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Original Article

Topical Adipose Mesenchymal Stem cell metabolites regulate the expression of MMP-1, MMP-9, EGF, TGF-β in oral mucosa ulcer rat model

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ABSTRACT

Objective: Matrix metalloproteinase (MMP-1 and −9), Epidermal Growth Factor (EGF), and Transforming Growth factor (TGF)-β are expressed in the oral ulcer wound-healing process. The Adipose mesenchymal stem cell metabolites (AdMSCMs) may accelerate wound-healing. This study aimed to investigate the expression of MMP-1, MMP-9, EGF, and TGF-β in the oral mucosa ulcer rat model treated with topical AdMSCMs.

Materials and Methods: Oral ulcer models were created in twenty healthy male Wistar rats (*Rattus novergicus*) divided into AdMSCMs and control groups. The oral ulcer model was treated topically using AdMSCMs oral gel three times daily for 3 and 7 days. The expression of MMP-1, MMP-9, EGF, TGF-β was evaluated through histological examination using the immunohistochemistry method. Independent *t*-test was used to compare the mean of expression of MMP-1, MMP-9, EGF, TGF-β between control and treatment groups (AdMSCMs), and paired *t*-test was used to analyze the mean between day 3 and day 7 of each group.

Results: A lower expression of MMP-1, MMP-9 in AdMSCMs group and higher expression EGF and TGF-β in AdMSCMs group compared to the control group in day 3 and day 7. Independent *t*-test results showed a significant difference in the expression of MMP-1, MMP-9, EGF between the control and AdMSCMs group in day 3 and day 7. Only TGF-β expression mean difference between day 3 and day 7 showed a significant difference compared to the control groups ($p < 0.05$)

Conclusions: AdMSCMs oral gel may accelerate oral ulcer healing models by reducing the expression MMP-1, MMP-9, and increasing EGF and TGF-β expressions during the wound-healing process.

1. Introduction

A wide range of diseases may have signs, such as oral ulcers in the oral cavity. The course of oral ulcers may be acute and chronic (Bruce and Rogers, 2003; Field and Allan, 2003; Muñoz-Corcuera et al., 2009). People may complain due to the discomfort when chewing, speech, and swallowing. Furthermore, oral ulcers may have a negative impact on the quality of life of people (Kozlak et al., 2010; Mortazavi et al., 2016; Porter and Leao, 2005; Rajan et al., 2014; Siu et al., 2015).

Controlling the inflammatory reaction and accelerating the healing process of oral ulcers are the main strategies for treating oral ulcers (Field and Allan, 2003; Siu et al., 2015). Hence, most drugs used are anti-inflammatory drugs. The steroid anti-inflammatory drugs are the gold standard of therapy for oral ulcers. The topical application may be the first choice of anti-inflammatory medications for oral ulcers (Bruce and Rogers, 2003; Gonzalez-Moles and Scully, 2005). Since the unfavorable side effects of extended duration of therapy using steroid anti-inflammatory drugs, non-steroid anti-inflammatory drugs may be the alternatives to oral ulcer treatment (Gonzalez-Moles and Scully, 2005; Mortazavi et al., 2016; Siu et al., 2015).

Besides, tissue regeneration is essential in the wound-healing process of oral ulcers. The most recent agents that have been studied for oral ulcer treatment are AdMSCMs. Topical AdMSCMs have been proven to increase the angiogenesis and pro-angiogenic factors expression in the

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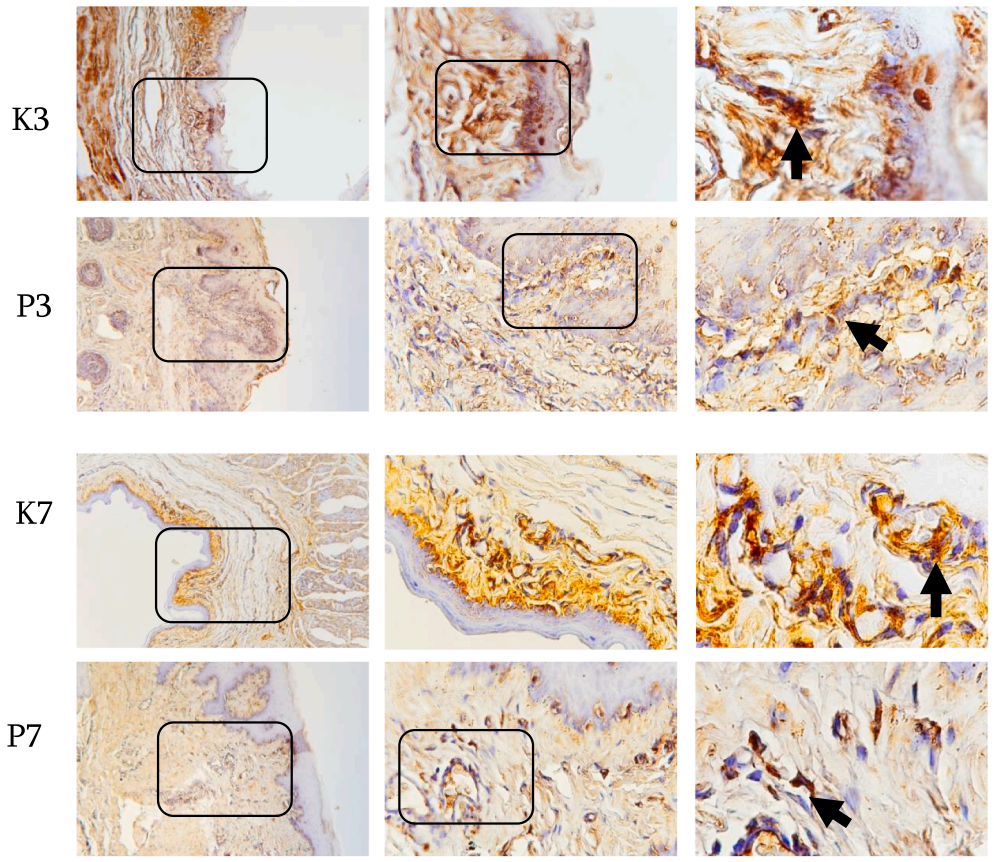
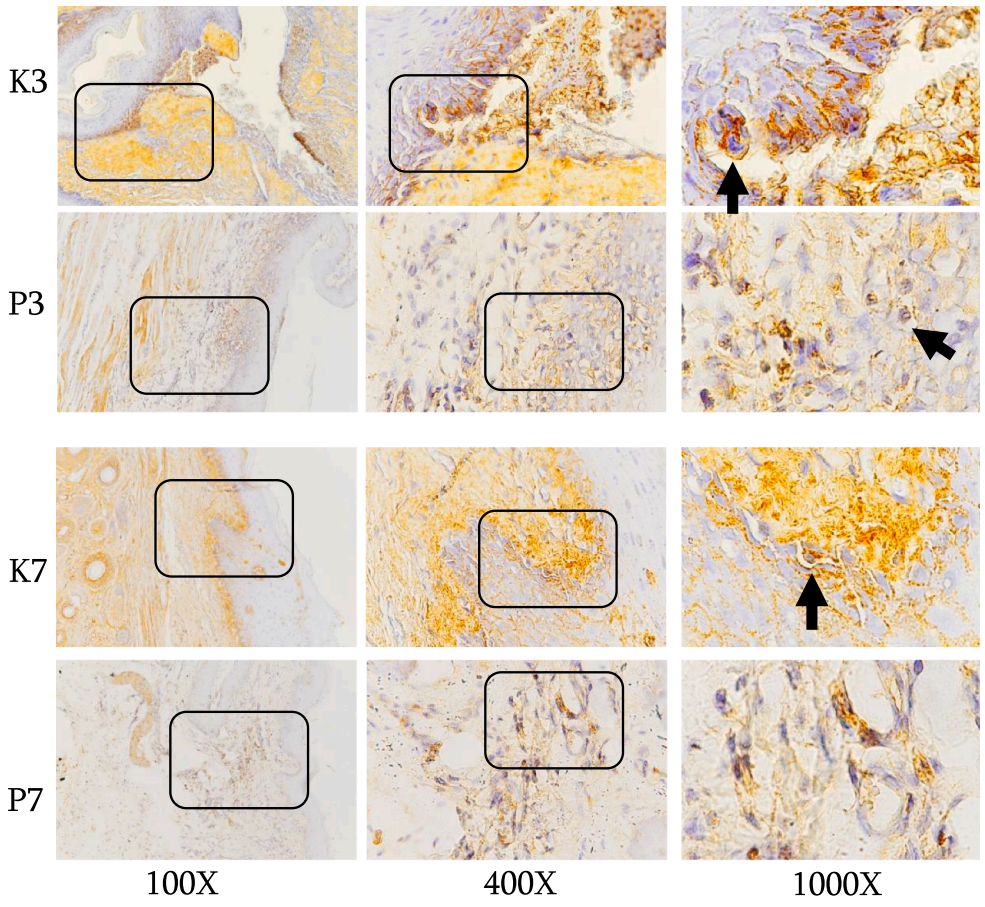


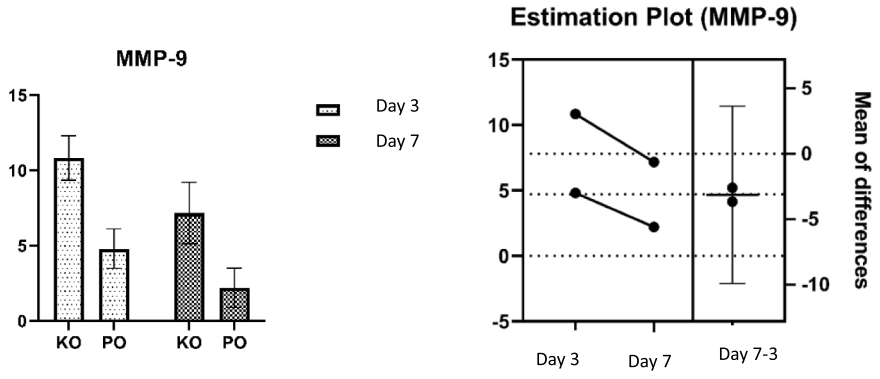
Fig. 1. MMP-1 expression in control (KO) and treatment group (PO) using AdMSCM on day-3 and day-7 rats buccal mucosal ulcer model with 100x, 400x dan 1000x magnificent. The brown colour showed cells with MMP-1 expression (arrow) (a). the graph of MMP-1 expression in treatment (P) and control (K) group day-3 & 7 (b).

oral ulcer model (Wicaksono et al., 2023). The AdMSCMs have many bioactives components that may have anti-inflammatory actions and promote tissue regeneration. The effect of AdMSCMs topically in the regulation of biomarkers such as matrix metalloproteinases/MMPs, and the expression of growth factors (transforming growth factors/TGF and epidermal growth factors/EGF) in oral ulcer wound-healing process may be needed to be investigated.

Matrix metalloproteinases (MMPs) have been known as calcium-dependent zinc-containing enzymes that are involved in the degradation of extracellular matrix (ECM). In the wound-healing process, MMPs are activated and expressed in several cells (fibroblast, keratinocytes, inflammatory cells, etc.) in response to several signals from cytokines and growth factors such as transforming growth factors (TGF-β), epidermal growth factors/EGF which expressed through several pathways. MMP-1 was immediately expressed in the basal area when the ECM was lost and migrated to the wound edges. MMP-9 contributed to the angiogenesis process of keratinocyte migration during the wound-healing process (Caley et al., 2015). A recent study



(a)



(b)

Fig. 2. MMP-9 expression in control (KO) and treatment group (PO) using AdMSCM on day-3 and day-7 rats buccal mucosal ulcer model with 100x, 400x dan 1000x magnificent. The brown colour showed cells with MMP-9 expression (arrow) (a). The graph of MMP-9 expression in treatment (P) and control (K) group day-3 & 7 (b).

showed that platelet-rich plasma treatment topically reduced MMP-9 expression in traumatic oral ulcers in diabetes mellitus models in animals (Radithia et al., 2023). TGF-β palys a significant role during the inflammatory, proliferation, and remodeling phases of wound healing, especially in fibroblast proliferation. The EGF and TGF-β, which are secreted by M2 macrophage, also contribute to cell migration of keratinocytes and fibroblast in wound bed in proliferation phases. (Toma et al., 2021; Waasdorp et al., 2021). After tissue injury, TGF-β1 is rapidly regulated and released by many cells, such as keratinocytes, platelets, monocytes, macrophages, and fibroblasts. TGF-β1 increases the expression of VEGF, which is needed for angiogenesis in wound healing. A study showed that TGF-β expression increased in the traumatic ulcer model in the Diabetes model *Rattus novergicus* after being treated using platelet-rich protein topically (Radithia et al., 2023).

Hence, this current study aimed to investigate the effect of Adipose Mesenchymal Stem Cell Metabolites topically regulating the expression of MMP-1, MMP-9, TGF-β, and EGF in the Oral Ulcer Rat Model.

2. Material and methods

This laboratory study received ethical clearance with Certificate Number 635/HRECC.FODM/V/2023 from the Ethical Clearance Commission at the Faculty of Dental Medicine, Universitas Airlangga, Indonesia. The research was conducted at the Laboratory of Medical Biochemistry, Dental Research Center, and Research Center for Vaccine Technology and Development Institute of Tropical Disease, all at Universitas Airlangga. Animal experimentation adhered to ARRIVE guidelines and institutional ethical protocols, following the principles of the Declaration of Helsinki.

2.1. AdMSCMs oral gel preparation

The AdMSCMs oral gel was prepared following our previously published protocol in Wicaksono et al. (2023) as follows: Culture medium was collected after the fourth passage of AdMSC culture, then centrifuged at 1,500 rpm for 3 min to remove debris and dead cells. Subsequently, the medium was filtered through a 0.45 μm syringe filter to isolate bioactive substances released by AdMSCs during growth. Finally, the purified culture medium was mixed with 5 % hydroxypropylmethylcellulose (HPMC) at a 1:3 vol ratio.

2.2. Experimental animal groups

This study included 20 *Rattus norvegicus* meeting inclusion criteria: aged 1–2 months, weighing 250–300 g, and without oral or systemic pathologies. All animals underwent oral ulcer induction using an 8 g/3 mm punch biopsy instrument with precise dissection at the base with No. 15 surgical blades. Clinical evaluation 24 h post-biopsy confirmed oral ulcers, identified by a white lesion surrounded by an erythematous border. The animal models were divided into four groups, each consisting of five rats, as described below.

1. Positive control group: Animals underwent oral ulcer induction via punch biopsy and received 5 % HPMC gel topically for three days (C3) and seven days (C7).
2. Treatment group: Animals underwent oral ulcer induction via punch biopsy and received AdMSCMs gel topically for three days (T3) and seven days (T7).

Animal euthanasia involved cervical dislocation after a single intraperitoneal injection of 50 mg/kg pentobarbital (pentobarbital solution, cat no: P-010, Sigma Aldrich), administered within the range of 20–40 mL just before euthanasia.

2.3. Tissue analysis

This study employed immunohistochemistry to assess wound-healing-associated variables. Ulcer and adjacent healthy tissue were excised via biopsy, processed into formalin-fixed paraffin-embedded (FFPE) specimens, and sectioned into slides. MMP-1, MMP-9, EGF, and TGF-β expressions were evaluated using immunohistochemistry. Horseradish peroxidase-labeled secondary antibodies (EGF polyclonal antibody (PeproTech® #500-P277-1MG), TGF-β1 monoclonal antibody (Invitrogen #MA5-16949), MMP-1 polyclonal antibody (Invitrogen #PA5-102381), and MMP-9 polyclonal antibody (Invitrogen #MA5-15886)) were used. Protein expression was examined by two observers under a light microscope (100x, 400x, and 1000x magnifications) across five fields of view in oral ulcer sites, identified by brown precipitate.

2.4. Analytical statistics

Statistical analysis utilized the Statistical Package for Social Sciences (SPSS version 25, IBM Corp, New York). Normality was tested with the Shapiro-Wilk test, and Homogeneity was assessed using Levene's tests.

Table 1
Independent *t*-test of the mean MMP-1 and MMP-9 expression value between the treatment group (AdMSCMs) and control group based on treatment duration.

Group	Mean Value ± SD	p-value
MMP-1 day 3		0.000*
Control	10.17 ± 2.04	
Treatment	4.20 ± 1.30	
MMP-1 day 7		0.001*
Control	7.67 ± 2.16	
Treatment	2.20 ± 1.30	
MMP-9 day 3		0.000*
Control	10.83 ± 1.47	
Treatment	4.80 ± 1.30	
MMP-9 day-7		0.001*
Control	7.67 ± 2.16	
Treatment	2.20 ± 1.30	

SD: standard deviation; p: probability.
* Significant with $p < 0.05$.

Parametric comparative analysis employed the independent *t*-test and paired *t*-test at a significance level of p-value < 0.05.

2.5. Matrix metalloproteinase (MMP)-1 and MMP–9 expression

AdMSCMs topically treated on the Wistar labial mucosa ulcer model revealed brown-colored cells indicating MMP-1 (Fig. 1) and MMP-9 (Fig. 2) expression in histopathological sections. Independent *t*-test results (Table 1) indicated a significant difference in MMP-1 and MMP-9 expression between day 3 and day 7 of AdMSCMs treatment. Paired *t*-test analysis showed no significant difference in MMP-1 expression ($p = 0.07$; $p > 0.05$) and non-significant MMP-9 expression between mean differences of day 3 and day 7 applications ($p = 0.11$; $p > 0.05$) (Fig. 1b).

2.6. Transforming growth factor (TGF)-β and epidermal growth factor (EGF) expression

EGF and TGF-β expression in the AdMSCMs-treated group on the labial mucosal ulcer models showed brown-colored cells in histopathological sections (Figs. 3 and 4). Independent *t*-test results indicated significant differences in EGF expression between the treatment and control groups after AdMSCMs application on days 3 and 7, and in TGF-β expression between the treatment and control groups after AdMSCMs application on day 3 ($p < 0.05$) (Table 2). Paired *t*-test analysis of mean differences in EGF and TGF-β expression after day 3 and day 7 applications showed no significant difference between the AdMSCMs-treated and control groups ($p = 0.25$; $p > 0.05$). However, a significant mean difference in TGF-β expression was observed between the AdMSCMs-treated group and the control group with $p = 0.02$ ($p < 0.05$) (Fig. 4b).

3. Discussion

This study results showed that topical AdMSCMs application to oral mucosal ulcers model of Wistar rats stimulated MMP-1, MMP-9, EGF, and TGF-β expression in oral mucosal epithelial tissue on both the 3rd and 7th days of treatment. MMP-1 and MMP-9 are expressed by various cells like inflammatory cells (lymphocytes, monocytes, macrophages), fibroblasts, and endothelial cells, including keratinocytes, to promote cell migration in wound healing. MMP-1 and MMP-9 are also activated by growth factors such as EGF and TGF-β. The expression of MMP is controlled by the inhibition of proteinase activity by plasma proteinase

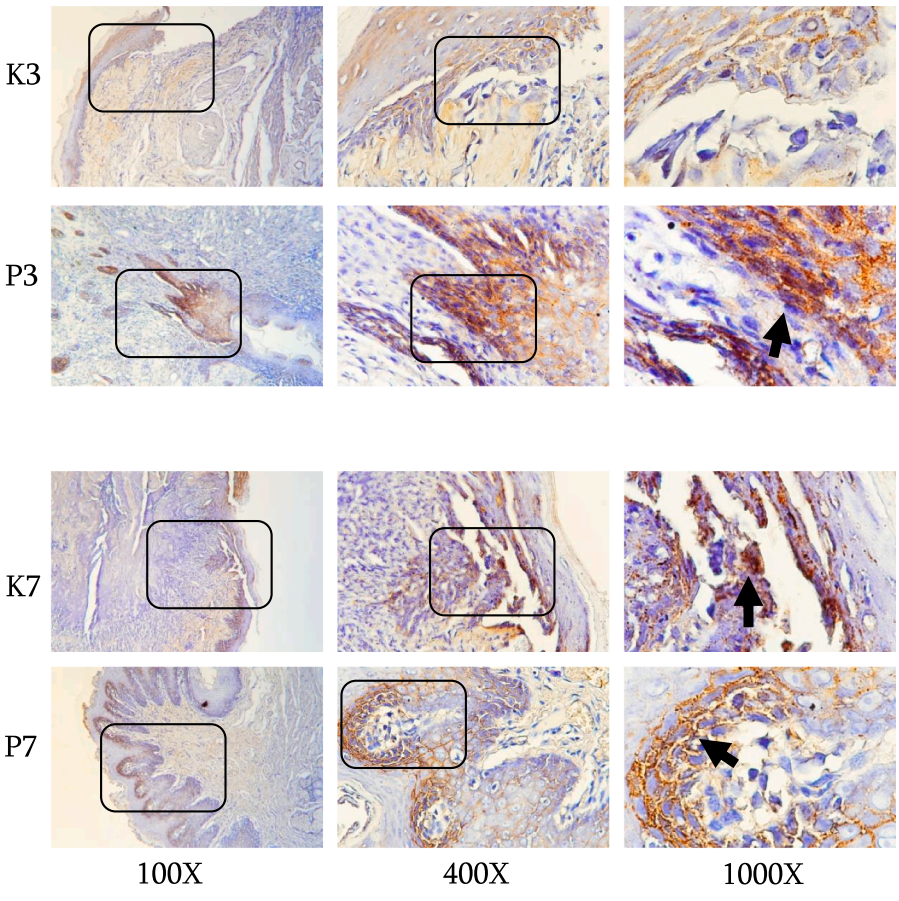


Fig. 3. EGF expression in control group (KO) and treatment group (PO) using AdMSCM on day-3 and day-7 rats buccal mucosal ulcer model with 100x, 400x dan 1000x magnificent. The brown colour showed cells with EGF expression (arrow) (a). the graph of EGF expression in treatment (P) and control (K) group day-3 & 7 (b).

inhibitors, such as a1-proteinase and a2-macroglobulin, or by MMP binding proteins, such as thrombospondin-1 and −2. MMPs are derived from latent pro-MMP, which is activated to form MMP by serine proteinases and other MMPs. The catalytic activity requires a zinc ion in the active site. A number signalling the pathway of MMP include activation of NF-κB, mitogen-activated protein kinase, Smad-dependent pathways by growth factors or cytokines, activation of focal adhesion kinase (FAK) by integrin activation, or activation of Wnt signaling (Caley et al., 2015).

In this study, the results showed that the lower number of MMP-1 and MMP-9 expressions in the treatment group could be caused by the role of AdMSCMs in oral ulcer mucosa model suppressed the greater number of MMP-1 and MMP-9 expression. The reduced expression of MMP-1 and −9 was slight between both days and may be caused by the duration of treatment between day 3 and day 7 that was only four days apart, and the inflammatory process decreased in the stage of the wound-healing process and continued to the epithelialization process. The expression of MMP-1 &-9 during the inflammation stage is important to ensure the process of wound healing proceeds to a further stage, the epithelization stage (Kandhwal et al., 2022) which both MMP-1 and MMP-9 are expressed to facilitate cell migration such as inflammatory cells, keratinocyte, endothelial cells on in wound edge collagen (Caley et al., 2015; Rohani and Parks, 2015). AdMSCMss can switch the macrophages to an anti-inflammatory phenotype, regulate fibroblasts to secrete more collagen to promote scarless wound healing, and complete

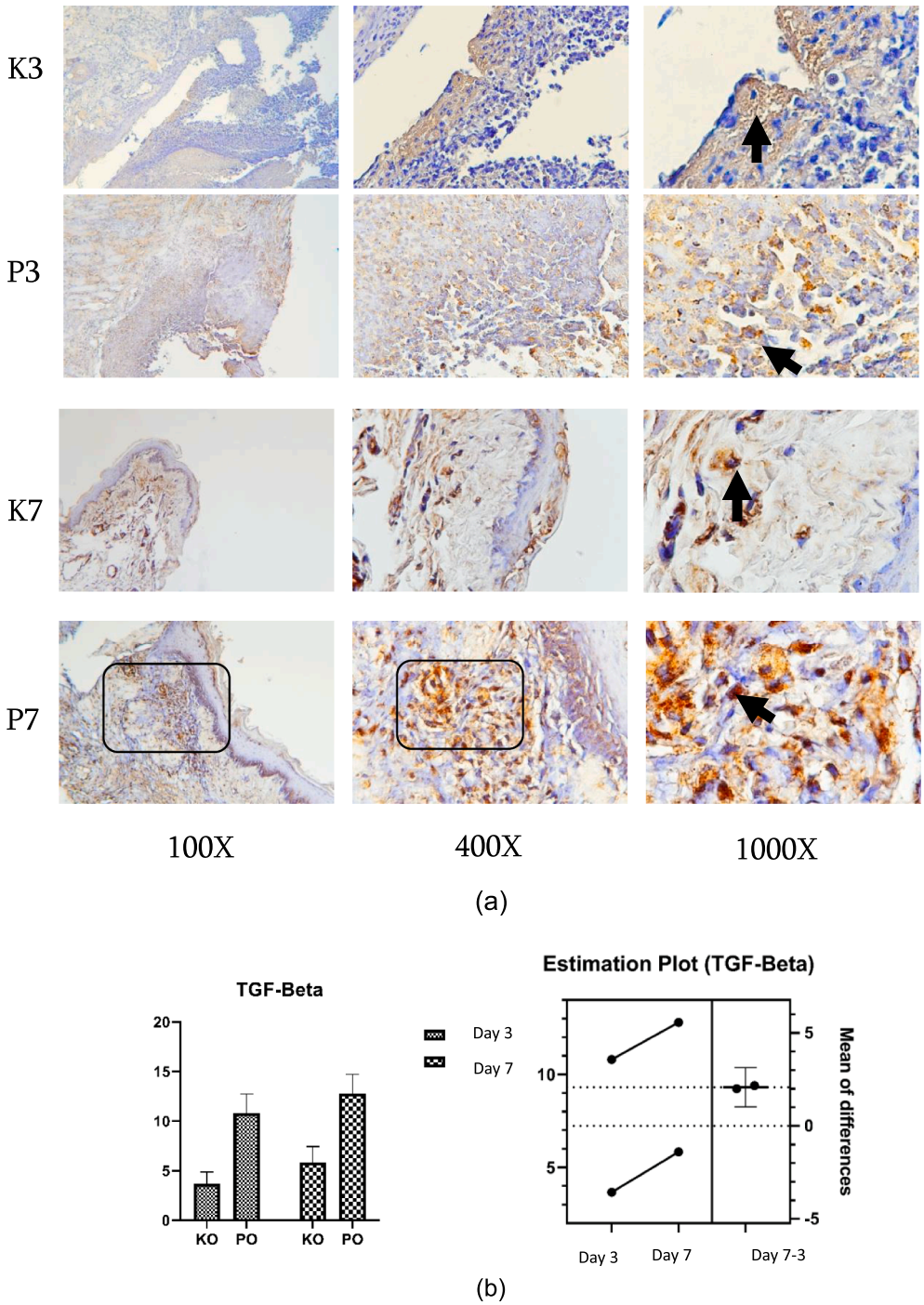


Fig. 4. TGF-β expression in control group (KO) and treatment group (PO) using AdMSCM on day-3 and day-7 rats buccal mucosal ulcer model with 100x, 400x dan 1000x magnificent. The brown colour showed cells with TGF-β expression (arrow) (a). The graph of EGF expression in treatment (P) and control (K) group day-3 & 7 (b).

the wound-healing process beyond the inflammatory phase (Ajit and Ambika Gopalankutty, 2021).

On the other hand, the AdMSCMs treatment group showed a higher mean value of expression EGF and TGF-β compared to the control group. The increase of EGF and TGF-β expression occurs because the epithelialization process in the stage of the wound-healing process was increased. Therefore, there was an increase of EGF and TGF-β expression in the oral ulcer mucosa model after AdMSCMs treatment. During the wound-healing process, a large number of TGF-β produced by inflammatory cells. Therefore, the high number of TGF-β expressed accelerates wound healing in the epithelization stage. TGF-β proteins are the TGF-β super-family which have three functional isoforms (TGFβ1, β2, β3). TGF-β predominantly released into the wound microenvironment from ECM secreted by fibroblasts, macrophages, and platelets in the oral mucosa ulcer wound-healing process (Schrementi et al., 2008; Yamakawa and Hayashida, 2019). Furthermore, TGF-β regulates keratinocytes, fibroblasts, endothelial cells, monocytes, and platelets, increasing the expression of VEGF to form new blood vessels. TGF-β also inhibits MMP-9 and results in collagen degradation inhibition (Radithia et al., 2023). The smad signaling pathway is the main TGF-β response. TGF-β also activates extracellular signal-regulated kinase (ERK)-1, ERK-2, p38, or c-Jun amino-terminal kinase (JNK), also known as mitogen-activated

Table 2
Independent *t*-test of the mean value of EGF and TGF-β expression between the treatment group (AdMSCMs) and control group based on treatment duration.

Group	Mean value ± SD	p
TGF-β day-3	3.67 ± 1.21	0.000*
control	10.80 ± 1.92	
treatment		
TGF-β day-7	5.83 ± 1.60	0.180
control	32.80 ± 45.97	
treatment		
EGF day-3	2.67 ± 1.03	0.000*
control	8.00 ± 1.58	
treatment		
EGF day-7	4.16 ± 1.16	0.000*
control	11.60 ± 2.07	
treatment		

SD: standard deviation; p: probability.
* Significant with $p < 0.05$.

protein kinases (MAPKs). TGF-β accelerates mucosal re-epithelization by strengthening the attachment of migrating epithelial cells to basement membrane collagens by regulating expression in epithelial cells of extracellular matrix receptor, triggering angiogenesis by inducing VEGF, formation of granulation tissue (Tanigawa et al., 2005). The AdMSCMs treatment increases TGF-β expression in oral mucosa ulcer wound-healing process indicated that the AdMSCMs increase the amount of TGF-β then transcribes NF-κB in the macrophages, reducing the pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, MMP-9).

A previous study showed that AdMSCMs gel topically increase FGF-2 and VEGFA in an oral mucosal ulcer rat model (Wicaksono et al., 2023). MMP-1 and MMP-9 have a role to mediate inflammatory cells to control inflammation response (Zhang et al., 2020). The immunomodulatory effects and various anti-inflammatory are from components of AdMSCMs (Ajit and Ambika Gopalankutty, 2021) by interacting with immune cells (Zriek et al., 2021). Further, MMP-1 facilitates the epithelization stage by mediating cell migration, including immune cells, fibroblast, and the degradation of collagens, which are essential components of extracellular matrix (ECM) in order to induce tissue remodelling and prevent over-expression of keratinocytes which may delay reepithelization (Caley et al., 2015). MMP-9 is also needed for the degradation of ECM; this degradation facilitates fibroblast and keratinocyte migration to the wound site, which is needed for the repairing process (Hariono et al., 2018).

This study showed that the AdMSCMs application increased TGF-β significantly, which indicated that the AdMSCMs involved in collagen turnover regulation and fibrogenesis and may accelerate collagen stabilization in the wound-healing process of oral ulcer (Kim et al., 2013; Pilloni et al., 2023). Collagen stabilization may facilitate nonscar formation in oral mucosal wound healing. The production of TGF-β continued in all phases in response to several stimuli to increase matrix protein and maintain increased expression throughout the wound-healing process, promoting downstream fibroblast proliferation (Schrementi et al., 2008). This result was similar to another study, which showed TGF-β expression increased in traumatic oral ulcers in the diabetes model *Rattus novergicus* after being treated using platelet-rich protein (Radithia et al., 2023).

This study result showed that EGF increased during the wound-healing process. EGF is also the key inducer of angiogenesis, which is an important step in the wound-healing process to obtain optimal reepithelization (de Sousa Sá et al., 2018). Another study showed that AdMSCMs increase vascular endothelial growth factor (VEGF) A and fibroblast growth factor (FGF), which is important for angiogenesis during the wound-healing process in oral ulcer (Wicaksono et al., 2023).

This study has some limitations. Our study only investigated the application of AdMSCMs in the wound-healing process with the cut-off time point on days 3 and 7. Since the wound-healing process of oral ulcers may take 10–14 days, it is important to investigate the expression of MMP-1, MMP-9, TGF-β, and EGF until day 14 in order to have a better explanation and understanding of the wound-healing process in oral ulcer.

4. Conclusion

AdMSCMs oral gel may accelerate oral ulcer healing models by reducing the expression of MMP-1, and MMP-9, and increasing EGF and TGF-β expressions during the wound-healing process.

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CRediT authorship contribution statement

Hendri Susanto: Conceptualization, Writing – original draft. **Ade Ratu Mas Saraswati:** . **Alexander Patera Nugraha:** Conceptualization, Methodology, Software, Writing – original draft. **Satutya Wicaksono:** Conceptualization, Investigation, Methodology, Software, Data curation. **Nanan Nur'aeny:** Writing – review & editing, Writing – original draft. **Diah Savitri Ernawati:** Supervision, Writing – review & editing.

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References

Ajit, A., Ambika Gopalankutty, I., 2021. Adipose-derived stem cell secretome as a cell-free product for cutaneous wound-healing. 3 Biotech. https://doi.org/10.1007/s13205-021-02958-7.
Bruce, A.J., Rogers, R.S., 2003. Acute oral ulcers. Dermatol Clin. 21, 1–15.
Caley, M.P., Martins, V.L.C., O'Toole, E.A., 2015. Metalloproteinases and Wound-healing. Adv. Wound Care (New Rochelle) 4, 225–234. https://doi.org/10.1089/wound.2014.0581.
de Sousa Sá, O.M., Lopes, N.N.F., Alves, M.T.S., Caran, E.M.M., 2018. Effects of glycine on collagen, PDGF, and EGF expression in model of oral mucositis. Nutrients 10. https://doi.org/10.3390/nu10101485.
Field, E.A., Allan, R.B.A., 2003. Review article: oral ulceration – aetiopathogenesis, clinical diagnosis and management in the gastrointestinal clinic. Alinment. Pharmacol. Their. 949–962 https://doi.org/10.1046/j.0269-2813.2003.01782.x.
Gonzalez-Moles, M.A., Scully, C., 2005. Vesiculo-erosive oral mucosal disease-management with topical corticosteroid: (2) Protocols, monitoring of effects and adverse reactions, and the future. J. Dent. Res. 84, 302–308.
Hariono, M., Yuliani, S.H., Istyastono, E.P., Riswanto, F.D.O., Adhipandito, C.F., 2018. Matrix metalloproteinase 9 (MMP9) in wound-healing of diabetic foot ulcer: molecular target and structure-based drug design. Wound Medicine. https://doi.org/10.1016/j.wndm.2018.05.003.
Kandhwal, M., Behl, T., Singh, S., Sharma, N., Arora, S., Bhatia, S., Al-Harrasi, A., Sachdeva, M., Bungau, S., 2022. Role of matrix metalloproteinase in wound-healing. Am. J. Transl. Res.
Kim, Y.J., Carvalho, F.C., Souza, J.A.C., Gonçalves, P.C.G., Nogueira, A.V.B., Spolidório, L.C., Roque-Barreira, M.C., Cirelli, J.A., 2013. Topical application of the lectin Artin M accelerates wound-healing in rat oral mucosa by enhancing TGF-β and VEGF production. Wound Repair Regen. 21, 456–463. https://doi.org/10.1111/wrr.12041.
Kozlak, S.T., Walsh, S.J., Lalla, R.V., 2010. Reduced dietary intake of vitamin B12 and folate in patients with recurrent aphthous stomatitis. J. Oral Pathol. Med. 39, 420–423. https://doi.org/10.1111/j.1600-0714.2009.00867.x.
Mortazavi, H., Safi, Y., Baharvand, M., Rahmani, S., 2016. Diagnostic features of common oral ulcerative lesions: an updated decision tree. Int J Dent 2016. https://doi.org/10.1155/2016/7278925.
Muñoz-Corcuera, M., Esparza-Gómez, G., González-Moles, M.A., Bascones-Martínez, A., 2009. Oral ulcers: clinical aspects. A tool for dermatologists. Part II. Chronic ulcers. Clin Exp Dermatol. 10.1111/j.1365-2230.2009.03219.x.

Pilloni, A., Marini, L., Gagliano, N., Canciani, E., Dellavia, C., Cornaghi, L.B., Costa, E., Rojas, M.A., 2023. Clinical, histological, immunohistochemical, and biomolecular analysis of hyaluronic acid in early wound-healing of human gingival tissues: a randomized, split-mouth trial. J Periodontol. https://doi.org/10.1002/JPER.22-0338.

Porter, S.R., Leao, J.C., 2005. Review article: oral ulcers and its relevance to systemic disorders. Aliment. Pharmacol. Ther. 21, 295–306. https://doi.org/10.1111/j.1365-2036.2005.02333.x.

Radithia, D., Yuliana, Y., Puspitasari, Y., Sismiyanti, R., Pratiwi, A.S., 2023. An in-vivo study of effects of platelet-rich plasma on transforming growth factor-β1 and matrix metalloprotein 9 expression in traumatic ulcers with diabetes mellitus. Eur. J. Dent. https://doi.org/10.1055/s-0043-1764429.

Rajan, B., Ahmed, J., Shenoy, N., Denny, C., Ongole, R., Binnal, A., 2014. Assessment of quality of life in patients with chronic oral mucosal diseases: a questionnaire-based study. Perm. J. 18 https://doi.org/10.7812/tpp/13-095.

Rohani, M.G., Parks, W.C., 2015. Matrix remodeling by MMPs during wound repair. Matrix Biol. https://doi.org/10.1016/j.matbio.2015.03.002.

Schrementi, M.E., Ferreira, A.M., Zender, C., DiPietro, L.A., 2008. Site-specific production of TGF-β in oral mucosal and cutaneous wounds. Wound Repair Regen. 16, 80–86. https://doi.org/10.1111/j.1524-475X.2007.00320.x.

Siu, A., Landon, K., Ramos, D., 2015. Differential diagnosis and management of oral ulcers. Semin. Cutan. Med. Surg. 34 https://doi.org/10.12788/j.sder.2015.0170.

Tanigawa, T., Pai, R., Arakawa, T., Higuchi, K., Tarnawski, A.S., 2005. TGF-β signaling pathway: its role in gastrointestinal pathophysiology and modulation of ulcer healing. J. Physiol. Pharmacol. 56, 3–13.

Toma, A.I., Fuller, J.M., Willett, N.J., Goudy, S.L., 2021. Oral wound-healing models and emerging regenerative therapies. Transl. Res. https://doi.org/10.1016/j.trsl.2021.06.003.

Waasdorp, M., Krom, B.P., Bikker, F.J., van Zuijlen, P.P.M., Niessen, F.B., Gibbs, S., 2021. The bigger picture: why oral mucosa heals better than skin. Biomolecules. https://doi.org/10.3390/biom11081165.

Wicaksono, S., Nugraha, A.P., Rahmahani, J., Rantam, F.A., Kuncorojakti, S., Susilowati, H., Riawan, W., Arundina, I., Lestari, P., Masya, R.N., Surboyo, M.D.C., Ernawati, D.S., 2023. Adipose mesenchymal stem cell metabolites oral gel enhance pro-angiogenic factors expression, angiogenesis, and clinical outcome of oral ulcer rat model. Eur. J. Dent. https://doi.org/10.1055/s-0043-1761192.

Yamakawa, S., Hayashida, K., 2019. Advances in surgical applications of growth factors for wound-healing. Burns Trauma. https://doi.org/10.1186/s41038-019-0148-1.

Zhang, H., Liu, L., Jiang, C., Pan, K., Deng, J., Wan, C., 2020. MMP9 protects against LPS-induced inflammation in osteoblasts. Innate. Immun. 26, 259–269. https://doi.org/10.1177/1753425919887236.

Zriek, F., Di Battista, J.A., Alaaeddine, N., 2021. Mesenchymal stromal cell secretome: immunomodulation, tissue repair and effects on neurodegenerative conditions. Curr. Stem. Cell. Res. Ther. 16, 656–669. https://doi.org/10.2174/1574888x16666210202145639.