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Mesenchymal stem cells from human umbilical cord decrease inflammation and increase vascularization of induced apical periodontitis model in diabetes mellitus rats

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Abstract

Objectives: to evaluate inflammation and vascularization in a model of apical periodontitis in diabetic Wistar rats through histopathological examination of blood vessels and immunohistochemical examination of interleukin 1b (IL-1b) and tumor necrosis factor-a (TNF-a). Methodology: Diabetes was induced in 20 Wistar rats using multiple low-dose injections of streptozotocin (STZ) until blood glucose levels stabilized above 300 mg/dL, confirmed by glucometer. Under anesthesia, apical periodontitis was induced in the right mandibular first molars. After preparing the access cavity and extirpating pulp and canal, the teeth were left open. Apical periodontitis was projected seven days afterwards and the Wistar rats were assigned in random into four groups, each group consisting of five rats. The first group (C14) was euthanized 14 days post-induction, while the second group (C28) was euthanized 28 days later, serving as controls. The third group (T14) received mesenchymal stem cells from human umbilical cords and was euthanized after 14 days, while the fourth group (T28) received mesenchymal stem cells from human umbilical cord and was euthanized after 28 days. The number of blood vessels and the expressions of IL-1b and TNF-a were analyzed. Data were evaluated with ANOVA and by Tukey's HSD test, with significance at $p<0.05$. Results: Both control and treatment groups showed a significant increase in vascularization in the apical periodontal area of apical periodontitis in the control and treatment groups at 14 and 28 days ($p<0.05$). A significant reduction of IL-1b and TNF-a levels was found in the mesenchymal stem cells treatment groups when compared to control groups ($p<0.05$). Conclusion: Our findings support the use of mesenchymal stem cells from human umbilical cords to decrease inflammation and increase vascularization in an induced apical periodontitis model in diabetes mellitus Wistar rats.

Keywords: Diabetes mellitus. Animal model. Inflammation. Vascularization. Mesenchymal stem cells.

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Introduction

Diabetes mellitus (DM) is a disease characterized by hyperglycemia, caused by insufficient insulin secretion or reactiveness. The global population affected by DM is expected to exceed 600 million by 2040.[1,2] It is an international issue affecting all age groups. Obstinate and unmedicated hyperglycemia can lead to micro- and macrovascular complications, which also affect the oral tissues.[3] Failure to manage hyperglycemia can exacerbate oral health problems, such as periodontal problems, caries, pulp necrosis, tooth loss, and apical periodontitis.[4,5]

Apical periodontitis (AP) is an inflammation in periradicular zone, leading to tissue destruction, inflammation, and alveolar bone resorption.[6] This condition involves host defense mechanisms and the production of inflammatory mediators.[7] Inflammatory cytokines focused in this study are interleukin 1b (IL-1b) and tumor necrosis factor-a (TNF-a).[8] These cytokines are associated with delayed apical tissue healing, chronic inflammation, and increased bone damage in hyperglycemic conditions.[9] In the progression of apical periodontitis in DM subjects, macrophages and neutrophils play crucial roles in driving inflammation and bone ruin, as well as the discharge of pro-inflammatory mediators that contribute to bone destruction.[10]

Regenerative endodontic therapy (RET) using stem cells has gained attention for their advantages in tissue regeneration. Mesenchymal stem cells (MSCs) from the umbilical cord are promising due to ease of isolation, high proliferation potential and growth capacity. MSCs from the human umbilical cord are more primitive and possess high differentiation potential, as well as immunosuppressive properties and greater stemness if compared to other stem cell sources from adult tissues. As such, these cells have potential for regenerative treatments.[11-14] Several *in vitro* studies have explored these cells' potential.[15-17] However, the use of mesenchymal stem cells from human umbilical cord for biological therapies in dentistry remains limited and therefore warrants further investigation.

This animal model was selected due to the anatomic similarity of periapical tissues between Wistar rats and humans. Rats induced with STZ presented similar symptoms to those found in diabetic humans, such as polyuria, polydipsia, and hyperglycemia. Despite some limitations, this animal model can be considered as the first stage to develop cell-based RET study in diabetes mellitus.[18]

Previous studies and clinical findings indicate that diabetes mellitus negatively impacts the success of endodontic treatments regarding vascularization and inflammation.[18-20] Our study sought to evaluate the effects of MSCs from the human umbilical cord on inflammation and vascularization in AP in diabetic rats through histopathological examination of blood vessels and immunohistochemical analysis of IL-1b and TNF-a after 14 and 28 days. We hypothesize that MSCs from human umbilical cord decrease inflammation and increase vascularization in an induced AP model in diabetic Wistar rats. This is crucial because a successful RET requires healthy apical periodontal support to reconstruct a functional pulp.

Methodology

Ethics approval and study design

All experimental procedures involving humans and animals were performed according to relevant regulations and guidelines. This study was approved by the Universitas Airlangga, Faculty of Dental Medicine Ethical Commissions regarding Animal Studies (Number 1170/2023). This study was prepared according to PRIASE 2021 guidelines on animal studies in endodontology.[21] This study had a true experimental design, using a post-test random control group.

A total of 20 adult male Wistar rats (Wistar strain of *Rattus norvegicus*) aged 10-12 weeks and weighing 250-300 g, in healthy conditions, with mature molar teeth (closed foramen) were used in this study. The sample size calculation was obtained from a previous study and confirmed with the Lemeshow formula.[8] The rats were distributed randomly into four groups with five rats each, comprising the four experimental groups (C14: control of 14 days, C28: control of 28 days, T14: MSCs treatment of 14 days, and T28: MSCs treatment of 28 days).

The rats were maintained at a steady temperature of 25°C, with a humidity of 45-55%, under diurnal lighting (12-hour dark/light cycle). They had free access to water and food (ad libitum) and were cared for by a team of veterinarians specialized in experimental animal models.

Diabetes mellitus preparation by streptozotocin induction

The diabetes mellitus rat model was conducted according to a previously published method.[8] Briefly, blood glucose levels were monitored using a digital glucometer (AccuCheck, Germany) before, during, and five days after the end of streptozotocin (STZ) induction. The streptozotocin (STZ) obtained from Bioworld (Ohio, USA) was dissolved in a 0.05 M citrate buffer (pH 4.5). The rats were fasted for six hours before receiving daily STZ peritoneal injections (20 mg/kg body weight) over consecutive days. A 5% glucose solution was provided for 24 h after STZ injections (Otsuka, Indonesia). One week after STZ injections, the efficacy of the diabetes model was validated by checking blood glucose levels exceeding 300 mg/dL

Apical periodontitis model by root canal exposure to oral cavity

After confirming DM induction, initiation for apical periodontitis model was conducted according to a modified previous method.[8] Adult Wistar rats weighing 250-300 g were anesthetized with intraperitoneal injections of xylazine 5 mg/kg (Xyla Interchemie, The Netherlands) and ketamine hydrochloride 40 mg/kg (Kepro B.V., The Netherlands). The right mandibular first molars were accessed with no. 1 round diamond bur (SS White, USA), while pulpectomy was performed with barbed broaches (Dentsply, USA). The teeth were left open for exposure to oral cavity microorganisms. After this procedure, phenylbutazone analgesics (Phenylject, TMC, Indonesia) 20 mg/kg body weight was administered intramuscularly. Apical periodontitis was confirmed by histopathological examination, showing periodontal tissue irregularities in the apical area.

Preparation of mesenchymal stem cells

Mesenchymal stem cells from umbilical cords were obtained according to previous method.[2] MSCs were characterized using flow cytometry (negative CD34, CD45 and positive CD90, CD105, CD73). Stem cells from passage 5 were used in the study. Gelatin solvent was used as scaffold to carry 500,000 cells for injection into the root canals and pulpal space. For injection, conical tubes containing 20 µL of MSCs in gelatin solvent were prepared.

MSCs transplantation into the root canals

Exogenous MSCs were transplanted into the pulp chamber to extrapolate a cell-based RET in this animal model, compensating for the inferior quality of endogenous MSCs caused by DM. MSC transplantation followed a modified clinical RET method.[22] After seven days of AP induction, root canal cleaning was completed using K-files (Dentsply, USA) nos. 8, 10 and 15, supported by an electronic apical locator (Dentsply, USA). Following root canal preparation, an irrigation procedure using sodium hypochlorite 1.5% (Hyposol, USA) was conducted. Then, final irrigation was performed using sterile saline (Otsuka, Indonesia) and Navi-tips (Ultradent, USA). The canals were dried with sterilized paper points (Dentsply, USA) number 15. A 20 µL solution of MSCs in gelatin scaffold was injected into the root canals using a microliter syringe (Hamilton, USA). The coronal part was sealed with bio-c repair (Angelus, Brazil) and protected with glass ionomer restoration (GC, Japan). The Wistar rats were euthanized 14 and 28 days after MSC transplantation by intraperitoneal overdose anesthesia with xylazine 30 mg/kg and ketamine hydrochloride 300 mg/kg according to AVMA euthanasia guidelines. The mandibles were separated and then immersed in 10% paraformaldehyde.

Histopathological and immunohistochemical analysis

Specimens were decalcified with a 5.0% ethylene diamine tetra acetic acid aqueous solution according to a previously described method.[8] All samples were fixed in paraffin chunks and cut into 5 µm sections using a microtome, following usual histological procedure. Hematoxylin-eosin (HE) staining was used for histopathological examination to assess vascularization. The focus was on sections comprising treated teeth with apical periodontitis.

Immunohistochemical staining was conducted using monoclonal primary antibodies for IL-1b (SC52012, Santa Cruz Biotechnology, USA) and TNF-a (SC52746, Santa Cruz Biotechnology, USA) to assess macrophage expression. Vascularization and inflammatory cell counts were determined using a standardized guide on captured images, following examination in 100 µm squares in five fields around periapical region.

Microscopically, vascularization was assessed in the apical area from HE-stained specimens, while

inflammatory cell counts were calculated from IHC stained samples (IL-1b and TNF-a). Histopathological and immunohistochemical analysis were conducted with a Nikon Eclipse Ci-E (Nikon Corporation, Japan) compound microscope at a 400× magnification, focusing in the apical area of AP.

Data analysis

Data were calculated and expressed as mean ± standard deviation. Statistical analysis was conducted using an SPSS software (SPSS, version 27, SPSS Inc., IBM, NY, USA). Statistical significancy was defined as $p<0.05$. Data were assessed using analysis of variance (ANOVA) followed by post-hoc comparisons between groups.

RESULTS

Blood glucose level

Prior to STZ induction, the blood glucose measurement of each Wistar rat was recorded as normal (70-130 mg/dL). Throughout the STZ induction period, blood glucose levels fluctuated (140-320 mg/dL), and after the end of the STZ injection period (5 days), blood glucose stabilized between 300-490 mg/dL. All 20 rats survived the multiple low-dose STZ injections.

Vascularization in the apical area

Histological examination on the number of blood vessels in the apical area was performed in the control and MSCs treatment groups. Hematoxylin-eosin staining is shown in Figure 1. All data followed a normal distribution, and variances were homogenous ($p>0.05$). The vascular count in the 14-day control group (C14) was 25.48±5.71; in the 28-day control group (C28) it was 28.84±7.81; in the 14-day MSCs treatment group (T14) it was 45.96±7.63; while in the 28-day MSCs treatment group (T28) it was 46.28±10.56. The MSCs treatment groups had a significant increase in vascularization, with more formation of blood vessels both in T14 and T28, while the control groups of C14 and C28 remained low ($p<0.05$). The number of blood vessels between the control groups (C14 and C28) were not significantly different ($p=0.912$) nor was there a significant difference between the MSCs treatment groups (T14 and T28) ($p=1.000$). Significant differences in blood vessels were found between C14 and T14 ($p=0.005$); C14 and T28 ($p=0.005$); C28 and T14 ($p=0.020$); and C28 and T28 ($p=0.017$). The significance of vascularization between groups is shown in Table 1.

Inflammatory expression in the apical area

Immunohistochemical staining was used to evaluate inflammatory expressions. Macrophage expression of IL-1b at 14 and 28 days is shown in

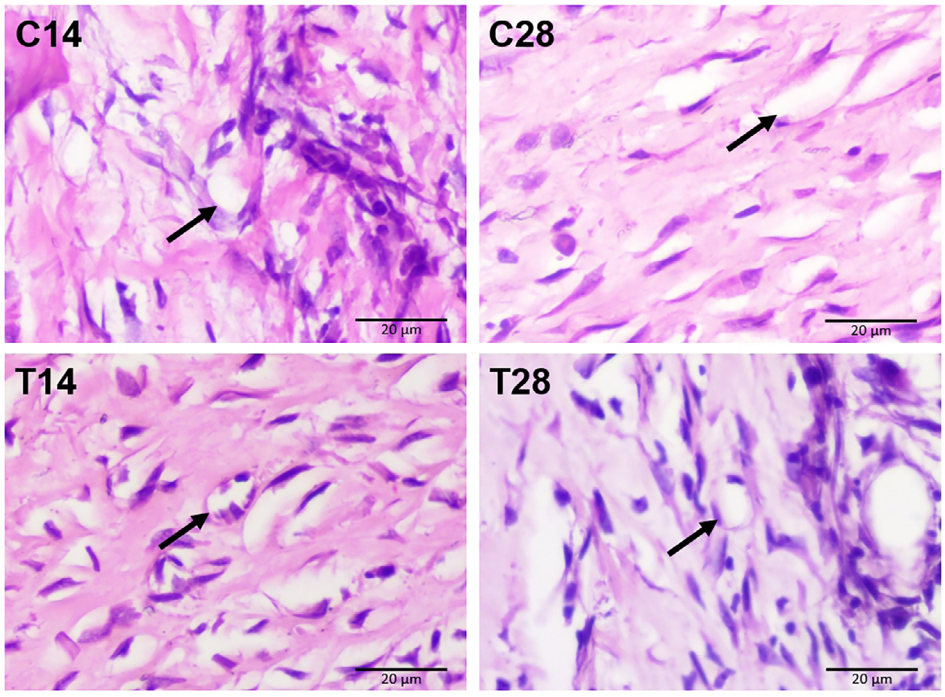

Figure 1- Histopathological evaluation on the number of blood vessels in the apical area, indicated by black arrows (C14: control group at 14 days, C28: control group at 28 days, T14: MSCs treatment group at 14 days, T28: MSCs treatment group at 28 days). Scale bar: 20 µm.

Figure 2, while that of TNF-a at 14 and 28 days is shown in Figure 3.

IL-1b expression in the control group C14 was 7.88±2.44, while in the C28 group it was 9.32±1.00. In the MSCs treatment group T14 it was 4.00±0.51, and in the MSCs treatment group T28 it was 4.88±1.52. MSCs treatment groups significantly reduced IL-1b expression both in T14 and T28, while the control groups of C14 and C28 remained high ($p<0.05$). There was no significant difference in IL-1b expression between the control groups C14 and C28 ($p=0.474$) or between groups T14 and T28 ($p=0.804$). Significant difference in IL-1b expression were found between C14 and T14 ($p=0.005$); C14 and T28 ($p=0.033$); C28 and T14 ($p=0.000$); C28 and T28 ($p=0.002$). The significance of IL-1b expression between groups is shown in Table 2.

TNF-a expression in the C14 group was 6.56±0.48; in C28 group it was 7.04±0.62; in the T14 group it was 2.64±0.43; and in the T28 group it was 3.80±0.97. The MSCs treatment groups significantly reduced TNF-a expression both in T14 and T28, while the control groups C14 and C28 remained high ($p<0.05$). There was no significant difference in TNF-a expression between control groups C14 and C28 ($p=0.666$) or between treatment groups T14 and T28 ($p=0.059$).

Table 1- Significance of vascularization among groups

Groups	Control 14 days (25.48+5.71)	Control 28 days (28.84+7.81)	MSC 14 days (45.96+7.63)	MSC 28 days (46.28+10.56)
Control 14 days (25.48+5.71)		p=0.912	p=0.005*	p=0.005*
Control 28 days (28.84+7.81)	p=0.912		p=0.020*	p=0.017*
MSC 14 days (45.96+7.63)	p=0.005*	p=0.020*		p=1.000
MSC 28 days (46.28+10.56)	p=0.005*	p=0.017*	p=1.000	

*Significant difference

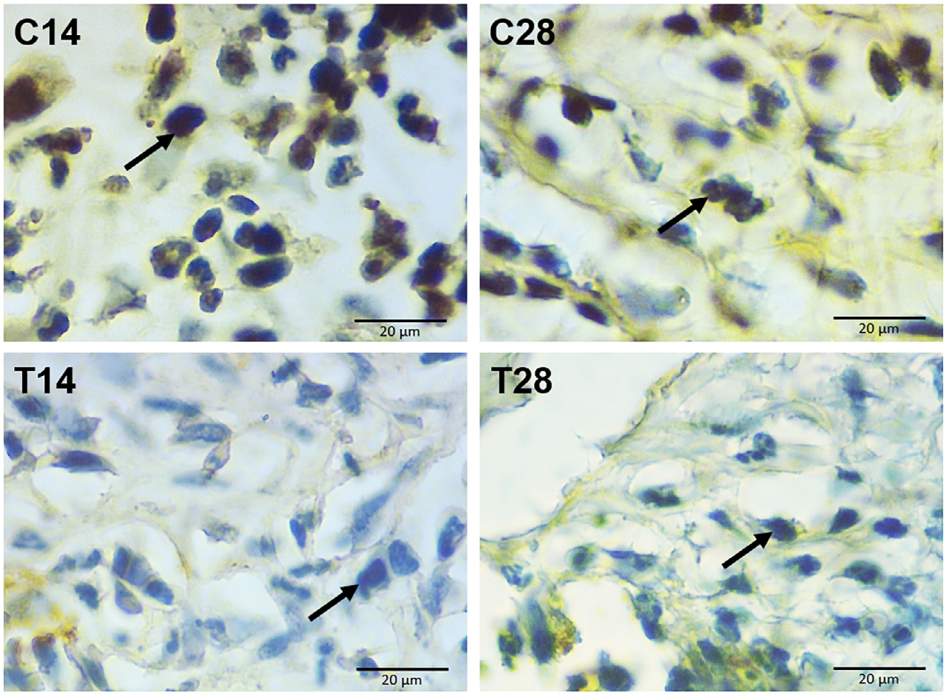

Figure 2- IL-1b expression in macrophages. The immune reactivity of macrophages in the apical area is indicated by black arrows (C14: control group at 14 days, C28: control group at 28 days, T14: MSCs treatment group at 14 days, T28: MSCs treatment group at 28 days). Scale bar: 20 µm.

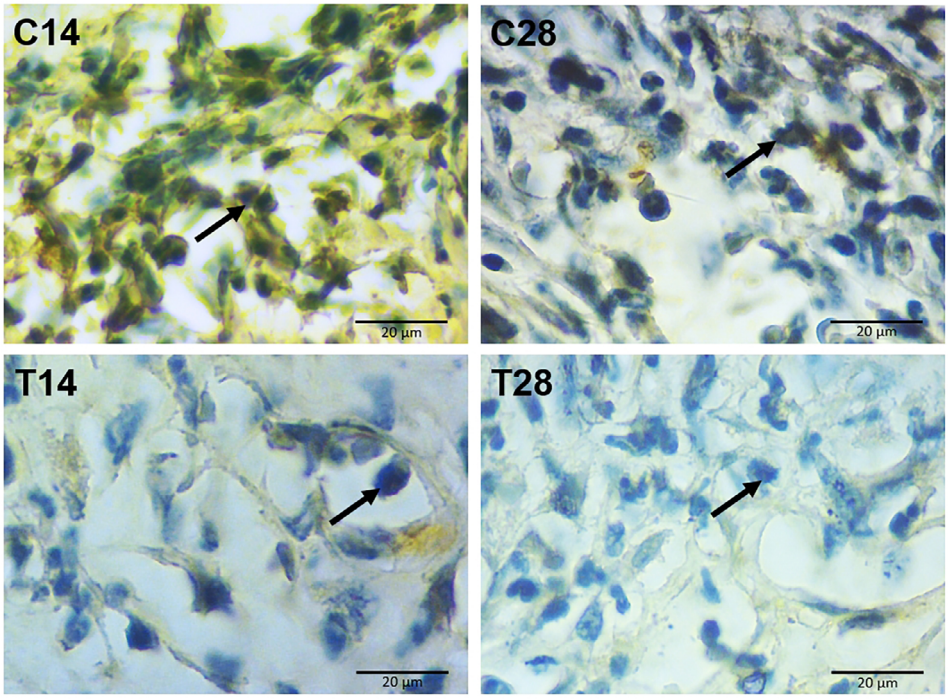

Figure 3- TNF-a expression in macrophages. The immune reactivity of macrophages in the apical area is indicated by black arrows (C14: control group at 14 days, C28: control group at 28 days, T14: MSCs treatment group at 14 days, T28: MSCs treatment group at 28 days). Scale bar: 20 µm.

Table 2- Significance of IL-1b expressions among groups

Groups	Control 14 days (7.88+2.44)	Control 28 days (9.32+1.00)	MSC 14 days (4.00+0.51)	MSC 28 days (4.88+1.52)
Control 14 days (7.88+2.44)		p=0.474	p=0.005*	p=0.033*
Control 28 days (9.32+1.00)	p=0.474		p=0.000*	p=0.002*
MSC 14 days (4.00+0.51)	p=0.005*	p=0.000*		p=0.804
MSC 28 days (4.88+1.52)	p=0.033*	p=0.002*	p=0.804	

*Significant difference

Significant differences in TNF-a expression were found between C14 and T14 (p=0.000); C14 and T28 (p=0.000); C28 and T14 (p=0.000); C28 and T28 (p=0.000). The significance of TNF-a expression between groups is shown in Table 3. The bar graph results of vascularization and inflammation between groups can be seen in Figure 4.

Discussion

The rat model to evaluate apical periodontitis and diabetes mellitus is well established. Diabetes mellitus induction using streptozotocin in animal models mirrors the mechanisms found in humans.[23] This is also true for the mechanism of apical periodontitis.[19] The use of Wistar rats in this study was advantageous due to the similarity of histological structures in rat and human teeth. Our study presents a comprehensive model using open pulp and root canal exposure to allow infection by oral microbes, leading to apical periodontitis in diabetic rats.

Inflammation in the apical area can be assessed through macrophage expression, which plays a crucial role in the progression of apical periodontitis, including

Table 3- Significance of TNF-a expressions among groups

Groups	Control 14 days (6.56+0.48)	Control 28 days (7.04+0.62)	MSC 14 days (2.64+0.43)	MSC 28 days (3.80+0.97)
Control 14 days (6.56+0.48)		p=0.666	p=0.000*	p=0.000*
Control 28 days (7.04+0.62)	p=0.666		p=0.000*	p=0.000*
MSC 14 days (2.64+0.43)	p=0.000*	p=0.000*		p=0.059
MSC 28 days (3.80+0.97)	p=0.000*	p=0.000*	p=0.059	

*Significant difference

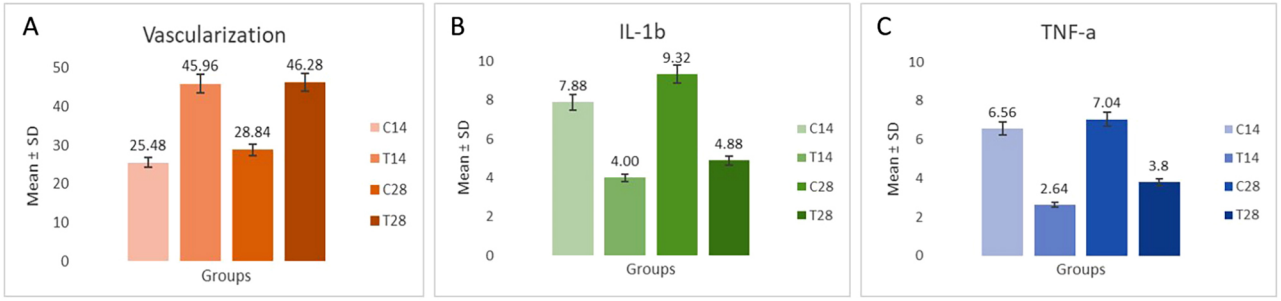

Figure 4- Bar graph results of vascularization and inflammation across control groups (C14 and C28) and MSCs treatment groups (T14 and T28). Vascularization (A), IL-1b (B), and TNF-a (C).

their migration and activation.[24] Macrophages, as dominant inflammatory cells, secrete and express proinflammatory cytokines such as IL-1b and TNF-a, which influence insulin sensitivity, signaling, and disrupt the M1/M2 macrophage equilibrium.[25]

Our findings attested that diabetes mellitus increases inflammation, as healing in the periapical tissues was not achieved, with IL-1b and TNF-a expression remaining stable at 14 and 28 days. The diabetic condition promotes the persistence of apical periodontitis. The consistent quantity of inflammatory cells is correlated to DM, in which constant hyperglycemia plays a critical role in tissue healing. Inflammation in diabetes may be activated by oxidative stress, due to prolonged hyperglycemia and high levels of advanced glycation end-products, which further activate pro-inflammatory pathways.[8]

IL-1b is more influential in the diabetic condition and is dominant in periapical lesions.[25,26] IL-1b expression in macrophages is triggered by endotoxins such as lipopolysaccharides.[27] Lipopolysaccharides, which are endotoxins of Gram-negative bacteria, activate pro-inflammatory cytokines such as IL-1b, IL-6 and IL-8, and lead to alveolar bone destruction periodontal tissue damage. IL-1b in apical periodontitis function in the inflammatory and healing phases, the immune response regulation, and bone resorption.[26] Most often, IL-1b expression decreases after endodontic treatment.[28] However, high macrophage expression of IL-1b in diabetic patients is closely associated with endodontic treatment failures.[29]

TNF-a, a key mediator released by initiated macrophages, is linked to the exacerbation and incidence of periodontitis in DM.[30] Similar to IL-1b, TNF-a is also triggered by endotoxins.[31] TNF-a expression correlates with macrophage levels in adjacent tissues and indicates the inflammation status.[32] TNF-a is well-known for its role in apical periodontitis, particularly in bone destruction and exacerbation of the condition.[33] It modulates cell differentiation, proliferation, autophagy, pro-inflammatory responses, and cell death.[31] TNF-a expression begins as early as day 7 after pulp exposure and is positively correlated with periodontal destruction in rats.[33]

Periodontal tissue repair in the apical area is a highly coordinated process involving growth factors, cytokine

release, as well as cell proliferation and migration.[8] Previous clinical studies and case reports in healthy subjects have shown promising results for allogenic cell-based RET in the treatment of periapical lesions and root perforations.[22,34] In our study with DM rats, MSC transplantation into the root canal significantly reduced IL-1b and TNF-a expressions. Without MSC transplantation, IL-1b and TNF-a expressions remained high despite root canal preparation and irrigation. The fact that macrophage expressions of IL-1b and TNF-a remained constant at 14 and 28 days is linked to the diabetic condition, in which continuous hyperglycemia plays an important part in the tissue healing mechanism. This is in line with a previous study in which the same bacterial stimulus resulted in more severe inflammation in diabetic animals when compared to normal ones, with diabetes significantly affecting susceptibility and severity of periodontal disease.[35] Another previous study found that higher IL-1b level in human apical periodontitis clinically lead to symptomatic lesions and histologically lead to the formation of radicular cysts.[36] In this study, decreased IL-1b expression would clinically and histologically give a positive impact on the healing of apical periodontitis.

MSCs from umbilical cords have several advantages, including their ability to proliferate, their primitive state, and their potential to differentiate into various progenitor cells.[37] A previous study with osteoporotic and diabetic animal models have shown improved bone condition with MSC treatment.[2,11] The biological mechanism of MSCs in inflammation involves increased PGE2 secretion to polarize the local macrophages from M1 (pro-inflammatory) to M2 (anti-inflammatory). MSCs also secrete IL-10, a key anti-inflammatory cytokine. Additionally, MSCs promote vascularization by activating β-catenin in endothelial cells through the Wnt signaling pathway. Other studies have demonstrated that MSCs from umbilical cords increase platelet-derived growth factors (PDGFs) and vascular endothelial growth factors (VEGFs), supporting angiogenesis for wound healing in DM patients.[38]

In our study, exogenous MSCs were transplanted into the root canal with the aim of simplifying their application into the apical area, potentially offering a useful approach for cell-based regenerative endodontic procedure in diabetic conditions. Exogenous MSC transplantation into the root canal addressed issues with the quality, quantity and differentiation ability of host MSCs in diabetes.[39] Prolonged hyperglycemia and high levels of advanced glycation end-products in diabetes mellitus negatively affect the alter proliferation and differentiation of host MSCs.[40]

Current therapies for diabetes mellitus involve insulin injections and glucose-controlling drugs. While these therapies are effective in controlling blood glucose levels, their impact on periodontal tissues requires further investigation.[41] A previous study stated that insulin does not improve metabolism, neuropathy, vascularization, and bone regeneration. Conversely, other studies attested that metformin might have a positive effect on apical lesions in animal models.[42] However, metformin has immunosuppressive properties and may lead to serious gastrointestinal side effects.[26,43] Despite endodontic treatment, diabetic patients often experience persistent periapical problems.[44]

Immune and systemic diseases are interconnected, sharing key mechanisms that influence the pathophysiology of apical periodontal tissues, including apical periodontitis. In our study, MSC treatment significantly increased vascularization in the apical area at both in 14 and 28 days. This finding may be related to the improved inflammatory condition, in which MSCs transplantation alter macrophages from the M1 to the M2 expression. With reduced inflammation, M2 macrophages produce and express anti-inflammatory mediators for instance transforming growth factor beta (TGFb), vascular endothelial growth factor (VEGF) and interleukin-10 (IL-10), as these mediators plays a crucial role in regeneration.[27,28]

This study focused in macrophage expression of IL-1b, TNF-a, and vascularization (blood vessels) at 14 and 28 days. Further studies ought to be conducted to explore the effects of MSC transplantation over longer observation periods and with other marker expressions. Future developments, including material enhancements and procedural improvements, are necessary for the clinical application of regenerative endodontic therapy. Clinically, these findings can serve as a reference for endodontists in the development of cell-based RET using MSCs from umbilical cords to treat persistent apical periodontitis in DM patients.

Conclusion

Our study confirmed that transplantation of MSCs from umbilical cords improves vascularization and

inflammation in apical periodontitis under diabetes mellitus conditions, specifically in terms of vasculature formation and the expression of interleukin 1b (IL-1b) and tumor necrosis alpha (TNF-a) at 14 and 28 days. Pulp revitalization will be our next focus of research, building on the positive outcomes from this study.

Conflict of interests

The authors declare no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Prasetyo, Eric Priyo: Conceptualization (Equal); Data curation (Equal); Formal analysis (Equal); Funding acquisition (Equal); Investigation (Equal); Methodology (Equal); Resources (Equal); Supervision (Equal); Writing - original draft (Equal); Writing - review & editing (Equal). **Juniarti, Devi Eka:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing - original draft (Equal). **Kuntjoro, Mefina:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing - original draft (Equal). **Hendrijantini, Nike:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing - original draft (Equal). **Putra, Aditya Arinta:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Software (Equal); Validation (Equal). **Oktaria, Wanda:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Software (Equal); Validation (Equal). **Hutapea, Zellita Frestica Rosmaida Devi:** Data curation (Equal); Formal analysis (Equal); Investigation (Equal); Software (Equal); Validation (Equal). **Tjendronegoro, Evelyn:** Conceptualization (Equal); Methodology (Equal); Resources (Equal); Writing - original draft (Equal); Writing - review & editing (Equal).

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