample is Premolar teeth from male patient, after extracted tooth to orthodontic treatment. It is premolar right side and left side from lower jaw.

Sample Preparation

Teeth splitting (cutting horizontal), and doing examination on dental layer (enamel, dentin, and pulp). The total sample is 6 from the left and the right teeth.

Raman Spectroscopy

This analysis use Raman Microconfocal Spectrocopy with laser wavelength 100-3400 cm^{-1}, in Laboratory of Analysis and Instrumental, Department of Chemical Engineering, Gadjah Mada University. The function of analysis is to see the micro structural of hidroxyapatite from dental layer (enamel, dentin, and pulp).

Concentration and purity of DNA amelogenin

Analysis of Concentration and purity in Institute of Tropical Diesease Airlangga University

Artificial intelligence (AI)

The data from the Raman Spectroscopy examination were then analyzed using Cemometric. The usefulness of this method is to group a layer of tooth from enamel, dentin and dental pulp. The potential layer to identify gender using the clustering method with the Principal Component Analysis (PCA) and continued with multivariant analysis using artificial intelligence (AI) (K-Means and Pearson Correlation).

3. Result

The laser of Raman Spectroscopy was analysis in the layer of teeth such as enamel, dentin and pulp. Showed in figure 1.

Figure 1: The part of examination of Raman spectroscopy.

The analysis of Raman Microconfocal Spectrocopy showed the result of the raman imagine view. That dentin area was the left and the right teeth have similar condition of the 3D view. Showed in **figure 2**. The result of the spectra from each area sample, showed in **figure 3**. The graphic of spectra showed that pulp area had flat spectra and enamel area had some high spectra. It is mean the area

doesn't have correct similar graphic. But different with dentin area had normal.

The chemometric analysis (Principal component analysis) showed that dentin area had significant clustering area from left and right teeth. Showed in **figure 4.**

Figure 2: The result of the 3D view from different layer of dental sample (enamel, dentin and pulp).

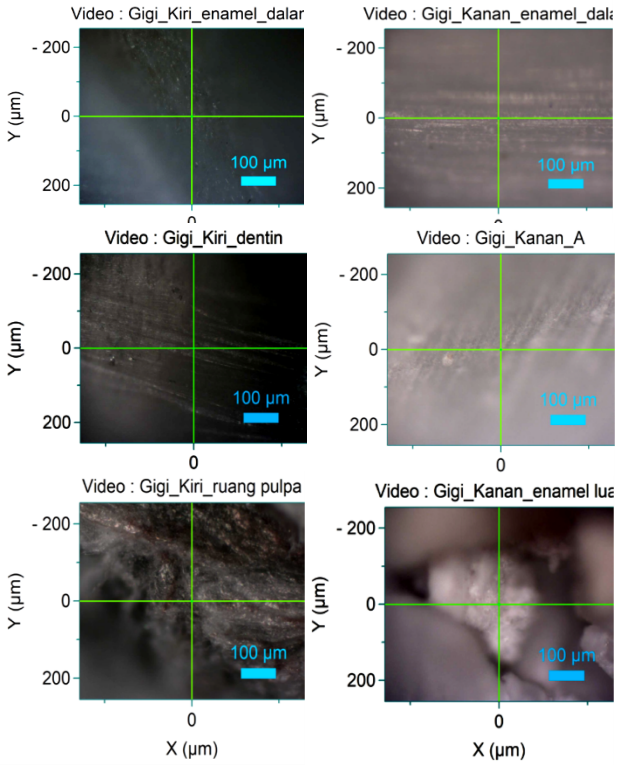
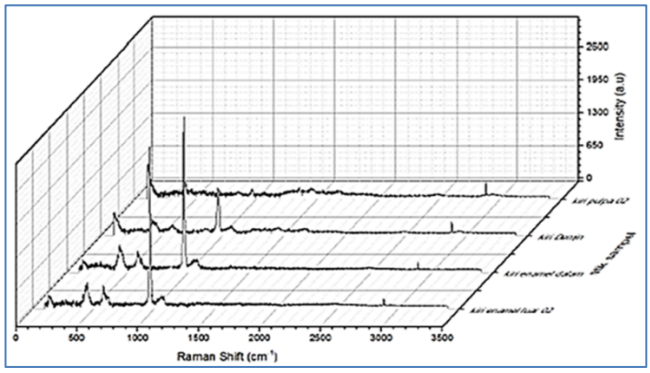
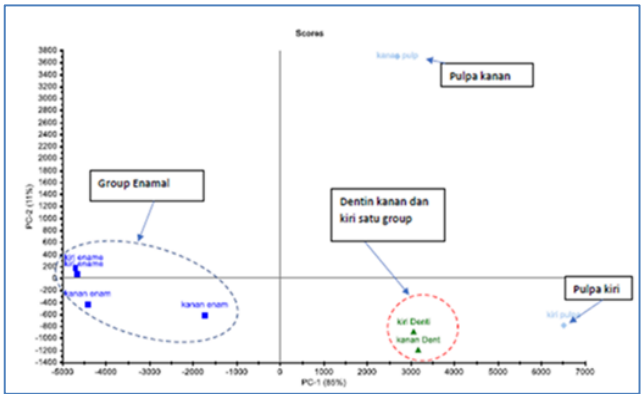


Figure 3: The result of spectra from Raman Microcnfocal spectroscopy.



Concentration of purity DNA showed two sample have less than normal standard. The ratio is appreciably lower (< 1.6). [11] A ratio of *1.8 is generally accepted as ''pure'' for DNA. Statistical analysis data showed that the correlation of Pearson had p > 0,05, it is mean the wavelength had correlation with layer of dental sample.

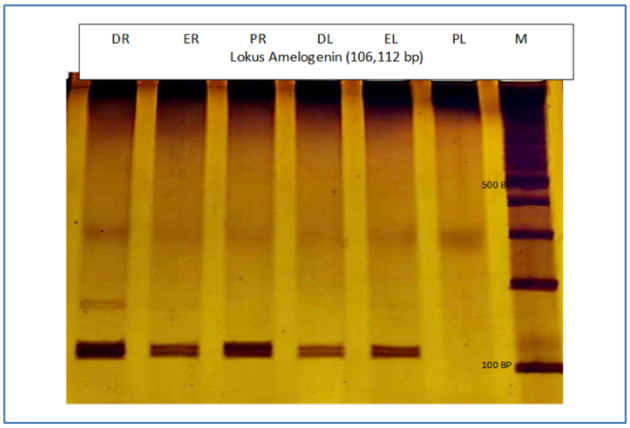
Figure 4: PCA analysis



4. Discussion

The effect of laser of Raman spectra with wavelength 100-3400 cm-1, in this research, shows side effects on dental samples, such as the concentration of purity to analysis DNA use ratio of absorbance at 260 and 280 nm is used to assess DNA purity, showed that area of the enamel layer, dentin left and pulp left was average concentration with of purity 1.83 - 1.87. Meanwhile, the sample of dentin left and pulp right had less accuracy of concentration which is 1.59 and 1.57.

Figure 5: The result of electrophoresis DNA Amelogenin



The results of DNA isolation are said to be pure if the absorbance ratio is at 1.8 – 2.0. The value of DNA purity was calculated by dividing the absorbance value at a wavelength of 260 nm by the absorbance value at a wavelength of 280 nm.[12]

In other conditions, the effect of low or normal DNA purity has an impact on the results of DNA electrophorensis analysis on Amelogenin DNA testing showed a negative result on the left pulp in **figure 5**, which is the dental sample from male patient. But the result of PL showed different thing.

Previous study Pfeiffer and Brenig (2005) if one band identifies the female gender and if two

bands identify the male gender.[6] This is also affected based on basepare length, male sex has two bands at ~1.3 kb and 1.5 kb, while for female gender there is only one band at 1.5 kb.[7]

De Luca (2014) showed the effect of laser of Raman Spectra use sperm sample [13], there was study on non-invasive sex assessment in bovine semen by Raman spectroscopy combined with PCA analysis had impact on quality DNA sperm. [14, 15]

In addition, Raman spectroscopy has detected oxidative DNA damage and mitochondrial damage caused by ultraviolet radiation.[15,16,17]

5. Conclusion

This preliminary study showed that there was had impact of laser of Raman spectroscopy on the concentration and purity of DNA amelogenin from dental samples, and also there was an impact on gender determination.

Contributor ship of Author: All authors equally contributed.

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