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Virgin Coconut Oil (Vco) As a Biomaterial in Regenerative Periodontal Therapy: Effects on Fgf-2 Expression

Hasanuddin Thahir[1*], Supiaty[1], Resky Ramadhani[2], Venda Novi Rianta[2], Davis Tunru[2]

[1]Department of Periodontology, Faculty of Dentistry,Universitas Hasanuddin Makassar, Indonesia
[2]Dental Hospital, Faculty of Dentistry, Universitas Hasanuddin Makassar, Indonesia

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ABSTRACT:
Background: Virgin Coconut Oil (VCO), derived from coconuts, is rich in beneficial properties, particularly in the treatment of periodontitis through its antibacterial and anti-inflammatory effects. Periodontitis is a destructive inflammatory disease affecting the supporting tissues of teeth, caused by specific microorganisms leading to destructive damage. In this context, Fibroblast Growth Factor-2 (FGF-2) is known to play a role in fibroblast migration coronally and enhance the proliferation of alveolar bone cells.
Objectives: This study aimed to assess the effectiveness of VCO gel on periodontitis-affected tissues by examining FGF-2 expression.
Methods: A post-test-only control group design was used for the laboratory experiment. VCO was obtained through coconut processing to produce oil and was further refined into a gel consistency expected to be beneficial in periodontitis management. The study samples were 24 male Wistar rats induced with period onto pathogenic bacteria P. gingivalis in gingival soft tissue. The sample was divided into 3 treatment groups, and observations were made on days 7 and 21. Immunohistochemical examinations were conducted on rat tissues, alongside radiographic assessments to compare bone mass density on days 7 and 21.
Results: The results showed that FGF-2 expression increased in all treatment groups, with the most significant rise observed in the group receiving the combination of SRP + VCO therapy on days 7 and 21.
Conclusions: VCO gel affected FGF-2 expression, showing its significant role in the inflammatory and proliferative stages of periodontitis healing.

1. Introduction

In the past few decades, biomaterials have marked significant advancements across various fields, including pharmaceuticals and medicine. These are substances designed to interact with biological tissues and are used for therapeutic and diagnostic purposes. Naturally derived biomaterials, biologically compatible with the human body, play a crucial role in supporting, replacing, and restoring the functions of damaged tissues [1]. Virgin Coconut Oil (VCO), derived from the Cocos nucifera plant prevalent in Southeast Asia, and islands around the Indian and Pacific Oceans, is known for its richness in lauric acid, polyphenols, and alpha-tocopherol. Furthermore, it possesses anti-oxidant, anti-thrombotic, hypolipidemic, antibacterial, antidermatophytic, antiviral, and immunostimulant properties [2]. The antibacterial properties are attributed to hydrophobic bacteriocins produced during fermentation with Lactic Acid Bacteria (LAB) [3]. Periodontitis is a chronic multifactorial inflammatory disease associated with the accumulation of dental plaque or biofilm, characterized by progressive damage to supporting dental tissues, including the periodontal ligament and alveolar bone. This disease entails dynamic and complex interactions among specific pathogenic bacteria, host immune responses, and environmental factors such as smoking. Common features of periodontitis include gingival inflammation, loss of clinical attachment, radiographic evidence of bone damage, significant probing depth, bleeding on probing, and pathological migration [4]. According to the FDI World Dental Federation in 2015, some countries worldwide had a high prevalence of periodontitis in 2010, with Australia and nearly all regions in Indonesia exceeding 15%. In 2018, Riskesdas data showed a 67.8% prevalence among the Indonesian population aged 15 years, suggesting that 7 out of 10 citizens were affected by the condition [5]. It is important to acknowledge that periodontal tissue regeneration is the goal of periodontal therapy [6]. Wound healing is a

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complex and dynamic process comprising 4 phases, namely hemostasis, inflammation, proliferation, and remodeling. Previous studies have shown the efficacy of VCO in wound healing compared to some commonly used standard drugs [7]. The regeneration of lost structures during periodontal disease is a complex biological process regulated by the interaction of cells and growth factors [8]. For over 20 years, various methods have been explored to achieve perfect restoration of both the structure and function of periodontal tissues. In this regard, biological mediators that regulate cell migration, proliferation, and attachment, such as growth factors, have been recently developed. Growth factors are key regulators of cellular events crucial for tissue repair by binding to specific cell surface receptors [10]. Neovascularization is essential to provide nutrition to damaged tissues and contribute to maintaining granulation tissue. Angiogenesis, the formation of new blood vessels, is associated with various molecules, including FGF, VEGF, and TGF-β [8]. Basic Fibroblast Growth Factor (FGF-2) belongs to the FGF family and plays a crucial role in tissue regeneration by promoting angiogenesis. Moreover, it can enhance the expression of osteogenic and mineralization markers, thereby facilitating bone regeneration [11]. Furthermore, it stimulates various cellular functions, induces strong proliferation and migration responses, as well as regulates the production of extracellular matrix by the periodontal ligament [12]. Previous studies explored the effectiveness of VCO in wound healing on the skin. However, reports on the impact of VCO on the expression of several growth factors in periodontal tissue healing remain limited. Therefore, this study aimed to investigate the effect of VCO application as an alternative therapy for periodontitis on tissue healing in periodontal disease by analyzing the expression of FGF-2 in *P.* gingivalis-induced Rattus norvegicus.

2. Method

A post-test-only control group design was adopted for the laboratory equipment, and the process commenced by preparing VCO in the Biology Laboratory of Universitas Negeri Makassar. Subsequent experimental procedures on laboratory animals were conducted at Docpet Makassar Animal Clinic. All study activities strictly adhered to the ethics issued by the Health Research Ethics Commission of Hasanuddin University Dental and Oral Hospital with No. 0136/PL.09/KEPK FKG-RSGM UNHAS/2023. VCO was produced from pure coconuts processed conventionally without heating and the addition of chemicals until oil was formed. The next step was the production of its Gel by mixing 1% NaCMC with 300 ml of distilled water, alongside other ingredients, until a homogenous mixture was achieved using a homogenizer. The study comprised Rattus norvegicus or Wistar rats, subjected to adaptation until ready for treatment. Inclusion criteria comprised a body weight of 200-250 grams, an age of approximately 6-8 months, male gender, as well as display of normal behavior and activity. Exclusion criteria included a 10% weight loss following the adaptation period. Wistar rats were induced using *P. gingivalis* periodontopathogenic bacteria, and silk ligature was inserted to modulate periodontitis in the anterior region of the lower jaw. The rats, divided into three groups, each receiving different treatments, were all given Scaling and Root planning (SRP) therapy. However, the second and third groups received additional VCO and Metronidazole gel application, respectively. On days 7 and 21, tissue sacrifice and bone density examination were performed on the samples. Two-dimensional radiographic analyses were conducted on each sample to compare bone density at the 2 observation times. Tissue sacrifice was performed after each rat was euthanized to facilitate tissue sample retrieval. Each tissue was prepared on microscope slides for Immunohistochemical examination to assess FGF-2 expression. The obtained data were tested for normality using the Kolmogorov-Smirnov test, followed by a homogeneity assessment using Levene's test. Differences in FGF-2 expression among the groups were then analyzed using the One-Way ANOVA test.

3. Results

Observations were made on 24 samples of Wistar rats induced with periodontitis using *P. gingivalis* bacteria and the installation of silk ligatures. After 7 days of bacterial induction, pockets and hyperemia formed on the gingiva. Treatment commenced on this day across all 3 groups, and observations were conducted on days 7 and 21. From the 7th day after periodontitis formation, initial therapy was administered in the form of SRP. The negative control group received SRP treatment only. Meanwhile, the positive control and the treatment group received SRP + Metronidazole as well as SRP + VCO gel, respectively, every day until the 7th day, before being sacrificed. Half of the total sample number was also given the same treatment until the 21st day before being sacrificed.

On days 7 and 21, each sacrificed sample was subjected to examination of bone density through two-dimensional radiographic analysis. Subsequently, immunohistochemical examinations were performed to observe FGF-2 expression in each group across different observation periods.

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Bone Density Analysis

Analysis of bone mass density was conducted to assess differences among the 3 samples. The two-dimensional radiographic examination, representing the radiographic image on day 7, showed that the SRP + VCO treatment group had more radioopaque results compared to the others.

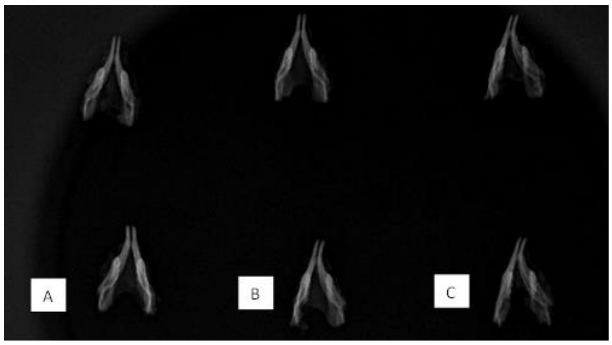

Figure 1. Radiographic image of samples from each group on day 7 for bone density analysis. A. Negative control group (SRP), B. Treatment group (SRP+VCO), C. Positive control group (SRP+Metronidazole) Density analysis on day 21 did not show differences in opacity among the 3 groups. This could be due to superimposed imaging, making it difficult to assess differences in bone density in each sample.

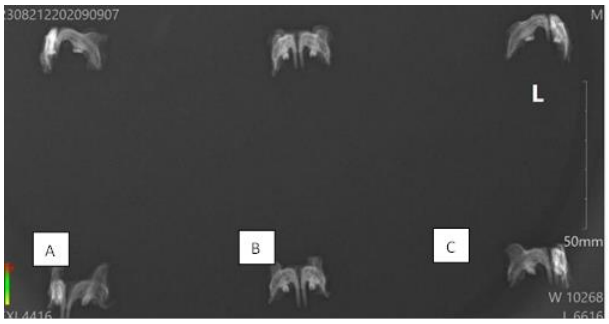

Figure 2. Radiographic image of samples from each group on day 21 for bone density analysis. A. Negative control group (SRP), B. Treatment group (SRP+VCO), C. Positive control group (SRP+Metronidazole).

FGF-2 Expression

The analysis of FGF-2 expression was conducted through immunohistochemical examination, with observations made on days 7 and 21 across 3 sample groups. The results of these examinations are summarized in Table 1, which shows the levels of FGF-2 expression in male Wistar rats induced with periodontitis. In the negative control group, there was no significant increase ($p > 0.05$) between days 7 and 21. Meanwhile, in the positive control and the treatment groups, a significant increase was detected ($p < 0.05$). Figure 3 shows the results of the sample examination with immunohistochemical staining using FGF-2 antibody at a magnification of 1000x. In Figure 4, a bar graph shows the variation in FGF-2 expression across each experimental group. All 3 groups showed an increase, but a significant rise was observed among animals receiving a combination of SRP and VCO. Despite an increase in expression observed in the combination of SRP and Metronidazole, it was not substantial compared to the SRP+VCO group. In the negative control, which was only given SRP therapy, there was an insignificant increase in expression. The bar graph in Figure 5 shows the increase in FGF-2 expression on days 7 and 21 within each group. On day 7, the elevated expression indicates an inflammatory response to periodontitis. The treatment group (SRP+VCO) showed relatively high results compared to the others, both on day 7 and 21. This suggests that the application of VCO gel plays a role in the improvement of periodontitis during the inflammatory and proliferative stages of tissue healing.

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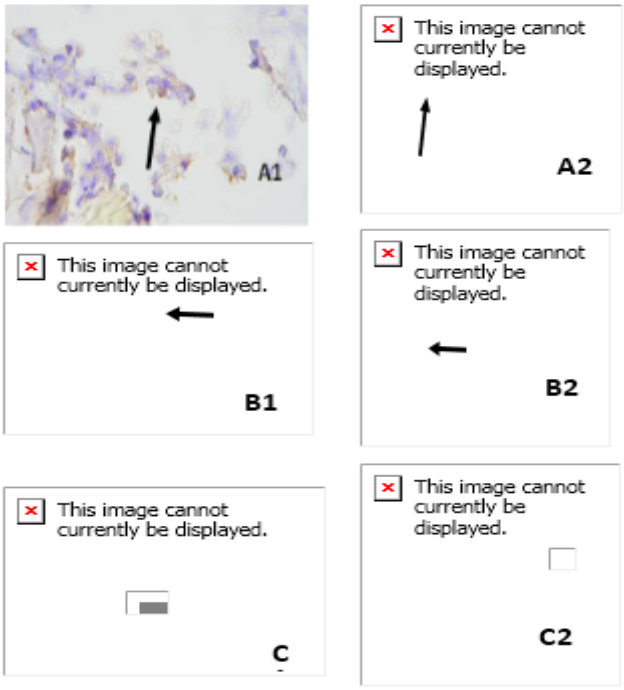

Figure 3. The results of the immunohistochemical examination showed the expression of FGF-2 in each sample group and observation time. A1. Negative Control Group (SRP) observed on Day 7. A2. A negative control group (SRP) was observed on Day 21. B1. The treatment group (SRP+VCO) was observed on Day 7. B2. The treatment group (SRP+VCO) was observed on Day 21. C1. Positive Control Group (SRP+Metronidazole) observed on Day 7. C2. Positive Control Group (SRP+Metronidazole) observed on Day 21.

Table 1. Mean values of FGF-2 expression on Day 7 and 21 in each treatment group.

Group	Observation time	Total Sample	Mean ± SD	P
Negative Control (SRP)	Day-7	5	5.20 ± 1.30	0.160
	Day-21	5	6.40± 1.14	
Positive Control (SRP+Metronidazole)	Day-7	5	6.60± 1.14	0.005
	Day-21	5	10.20± 1.92	
Treatment (SRP+ VCO)	Day-7	5	9.40 ± 1.14	0.007
	Day-21	5	13.40 ±2.07	

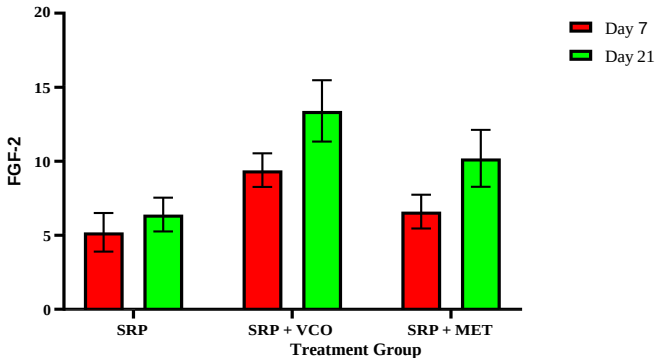

Figure 4. The increase in FGF-2 expression in each experimental group.

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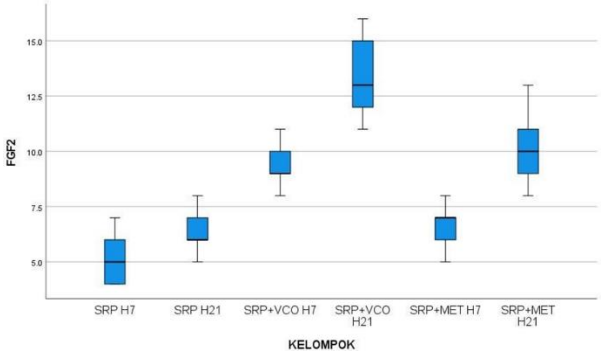

Figure 5. FGF-2 expression on Day 7 and Day 21.

4. Discussion

Periodontitis is a multifactorial inflammatory disease characterized by the destruction of both soft and hard tissues around the teeth. The inflammatory response associated with periodontal disease is closely correlated with an increased prevalence of specific bacteria. These bacteria instigate the recruitment of inflammatory immune cells aimed at eliminating the pathogenic bacterial population [13]. Furthermore, extensive evidence indicates that P. gingivalis, a gram-negative anaerobic bacterium, plays a key role in the development of chronic periodontitis [14].

The primary objective of periodontal therapy is to achieve simultaneous regeneration of alveolar bone, cementum, and lost periodontal soft tissues. However, current treatment options such as SRP, periodontal debridement, and periodontal tissue regeneration, applied either separately or in combination, have limited success in inhibiting periodontitis. With the advancement of periodontal tissue engineering and the development of extensively studied biomaterials, new strategies are being explored to regenerate damaged periodontal [15]. VCO, rich in various active components, has been identified as a promising therapeutic agent in periodontal tissue healing. This was supported by studies reporting its potential to accelerate the healing process [16]. In this study, radiographic examination was performed to assess bone density on Day 7 and Day 21 of observation. The results showed differences in opacity on Day 7 within the SRP+VCO group, where a more radioopaque image of bone was observed compared to the others. However, on Day 21, no differences were observed among the 3 groups. Hayatullina et al. conducted a study evaluating the effect of VCO supplementation on preventing bone loss in rats. The results showed that the supplementation yielded better results on bone microarchitecture compared to treatment with calcium alone. This could be attributed to the high content of medium-chain triglycerides, essential for calcium absorption from the intestines [17] [18]. This study shows an increase in FGF-2 expression in the group that received SRP+VCO treatment on Day 7 and 21 compared to others. The wound healing process consists of 4 interconnecting phases, including hemostasis and coagulation, inflammation, proliferation, remodeling, as well as wound maturation. The general principles of wound healing are also applicable in the context of periodontal [19]. Furthermore, the proliferation phase occurs from 3 days to 2 weeks after tissue injury. Angiogenesis is a complex process of growing new blood vessels from existing vessels. This process begins early in the healing process, with platelets releasing several growth factors, including FGF-2, to stimulate tissue regeneration [20]. This rationale guided the selection of observation periods of 7 and 21 days, during which tissue proliferation takes place. FGF-2, an essential growth factor, serves as a mitogen influencing angiogenesis and inducing differentiation stimuli for mesodermal cells [21]. Furthermore, it shows strong angiogenic activity and mitogenic capabilities in mesenchymal cells. Previous studies have presented the efficacy of FGF-2 in regenerating periodontal tissues, particularly in artificially created defects in the periodontal tissues of beagle dogs [22]. FGF-2 is a growth factor family that binds to heparin with various physiological actions and is considered to be included in the early stages of the wound-healing process [23].

VCO, based on the fatty acid content, falls into the category of lauric acid oil. This is because it has the highest content compared to other fatty acids. The constituents, including Myristic Acid (16-21%) and Lauric Acid content (43-53%), have demonstrated effectiveness in killing and weakening P. gingivalis bacteria. Additionally, VCO can reduce transudate formation, granuloma formation, and alkaline phosphatase serum activity [24].

The mechanism of increased expression in this study was in line with Shaofeng et al., who investigated the effect of FGF-2 on the growth and osteoblastic phenotype of hPDLC with or without osteogenic inducers, namely dexamethasone and β-glycerophosphate. The results

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showed a significant increase in FGF-2 during hDPLC proliferation [15].

Sunil et al. also mentioned that components discovered in VCO, such as polyphenols, are proven to inhibit the expression of pro-inflammatory molecules. The positive effects of this biomaterial are attributed to its capacity to mediate the modulation of the immune system through regulation by the neuroendocrine system. The thymus, a primary lymphoid organ, is crucial for producing various subsets of T cells to maintain immunocompetence. Meanwhile, lymph nodes and secondary lymphoid organs, are essential for immunosurveillance. Sympathetic noradrenergic innervation in lymphoid organs, mediated by norepinephrine release, regulates thymopoiesis and immune responses. Growth factors and pro/anti-oxidant status within these lymphoid organs are crucial for the survival of sympathetic neurons and modulation of the immune system response. Finally, this mechanism may also play a role in periodontal tissue healing [25].

5. Conclusion

In conclusion, the application of VCO gel in a periodontal defect showed excellent outcomes, as evidenced by the expression of FGF-2 as a marker of periodontal tissue regeneration

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