ORIGINAL ARTICLE

# Wound Healing Effects of Nanotransfersome Gel of Lime Peel Extract (*Citrus aurantifolia* Swigle) on Mucosa Labial Ulcer of Wistar Rats

Nenny Prasetyaningrum[1], Diena Fuadiyah[1], Khusnul Munika Listari[2], Qaulan Hadits Tsaqila[3], Sonia Priscilla Zaluchu[3], Sofi Isna Marwati[3]

[1] Department of Oral Biology, Faculty of Dentistry, Brawijaya University, Malang 65145, Indonesia.
[2] Department of Periodontology, Faculty of Dentistry, Brawijaya University, Malang 65145, Indonesia
[3] Dentistry Education Program, Faculty of Dentistry, Brawijaya University, Malang 65145, Indonesia

**ABSTRACT**

**Introduction:** The ulcer wound healing process is controlled by several growth factors, including FGF and VEGF. Quercetin, the major flavonoid in lime peel has an anti-inflammatory that stimulates macrophages to produce cytokines which can accelerate the wound healing process. Transfersome gel is one of the nano-drug delivery systems, it is biocompatible, and could deliver drugs through the small gap between cells. This study aimed to determine the effect of nanotransfersomes gel of lime peel extract on the expression of FGF and VEGF by macrophage in wound healing ulcers. **Methods:** Thirty-two Wistar rats were induced by heat in the lower labials, divided into four groups with 2-time series: the untreated group, the group with lime peel extract gel, nanotransfersome gel with lime peel extract, and aloe vera-based herbal gel. The ulcer area in the treatment was applied twice a day until the third and seventh day. Identification of macrophages expressing FGF and VEGF using immunohistochemical staining. **Results:** The results of the data analysis with One Way ANOVA showed a significant difference in the amount of FGF and VEGF expression in each group ($p<0.05$) with a significant and very strong correlation and the highest average was on the third day and the lowest on the seventh day in the nanotransfersome gel group lime peel extract. **Conclusion:** The study concludes that there is an effect of nanotransfersome gel of lime peel extract on increasing FGF and VEGF expressed by macrophages on the healing of labial mucosal wounds of Wistar rats.

**Keywords:** FGF; Lime peel; VEGF; Nanotransfersome gel; Wound healing

**Corresponding Author:**
Nenny Prasetyaningrum, M.Ked
Email: n3ny.fk@ub.ac.id
Tel: +(0341) 576161

## INTRODUCTION

Oral ulceration is common in adults causing pain and discomfort to the patient.[1] An Ulcer is a pathological condition in which epithelial tissue disappears due to the exfoliation of necrotic tissue that has extended to the underlying lamina propria. One of the causes of ulcers is trauma, which is called a traumatic ulcer. Oral mucosal injury is caused by physical, chemical, or thermal trauma. Typically, the traumatic ulcer is a single, oval, sunken ulcer with a yellow-gray or gray-white center with an erythematous border with irregular edges. [2,3] Wound healing in ulcers can take a long time due to the influence of salivary conditions and microorganisms that cause a secondary infection. Therapy of oral mucosal wounds such as traumatic ulcers is given with anti-inflammatory and antiseptic drugs, even though this treatment has not been able to stimulate growth factors to accelerate the healing process. Growth factors are proteins released by immune cells to initiate oral wound healing. [4]

The wound healing process is a complex process that is divided into several interrelated phases, including coagulation, inflammation, epithelialization, formation of granulation tissue, and tissue remodeling. Prolonged inflammation can lead to longer wound healing. [5] The wound healing process is controlled by several growth factors and cytokines released at the wound site. Several growth factors expressed by macrophages play an important role in proliferation, angiogenesis, and extracellular matrix synthesis. Macrophages show two phenotypes, namely M1 macrophages which are activated in the inflammatory

stage of wound healing, and mainly secrete proinflammatory factors (TNFα, IL-6, IL-1β, etc) and M2 macrophages are anti-inflammatory macrophages that secrete anti-inflammatory cytokines (IL-4, IL10, IL-12, etc.), growth factor (IGF-1, VEGF, TGF, FGF, etc.). Polarization from macrophage M1 to M2 phenotype is required in the wound healing process.[6-8] FGF's mitogenic and angiogenic properties can induce tissue remodeling, wound healing, and neovascularization. The role of VEGF in the wound healing process by stimulating the formation of granulation tissue, re-epithelialization, and angiogenesis by stimulating endothelial cells to proliferate and form new blood vessels (neovascularization). [7,8]

Lime (Citrus aurantifolia Swingle) is one of the toga plants used in the community, both for cooking spices and medicine from the juice of the lime juice. For medicine, lime is used as an appetite enhancer, fever reducer (antipyretic), diarrhea, slimming, anti-inflammatory, and antibacterial. The phytochemical constituents of C. aurantifolia Swingle extract of peel and leaves identified bioactive compounds including flavonoids, limonoids, coumarins, and phytosterols. In particular, flavonoids identified in C. aurantifolia extract (rutin, apigenin, quercetin, kaempferol, nobiletin, tangeretin, and hesperidin) are known for their antioxidant and anti-inflammatory properties. [9,10] As an anti-inflammatory, flavonoids can later stimulate macrophages to produce growth factors in the inflammatory and proliferative phase of the wound healing process. Quercetin, one of the flavonoid compounds, is known to improve the wound healing process by increasing fibroblast proliferation while reducing fibrosis and scar formation. Quercetin induces macrophage phenotype polarization from M1 to M2 in spinal cord injury in mice. [11,12]

Transfersome is a lipid vesicle that has the best deformability among other nanovesicles. Generally, transfersomes are used topically. These vesicles consist of phospholipids and an edge activator (EA), a single-chain surfactant. Transfersomes have several advantages, namely biocompatible, biodegradable, easy to manufacture, can protect drugs from environmental degradation, can deliver drugs through narrow gaps between cells well, and have been used for various ingredients such as peptides, proteins, analgesics, and natural compounds to protect the packaged drugs from metabolic degradation. [13] Transfersome can penetrate the stratum corneum by two routes in intracellular lipids that differ in their bilayer properties. Transfersomes can penetrate through intracellular and transcellular routes of the barrier in all parts of the body's skin. The transfersome penetration mechanism follows an osmotic gradient. Because of their elasticity, vesicles can conform to the shape of the pores or gaps in the stratum corneum. The size of the gap is about one to ten times smaller than the diameter of the vesicle. Liposomes are drug delivery systems by encapsulating materials with different properties, polarities, and sizes. Lecithin consists of which are often used in the manufacture and formulation of the liposome. [14,15]

Based on the explanation above, this study aims to determine the effect of the nanotransfersomes gel of lime peel extract on the expression of FGF and VEGF in accelerating the healing of traumatic ulcers in vivo in Wistar rats.

## MATERIALS AND METHODS

### Animals

In this study, 32 healthy adult male Wistar rats aged 2-3 months were used with an average body weight of 180-200 grams. This research was conducted after obtaining an ethical license at Brawijaya University (No:087-KEP-UB-2020). All samples were acclimatized for seven days. Animals were given standard laboratory food and drink and weighed at the end of acclimatization. All animals used in this study were treated and treated humanely following international guidelines. [16]

### Lime peel extract preparation and phytochemical analysis

The plant was identified and prepared at Materia Medika, Batu City, East Java, Indonesia. Lime peel extract was made using the maceration method using an ethanol solution. Lime peel in the oven at 60°C until dry, in a blender into a fine powder. The powder was wrapped in filter paper and soaked in 96% ethanol. Continue the evaporation process at a temperature of 30-40°C. [17] Characteristic test of lime peel extract using qualitative screening and quantitative levels of flavonoid (quercetin) with Liquid chromatography-mass spectrometry (LCMS) test. [18]

### Gel preparation

The nanotransfersome gel of lime peel extract (Citrus aurantifolia Swingle) is made from lime peel extract which is processed using transfersome encapsulation by sonication method. The formula used is a mixture of Lecithin and Tween 20 (1:2). Then it is dissolved in chloroform and Phosphate Buffer Saline (PBS) solution of pH 7.4 and then put into a sonicator to change the size to nano, then the process of changing the nanotransfersome preparation of lime peel extract is carried out into a gel preparation. [19]

The process continued with the manufacture of a gel, starting with dissolving Carboxymethyl Cellulose Sodium (CMC-Na) in water, then grinding

until homogeneous. Glycerin is weighed and then put into a mortar. All ingredients are ground until homogeneous. Furthermore, a gel of lime peel extract was made by adding lime peel extract to the CMC-Na solution and adding water up to 100 ml. A gel of nanotransfersome lime peel extract was made by adding lime peel extract to the CMC-Na solution and adding water up to 100 ml. [20] The final results obtained nanotransfersome gel lime peel extract with a concentration of 44% and continued with Particle Size Analyzer (PSA) and Zeta Potential tests.

**Traumatic Ulcer Creation**

This study used the lower labial mucosa as the area to be ulcerated. Anesthetize the mucosal area to be ulcerated with 0.2 ml of ketamine intramuscularly. Next, the ulcer was made by heating the burnisher instrument 2 mm with boiling water (100°C). The heated burnisher was then attached for 10 seconds to the labial mucosa of the rat. On the second day of the study, observations were made on clinically reddish ulcers with a yellowish base, a more concave area than the surrounding mucosa. [21]

**Treatment**

Wistar rats were randomly divided into four groups (8 rats in each group) with time series on the third and seventh days, namely untreated group, group A was given lime peel extract gel, group B was given nanotransfersome gel of lime peel extract, and group C was given aloe vera-based herbal medicine was applied to the traumatic ulcer area 2 times a day until the third and seventh day according to the treatment group. [21]

**Histomorphological Evaluation**

On days 3 and 7, all mice in the control and treatment groups were euthanized by cervical dislocation. Tissue preparations on rat labial mucosal tissue with fixation solution (10% formalin buffer) and IHC (Immunohistochemistry) staining at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya. Identification of macrophages assessed as a positive expression on IHC staining showed the presence of large round to oval cells with brown and foamy cytoplasm. The total expression of VEGF and FGF was calculated using a light microscope (Olympus, Japan) with 400x magnification for 5 fields of view. [22]

**Statistical analysis**

Quantitative data was obtained from the calculated number of macrophages expressing VEGF and FGF, then averaged per group. Statistical analysis using SPSS, and all data represented as mean±SD (standard deviation) was performed to test the comparative and correlative hypotheses, using a significance level of 0.05 (p=0.05) and 95% confidence level (α=0.05). The test was carried out with the data normality test and homogeneity test followed by the one-way analysis of variance (ANOVA), Tukey post hoc test, and Pearson correlation test.

## RESULTS

The results of the phytochemical screening tests that have been carried out are positive lime peels containing flavonoids, saponins, tannins, and alkaloids. Based on the results of the LCMS test, it can be seen that lime peel extract contains quercetin (one of the active substances in the flavonoid class). Quantitative results with quercetin standardization showed that the quercetin content in lime peel extract is 37.13 g/ml. Size and charge analysis of the nanotransfersome gel of lime peel extract obtained a PSA of 295.26 nm and a Zeta Potential of -9.59 mV.

Table I showed the results of the normality test showed that the significance value of the number of FGF and VEGF in the control and treatment groups on day 3 and day 7 had significant (p>0.05), so it can be concluded that the research data were normally distributed. The results of the homogeneity test show that Levene's Test sig value is p>0.05, so it can be concluded that all groups are homogeneous.

**Table I : Normality Test Results of FGF and VEGF**

| Variable | Sig *Shapiro-Wilk* | Decision |
|---|---|---|
| FGF Day-3 | 0.466 | Normal |
| FGF Day-7 | 0.525 | Normal |
| VEGF Day-3 | 0.243 | Normal |
| VEGF Day-7 | 0.753 | Normal |

* significant = p > 0.05

Table I shows the results of the One Way ANOVA test on the amount of FGF showed that on the 3rd day a sig value (p<0.05) was obtained as well as on the 7th day also obtained a significance (p<0.05) which indicated that there was a difference in the amount of FGF in the wound healing of traumatic ulcers between the control group, lime peel extract gel, nanotransfersome gel of lime peel extract and gel Aloe vera-based herbal medicine. Therefore, it will be continued with post hoc Tukey. The difference in the mean of FGF cells in the control group, treatment using lime peel extract gel, nanotransfersome gel of lime peel extract, and gel Aloe vera-based herbal medicine after day 3 and day 7. The mean of FGF in the nanotransfersome gel of the lime peel extract group on day 3 was known to be the highest with a mean of 24.20 and the lowest was the control group with a mean of 17.75. On the 7th day, there was a decrease in FGF in each group the lowest mean being

**Table II : Results of One Way ANOVA Test of FGF between Groups**

| Time | Group | Mean | SD | Sig |
|---|---|---|---|---|
| Day-3 | Control (K3) | 17.75 | 0.64 | 0.000 |
| | Lime peel extract (P3A) | 19.85 | 0.66 | |
| | Nanotransfersome lime peel extract (P3B) | 24.20 | 0.20 | |
| | Herbal medicine with aloe vera standard (P3C) | 21.93 | 0.12 | |
| Day-7 | Control (K7) | 17.40 | 0.92 | 0.000 |
| | Lime peel extract (P7A) | 14.33 | 0.42 | |
| | Nanotransfersome lime peel extract (P7B) | 10.93 | 0.31 | |
| | Herbal medicine with aloe vera standard (P7C) | 12.67 | 0.58 | |

*Data are presented as the mean ± SD. There are significant differences between groups = $p < 0.05$

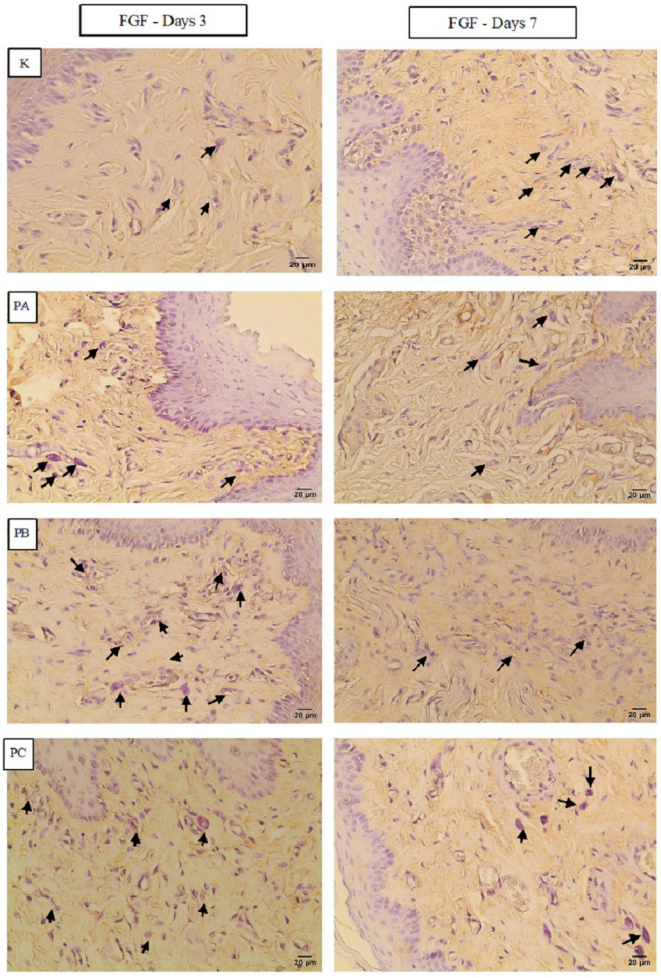


**Fig. 1 :** Reprensentative images of immunohistochemical (IHC) staining of the number of FGF on day 3 and 7 at 400x magnification. K (Control); PA (Lime peel extract gel); PB (Nanotransfersome gel of lime peel extract); PC (gel Aloe vera-based herbal medicine). Black arrows represent macrophages with positive stained.

nanotransfersome gel of lime peel extract with a mean of 10.93 and the highest was the control group with a mean of 17.40. On fig. 1 shows that the expression of FGF in macrophages in the nanotransfersome group was significantly higher than that in the other treatment groups and on the seventh day there was a lot of extracellular matrix tissue showing the healing process.

Table III shows the results of the One Way ANOVA test on the amount of VEGF showed that on the 3rd day a sig value ($p<0.05$) was obtained as well as on the 7th day ($p<0.05$) was obtained which indicated that there was a difference in the amount of VEGF in the wound healing of traumatic ulcers between the control group, lime peel extract gel, nanotransfersome gel of lime peel extract and gel Aloe vera-based herbal medicine. The difference in the mean of VEGF cells in the control group, treatment using gel extract, nanotransfersome gel of lime peel extract, and gel aloe vera-based herbal medicine after day 3 and day 7. The mean of VEGF on day 3 was known to be the highest in the nanotransfersome gel of the lime peel extract group with a mean of 20.47 and the lowest in the control group with a mean of 14.15. On the 7th day, there was a decrease in VEGF in each group with the lowest in nanotransfersome lime peel extract with a mean of 6.67 and the highest was the control group with a mean of 12.47. On fig.2 shows that the VEGF expression in macrophages in the nanotransfersome group was significantly higher than in the other treatment groups and on the seventh day indicated the healing process.

The results of the Post Hoc Tukey test (Table IV and V) on the amount of FGF and VEGF showed that on day 3 and day 7 there was a significant difference between the treatment groups ($p<0.05$).

**Table III : Results of One Way ANOVA Test of VEGF between Groups**

| Time | Group | Mean | SD | Sig |
|---|---|---|---|---|
| Day-3 | Control (K3) | 14.15 | 0.34 | 0.000 |
| | Lime peel extract (P3A) | 15.90 | 0.53 | |
| | Nanotransfersome lime peel extract (P3B) | 20.47 | 0.31 | |
| | Herbal medicine with aloe vera standard (P3C) | 18.40 | 0.20 | |
| Day-7 | Control (K7) | 12.47 | 0.64 | 0.000 |
| | Lime peel extract (P7A) | 10.53 | 0.12 | |
| | Nanotransfersome lime peel extract (P7B) | 6.67 | 0.61 | |
| | Herbal medicine with aloe vera standard (P7C) | 8.47 | 0.31 | |

* Data are presented as the mean ± SD. There are significant differences between groups = $p < 0.05$

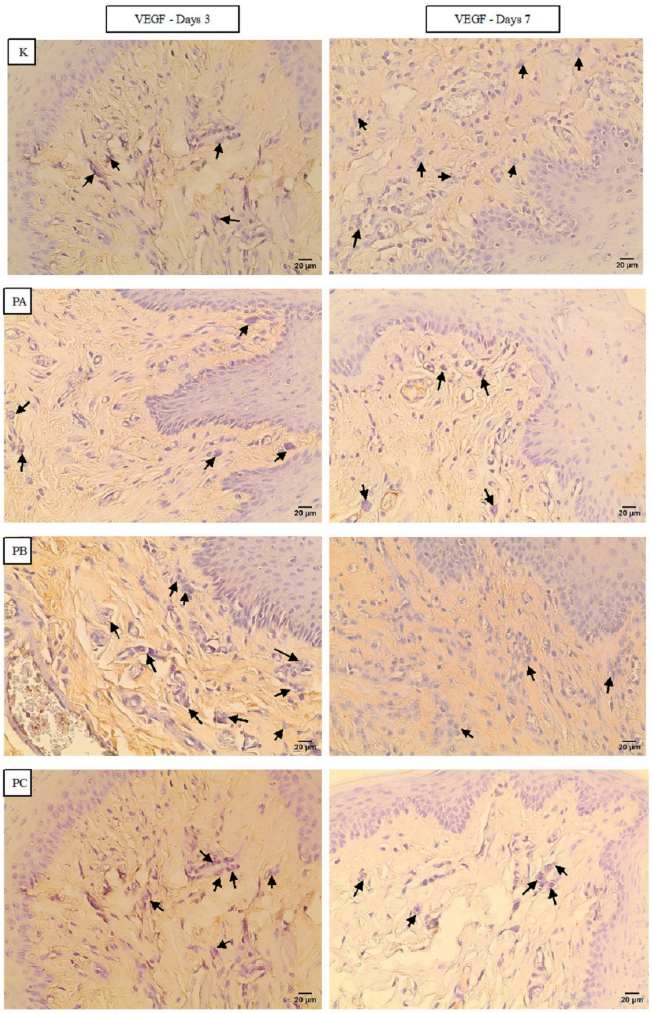


**Fig. 2 :** Reprensentative images of immunohistochemical (IHC) staining of of VEGF on day 3 and 7 at 400x magnification. K(Control); PA (Lime peel extract gel); PB (Nanotransfersome gel of lime peel extract); PC (gel aloe vera-based herbal medicine). Black arrows represent macrophages with positive stained.

The correlation test (Table VI) between the amount of FGF on day 3 and day 7 with treatment, $p<0.05$, indicates that there is a significant correlation. The magnitude of the correlation coefficient obtained on day 3 is 0.802 (positive) which indicates the average amount of FGF is increasing along with the treatment given with a very strong correlation strength. While the 7th day obtained a correlation coefficient of -0.808 (negative) which shows the average number of FGF decreases as the treatment is given with a very strong correlation strength.

In the relationship between the amount of VEGF on day 3 and day 7 with the treatment, the sig value <0.05, indicates that there is a significant correlation (Table V). The magnitude of the correlation coefficient obtained on day 3 is 0.822 (positive) which indicates the average amount of VEGF is increasing as the treatment is given with a very strong correlation strength. While on day 7, the correlation coefficient is -0.802 (negative) which indicates the average number of VEGF decreases as the treatment is given with a very strong correlation strength.

## DISCUSSION

Based on phytochemical results, lime peels contain flavonoids, saponins, tannins, and alkaloids. The quercetin content in lime peel extract is 37.13 g/ml. Krismaya et al (2019) found that the highest content of lime peel is 3.05% saponin and 2.78% flavonoid. The flavonoids contained in lime affect wound healing due to their anti-inflammatory effects, which affect the healing time. [9] The PSA test results for lime peel gel nanotransfersome particles were 295.26 nm. The results of the zeta potential test of lime peel gel nanotransfersomes showed a value of -9.59 mV. The new nanoparticles show their characteristic properties at diameters below 100 nm, but this limitation is

**Table IV : Results of Tukey Post Hoc of FGF between Groups**

| Time | Comparison | Treatment | Sig | Decision |
|---|---|---|---|---|
| Day-3 | Control, K(-)3 | Extract (P3A) | 0.001 | Significant |
| | | Nanotransfersome (P3B) | 0.000 | Significant |
| | | Herbal medicine with aloe vera standard (P3C) | 0.000 | Significant |
| | Extract (P3A) | Nanotransfersome (P3B) | 0.000 | Significant |
| | | Herbal medicine with aloe vera standard (P3C) | 0.002 | Significant |
| | Nanotransfersome (P3B) | Herbal medicine with aloe vera standard (P3C) | 0.001 | Significant |
| Hari-7 | Control K(-)7 | Extract (P7A) | 0.001 | Significant |
| | | Nanotransfersome (P7B) | 0.000 | Significant |
| | | Herbal medicine with aloe vera standard (P7C) | 0.000 | Significant |
| | Extract (P7A) | Nanotransfersome (P7B) | 0.001 | Significant |
| | | Herbal medicine with aloe vera standard (P7C) | 0.038 | Significant |
| | Nanotransfersome (P7B) | Herbal medicine with aloe vera standard (P7C) | 0.031 | Significant |

* significant differences between groups = $p < 0.05$

**Table V : Results of Tukey Post Hoc of VEGF between Groups**

| Time | Comparison | Treatment | Sig | Decision |
|---|---|---|---|---|
| Day-3 | Control K(-)3 | Extract (P3A) | 0.000 | Significant |
| | | Nanotransfersome (P3B) | 0.000 | Significant |
| | | Herbal medicine with aloe vera standard (P3C) | 0.000 | Significant |
| | Extract (P3A) | Nanotransfersome (P3B) | 0.000 | Significant |
| | | Herbal medicine with aloe vera standard (P3C) | 0.000 | Significant |
| | Nanotransfersome (P3B) | Herbal medicine with aloe vera standard (P3C) | 0.000 | Significant |
| Day-7 | Control, K(-)7 | Extract (P7A) | 0.005 | Significant |
| | | Nanotransfersome (P7B) | 0.000 | Significant |
| | | Herbal medicine with aloe vera standard (P7C) | 0.000 | Significant |
| | Extract (P7A) | Nanotransfersome (P7B) | 0.000 | Significant |
| | | Herbal medicine with aloe vera standard (P7C) | 0.003 | Significant |
| | Nanotransfersome (P7B) | Herbal medicine with aloe vera standard (P7C) | 0.007 | Significant |

* significant differences between groups = $p < 0.05$

difficult to achieve for nanoparticle systems as drug delivery systems. In general, drug nanoparticles must contain a sufficient amount of drug in the matrix for each particle, thus requiring a relatively larger size than non-pharmaceutical nanoparticles. Nanoparticles are particles that have a size below 1 micron in test animals. This is determined according to the theory that the zeta value is said to be stable when away from the isoelectric point which is 0, so that repulsion occurs, while a dispersion system with a low zeta potential value is easier to form aggregates along with Van der Waals forces in particle interactions. Kateh et al (2019) prove that vesicles with a size of less than 500 nm can pass through the skin by penetrating through pores five times smaller than their size. [15,16,18]

In natural wound healing (without intervention), the role of M1 and M2 macrophages is very important for wound healing. Monocytes to the wound tissue after 48 hours which transition to macrophages as the main cells in the wound. The four-phase natural wound healing process is characterized by a lag of about 3-5 days and prolonged inflammation can lead to fibrotic wound healing. The results showed that all groups experienced a decrease in the average amount of FGF and VEGF expression on the 7th day compared to the 3rd day. Macrophages M2 produce PDGF, TGF-β, FGF, and VEGF which can stimulate the formation of granulation tissue. Observations on day 7 were carried out based on the angiogenesis process stimulated by VEGF, mostly found in the proliferative phase, namely on days 3 to 21. [6,18]

The group treated with nanotransfersomes gel with lime peel extract had the highest average number on day 3. This can be caused by quercetin as a natural flavonoid that has an anti-inflammatory effect on the drug delivery system used, namely nanotransfersomes. Quercetin has been reported to modulate M2 polarization. As reported, M1 macrophages secrete a large number of pro-inflammatory cytokines, while M2 macrophages can downregulate pro-inflammatory cytokines and secrete growth factors (VEGF, FGF, TGF, etc.). The structure of the lipid bilayer membrane in the transfersomes is also able to support the penetration of the wound area through smaller pores, besides that the elasticity of the transfersomes membrane also minimizes the risk of rupture of vesicles in the skin/mucosa. Kant et al (2020) reported the results of studies on wounds of diabetic rats showing that quercetin treatment increased the expression of IL-10, VEGF, and TGF-β1 and decreased the expression of TNF-α, IL-1β, and MMP-9. [20,21,25]

The average amount of FGF and VEGF in the lime peel extract gel treatment group had the lowest mean on the 3rd day and the lowest on the 7th day compared to other treatment groups. This group has the same basic ingredients as the nanotransfersomes but slow progress in total FGF and VEGF expression. This can be influenced by the particle size of the lime peel extract gel which is larger than the lime peel extract gel nanotransfersomes so that it cannot penetrate faster on the target cells. Another factor that can affect the penetration of lime peel extract gel is mucosal permeability including the amount of salivary flow, where increased salivary secretion can decrease mucosal permeability resulting in rapid gel degradation. Nanocarriers are generally the preferred form of drug delivery due to the nature of mucoadhesion, in which the adhesion of the drug carrier occurs to the mucous membranes of the mucosa. [13, 22]. The administration of Aloe vera-based herbal medicines had the second average amount of FGF and VEGF on the 3rd day and the second lowest on the 7th day, this is following the theory which states that the content of Acemannan (mannose-6-phosphate) on Aloe vera can accelerate the wound healing process in diabetic ulcers in experimental animals by stimulating KGF-1, VEGF production, and collagen synthesis. [30]

The average amount of FGF and VEGF expression in each group on the 3rd day was found to decrease on the 7th day. The angiogenesis process will occur as long as macrophages are still in the wound area because macrophages are the key to the vascular formation by producing growth factors such as VEGF, TGF-β, and PDGF. Macrophages decrease when the injured tissue is going through a healing or remodeling phase. This indicates that the faster VEGF expression decreases, the faster the wound healing process run. [4,31]

On the 7th day, all treatment groups showed a decrease in the expression of FGF and VEGF. This indicates a decreased inflammatory phase and increased proliferation to form granulation tissue. Only the control group was still high because the control group was self-healing. Angiogenesis is important for wound healing because it involves the growth of new capillaries to form granulation tissue that occurs on the third to fifth day after tissue injury. Several growth factors produced by macrophages (PDGF, VEGF, and FGF) synergize in their ability to vascularize tissues. In the proliferative phase, an increase in the number of cells and wound healing factors will be seen, one of which is the proliferation of fibroblasts. The role of fibroblasts is very large in the repair process, which is responsible for the preparation of producing protein structure products that will be used during the tissue reconstruction process. The proliferation of fibroblasts in the wound healing process is naturally stimulated by IL-Ib, PDGF, and fibroblast FGF. The wound healing process is strongly influenced by the role of fibroblast migration and proliferation in the wound area. [25,32].

## CONCLUSION

The nanotransfersome gel of lime peel extract has the potential to accelerate wound healing by increasing the amount of FGF and VEGF in the labial mucosal wounds of Wistar rats. On day 3, the treatment group with lime peel extract nanotransfersome gel had the highest average VEGF and FGF values.

Research based on nanotransfersome gel of lime peel extract has the potential to be developed as herbal medicine by utilizing orange peel waste. This research is still limited to the microscopic figure of wound healing. Suggestions in this study need to be further

tested on the porosity of the lime peel extract nanotransfersome gel, immunohistochemical analysis to explore growth factors and anti-inflammatory cytokines, as well as antimicrobial tests to support the mechanism of healing effect on the administration of lime peel extract nanotransfersome gel.

**Abbreviation**
**FGF:** Fibroblast growth factors
**VEGF:** Vascular endothelial growth factor
**TNF-α:** Tumor necrosis factor-α
**IL:** Interleukin
**IGF:** Insulin-like growth factor
**TGF:** Transforming growth factor
**PDGF:** Platelet-derived growth factor
**MMP-9:** Matrix metalloproteinase-9

## ACKNOWLEDGMENT

We acknowledge the Dean Faculty of Dentistry Universitas Brawijaya for the financial support for this research in the form of a research grant.

## REFERENCES

1. Tsai HC, Li YC, Young TH, Chen MH. Citrus polyphenol for oral wound healing in oral ulcers and periodontal diseases. J Formos Med Assoc [Internet]. 2016;115(2):100–7. Available from: http://dx.doi.org/10.1016/j.jfma.2015.01.003
2. Mortazavi H, Safi Y, Baharvand M, Rahmani S. Diagnostic Features of Common Oral Ulcerative Lesions: An Updated Decision Tree. Int J Dent. 2016;14.
3. Nelonda R, Setiadhi R. Management of chronic traumatic ulcer mimicking oral squamous cell carcinoma on the tongue. Dent J (Majalah Kedokt Gigi). 2018;51(2):76.
4. Arundina I, Diyatri I, Surboyo MDC, Monica E, Afanda NM. Growth factor stimulation for the healing of traumatic ulcers with liquid rice hull smoke. J Taibah Univ Med Sci [Internet]. 2021;16(3):431–9. Available from: https://doi.org/10.1016/j.jtumed.2021.01.003
5. Farahpour MR, Pirkhezr E, Ashrafian A, Sonboli A. Accelerated healing by topical administration of Salvia officinalis essential oil on Pseudomonas aeruginosa and Staphylococcus aureus infected wound model. Biomed Pharmacother. 2020;128(September 2019).
6. Vaidyanathan L. Growth factors in wound healing ⇓ a review. Biomed Pharmacol J. 2021;14(3):1469–80.
7. Minutti CM, Knipper JA, Allen JE, Zaiss DMW. Tissue-specific contribution of macrophages to wound healing. Semin Cell Dev Biol [Internet]. 2017;61:3–11. Available from: http://dx.doi.org/10.1016/j.semcdb.2016.08.006
8. Fu J, Huang J, Lin M, Xie T, You T. Quercetin Promotes Diabetic Wound Healing via Switching Macrophages From M1 to M2 Polarization. J Surg Res [Internet]. 2020;246:213–23. Available from: https://doi.org/10.1016/j.jss.2019.09.011
9. Krismaya DG d. AN, Pramudya R, Sati PYI, Kamadjaja DB. Effects of lime (Citrus aurantifolia christm. swingle) peel extract on fibroblast proliferation and angiogenesis in rat's tooth extraction sockets. Biochem Cell Arch. 2019;(December 2019):4917–9.
10. Loizzo MR, Tundis R, Bonesi M, Menichini F, De Luca D, Colica C, et al. Evaluation of Citrus aurantifolia peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. J Sci Food Agric. 2012;92(15):2960–7.
11. Gopalakrishnan A, Ram M, Kumawat S, Tandan SK, Kumar D. Quercetin accelerated cutaneous wound healing in rats by increasing levels of VEGF and TGF-β1. Indian J Exp Biol. 2016;54(3):187–95.
12. Tsai CF, Chen GW, Chen YC, Shen CK, Lu DY, Yang LY, et al. Regulatory effects of quercetin on M1/M2 macrophage polarization and oxidative/antioxidative balance. Nutrients. 2022;14(1):1–21.
13. Opatha SAT, Titapiwatanakun V, Chutoprapat R. Transfersomes: A promising nanoencapsulation technique for transdermal drug delivery. Pharmaceutics. 2020;12(9):1–23.
14. Hosny KM, Rizg WY, Khallaf RA. Preparation and optimization of in situ gel loaded with rosuvastatin-ellagic acid nanotransfersomes to enhance the antiproliferative activity. Pharmaceutics. 2020;12(3):1–19.
15. Le NTT, Cao V Du, Nguyen TNQ, Le TTH, Tran TT, Thi TTH. Soy Lecithin-Derived Liposomal Delivery Systems: Surface Modification and Current Applications. Int J Mol Sci. 2019;20(19).
16. Dahmani S, Chair R, Errachidi F, Berrada W, Ansari H, Benidir M, et al. Evaluation of in vivo wound healing activity of Moroccan Citrus reticulata peel extract. Clin Phytoscience. 2020;6(1).
17. Widyaningrum I, Wibisono N, Kusumawati AH. Effect of Extraction Method on Antimicrobial Activity Against Staphylococcus Aureus of Tapak Liman (Elephantopus Scaber L.) Leaves. Int J Heal Med Sci [Internet]. 2020;3(1):105–10. Available from: https://www.neliti.com/publications/329617/effect-of-extraction-method-on-antimicrobial-activity-against-staphylococcus-aur
18. Chien WJ, Saputri DS, Lin HY. Valorization of Taiwan's Citrus depressa Hayata peels as a source of nobiletin and tangeretin using simple ultrasonic-assisted extraction. Curr Res Food Sci [Internet]. 2022;5:278–87. Available from: https://doi.org/10.1016/j.crfs.2022.01.013
19. Marie NK. Preparation and in Vitro Evaluation of Soya Lecithin Based Nano Transfersomal Dispersion for Loxoprofen Sodium. Al Mustansiriyah J Pharm Sci. 2019;(December 2019):102–15.

20. Badekova KZ, Atazhanova GA, Kacergius T, Akhmetova SB, Smagulov MK. Formulation of an Origanum vulgare-based dental gel with antimicrobial activity. J Taibah Univ Med Sci [Internet]. 2021;16(5):712–8. Available from: https://doi.org/10.1016/j.jtumed.2021.05.009
21. Titiek Berniyanti, Ira Arundina, Jan Terrie RP. Phytochemicals potential of Cyperus rotundus Linn. root extracts Kalimantan for treatment of Oral mucosa traumatic ulcer : "Healing process enhancement with Cyperus rotundus L. root" Titiek. J Res Heal Sci 2018 1 [Internet]. 2018;1(3):90–7. Available from: www.journalofresearch.org;info@journalofresearch.org
22. Yuanita T, Hotmaria H, Effendy R, Suardita K. Ekspresi Tgf-B Dan Mmp-1 Pada Periodontitis Apikalis Kronis Akibat Induksi Bakteri Enterococcus Faecalis Tikus Wistar. Conserv Dent J. 2019;7(2):86.
23. Abdassah M. Nanopartikel dengan gelasi ionik. J Farmaka. 2017;15(1):45–52.
24. Tiyaboonchai W. Chitosan Nanoparticles : A Promising System for Drug Delivery. Chem Pharm Bull [Internet]. 2003;11(3):51–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21048331
25. Kateh Shamshiri M, Momtazi-Borojeni AA, Khodabandeh Shahraky M, Rahimi F. Lecithin soybean phospholipid nano-transfersomes as potential carriers for transdermal delivery of the human growth hormone. J Cell Biochem. 2019;120(6):9023–33.
26. Kotwal JG, Chien S. Macrophage Differentiation in Normal and Accelerated Wound Healing Girish. Results Probl Cell Differ [Internet]. 2017;62:3–22. Available from: http://link.springer.com/10.1007/978-3-319-54090-0
27. Kant V, Jangir BL, Sharma M, Kumar V, Joshi VG. Topical application of quercetin improves wound repair and regeneration in diabetic rats. Immunopharmacol Immunotoxicol [Internet]. 2021;43(5):536–53. Available from: https://doi.org/10.1080/08923973.2021.1950758
28. Hu Y, Gui Z, Zhou Y, Xia L, Lin K, Xu Y. Quercetin alleviates rat osteoarthritis by inhibiting inflammation and apoptosis of chondrocytes, modulating synovial macrophage polarization to M2 macrophages. Free Radic Biol Med [Internet]. 2019;145(September):146–60. Available from: https://doi.org/10.1016/j.freeradbiomed.2019.09.024
29. Pandey M, Choudhury H, Ngu J, Ying S, Foo J, Ling S, et al. Mucoadhesive Nanocarriers as a Promising Strategy to Enhance Intracellular Delivery against Oral Cavity Carcinoma. 2022;1–20.
30. Sargowo D, Handaya AY, Widodo MA, Lyrawati D, Tjokroprawiro A. Aloe Gel Enhances Angiogenesis in Healing of Diabetic Wound. Indones Biomed J. 2011;3(3):204.
31. Wang PH, Huang BS, Horng HC, Yeh CC, Chen YJ. Wound healing. J Chinese Med Assoc [Internet]. 2018;81(2):94–101. Available from: https://doi.org/10.1016/j.jcma.2017.11.002
32. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. Expert Rev Mol Med. 2011;13(July 2011):1–12.
33. Toma AI, Fuller JM, Willett NJ, Goudy SL. Oral wound healing models and emerging regenerative therapies. Transl Res [Internet]. 2021;236(404):17–34. Available from: https://doi.org/10.1016/j.trsl.2021.06.003