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ORIGINAL ARTICLE

Effect of Robusta Coffee Bean Extract as Immunoregulator on Dental Pulp Mesenchymal Stem Cells via Paracrine Pathway

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ABSTRACT

Objective: To analyze the effect of Robusta Coffee Bean Extract (RCBE) on Dental Pulp Mesenchymal Stem Cells (DPSCs) culture in secreting cytokines, growth factors, and cell differentiation. **Material and Methods:** DPSCs culture from premolar human teeth only and DPSCs culture from premolar human teeth given RCBE with concentrations of 0.0625%, 0.125%, 0.25%, and 0.5% for 24 hours, 48 hours, and 72 hours. The secretome of the DPSCs culture was examined for TNF-α, IFN-γ, IGF, and VEGF, examination of SOX2 and Oct4, and Wnt differentiation markers by ELISA. Statistical analysis used ANOVA and continued with LSD. **Results:** There was a significant reduction in the levels of TNF-α and IFN-γ at 0.25% RCBE concentration at 72 hours of immersion ($p<0.05$). Growth factor levels of IGF and VEGF increased when given 0.25% RCBE compared to the other groups, and the differentiation markers SOX2 and Oct4 and Wnt also increased at a concentration of 0.25% at 72 hours of immersion ($p<0.05$). **Conclusion:** Giving RCBE with a concentration of 0.25% can reduce inflammatory cytokines and increase growth factors and differentiation markers in DPSCs cultures.

Keywords: Dental Pulp; Mesenchymal Stem Cells; Secretome; Herbal.

Introduction

The use of mesenchymal stem cells with herbal active ingredients is gaining popularity because of its potential in repairing and regenerating periodontal tissue [1-3]. Mesenchymal Stem cell secretion bioactive ingredients such as cytokines, chemokines, and growth factors can accelerate the healing process [4,5]. Herbal active ingredients such as Robusta Coffee Bean Extract (RCBE) contain flavonoids, caffeine, phenolic compounds, trigonelline, and chlorogenic acid. Indonesia is the 3rd largest coffee producer in the world, and nearly 64.4% of Indonesia's population drinks coffee every day [1,6,7].

RCBE is an immunoregulatory agent mesenchymal stem cell (MSCs) that regulates and stimulates the host's immune system to be active, as well as the active ingredients of RCBE as anti-inflammatory, antioxidant, and antibacterial [8].

Recently, research on MSCs has shown that they play an important role in regulating the microenvironment as an immunoregulator of immune cells through a paracrine pathway [9,10]. MSCs secrete various cytokines and growth factors that modulate adaptive and innate immune cells [11,12]. Dental pulp Mesenchymal Stem Cells (DPSCs) culture that is added by RCBE generates secretome, which decreases inflammatory cytokine and growth factor and differentiation [13,14]. Those results will replace damaged cells and activate communication between cells [15]. This paracrine pathway will stimulate stem cells' endogenous by secreting soluble factors or bioactive ingredients in the periodontal tissue so it can accelerate healing [16,17].

Previous research stated that robusta coffee bean extract (RCBE) did not cause toxicity in DPSCs cultures with a concentration of 0.0625% and 0.125% for 72 hours with the MTT test [13]. Martin et al. [1] stated that the combination of the DPSCs stem cell secretome and robusta coffee bean extract can enhance the markers osteocalcin (OCN) and alkaline phosphatase (ALP) in periodontitis-model rats. Robusta coffee bean extract can accelerate wound healing on diabetic ulcers, reduce neutrophil cells, and increase fibroblast cell ulcers [18].

DPSC had a fusiform shape, with a vast cytoplasm, a cell nucleus in the center, and a tapering tip. DPSC surface expression indicators were observed using a flow cytometry (FCM) test to quantify and examine the surface properties of the stem cells used in this study. The mesenchymal stem cell lineage markers CD90 and CD105 were used as positive controls. In addition, the hematopoietic stem cell lineage markers CD34 and CD45 were used as negative controls [13]. This study aimed to analyze RCBE as an immunoregulator on DPSC secretion cytokine and differentiation through the paracrine pathway.

Material and Methods

Ethical Clearance

This research was approved by The Ethical Committee of Medical Research Faculty of Dentistry University of Jember (No.2145/UN.25.8/KEPK/DU 2023).

Sample Criteria (Pulp Extraction from Human)

Human dental pulp with inclusion criteria: patients aged between 19-29 years, 1st premolars or 2nd premolar for orthodontic treatment indications or 3rd mandibular molars for odontectomy indications, healthy patients, free from infectious and contagious diseases.

DPSCs Culture Research Procedures

Extracted intact teeth (less than 6 hours), washed 3 times with phosphate-buffered saline (PBS) (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA), cut on the cementum-enamel junction with dental carbide bur (SS White Dental, Lakewood, NJ, USA) and high-speed (W&H Group, Bürmoos, Austria) until the pulp chamber opens, immersing the tooth in cold PBS (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA) intermittently to avoid heating while cutting the tooth. Separate the pulp tissue from the pulp chamber with a dental excavator. The dental pulp tissue is taken and transferred inside a tube containing a complete culture medium. The intercellular bonds are then separated using pipetting. Furthermore, the pulp tissue was washed twice by centrifugation (2000 g/min) for 10 minutes. After that, the supernatant was removed and the pellet dissolved in a complete culture medium. Then put into the media solution containing collagenase type I (Worthington USA) dispase and gentamycin were added (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA). MSCs were cultured with α-modified minimum essential medium eagle (αMEM) (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA) plus 15% fetal bovine serum (Biowest LCC, Riverside, MO, USA), 2 mM of L-glutamine (Thermo Fisher Scientific Inc., Waltham, MA, USA), 100 mg/mL streptomycin (Thermo Fisher Scientific Inc., Waltham, MA, USA), 2.5 µg/mL fungizone (Thermo Fisher Scientific Inc., Waltham, MA, USA), and 100 IU/mL penicillin (Gibco Thermo Fisher Scientific Inc., Waltham, MA, USA) before being incubated at 37°C with 5% CO_2.

The cells were grown in six wells on a tissue culture plate at a concentration of 107 in each well. The medium was changed on the 7th day and every 3 days thereafter. Observation of the cells was performed using an inverted microscope (80× magnification). The cell culture used in the experiment was a cell culture that had been harvested 2-3 times (passages). Then, do the calculation of the number of cells/ml with trypan blue exclusion test using a hemocytometer (Assistant, Copens Scientific, Selangor, Malaysia) observed under a microscope Leica DM500 (Leica Microsystems Inc., Wetzlar, Germany) with a 20x objective. Cells were cultured on 96well plates, and every well contained 1 x 106 cells/ml and incubated at 37°C and 5% CO_2 for 24 hours. Immunofluorescence characterization was performed with CD105 FITC and CD35 FTIC (Becton, Dickinson and Company, Franklin Lake, NJ, USA).

Preparation of Robusta Coffee Bean Extract

Production of coffee bean extract. Dried coffee beans that are in the city of Jember, Indonesia, then aerated briefly and dried/dried in the sun. After that, the coffee beans were ground using a blender to obtain coffee bean extract and then sieved to obtain a uniform 100 mesh size powder. For seventy-two hours, the powder was immersed in a 1:5 ratio of 96% ethanol and mixed twice daily. The filter paper was used to create filtrate and residue from the immersion results. A rotary evaporator was used to evaporate the filtrate and produce a viscous extract. The extract with a concentration was diluted using aquadest and stored in a tightly closed container and divided into five groups: 1) Control (DPSCs), 2) DPSCs with 0.0625% RCBE, 3) DPSCs with 0.125% RCBE, 4) DPSCs with 0.25% RCBE, and 5) DPSCs with 0.5% RCBE.

Inflammation Test

TNF-α and IFN-γ inflammatory assay and IGF and VEGF. Secretome stem cells plus robusta coffee bean extract with various concentrations were put in 24-well plates and then incubated for 24 hours, 48 hours, and 72 hours at 37 °C with 5% CO_2. The supernatant from the media was taken and analyzed for TNF-α, IFN-γ, IGF and VEGF levels using the ELISA kit.

The ELISA kits are coated with monoclonal antibody specific, standards and test samples are added. 50 µL of standard solution is added to the standard well. Then, 50 µL of streptavidin-HRP is added to the sample well and standard well. The plate is incubated for 60 minutes at 37 °C. Then, the seal is opened, and the plate is washed using wash buffer. Add 50 µl of stopping solution to each well. Colorimetric absorption was recorded at a wavelength of 450 nm with an ELISA reader.

Test differentiation

The differentiation test looked at the SOX2 and Oct4 and Wnt signal markers. Robusta coffee bean extract with various concentrations added to stem cell media. Dental pulp stem cells were grown at a density of $2x10^4$ cells and up to 80% growth and then fixed with 3% paraformaldehyde (Millipore) for 15 minutes. Then incubated with Oct4 (1:100 dilution), SOX2 (1:50 dilution), and Wnt (1:100 dilution) overnight at 4 °C. Samples were washed three times in PBS (Gibco) and incubated with secondary antibody for 3 hours at room temperature. Sections were washed thoroughly in PBS (Gibco) and counterstained with hematoxylin. The primary antibody was replaced with PBS (Gibco) as a negative control.

Statistical Analysis

Data were analyzed using SPSS 26 software, with the ANOVA test followed by LSD with a significance value of $p>0.05$.

Results

This study confirmed that DPSCs have a fusiform shape with a tapered tip, a cell nucleus in the middle, and a large cytoplasm commonly referred to as a fibroblast-like spindle formation based on observations of morphological characteristics. These outcomes follow the past hypothesis, which affirmed that DPSCs, which are mesenchymal foundational microorganisms, have a morphology like fibroblast cells (Figure 1).

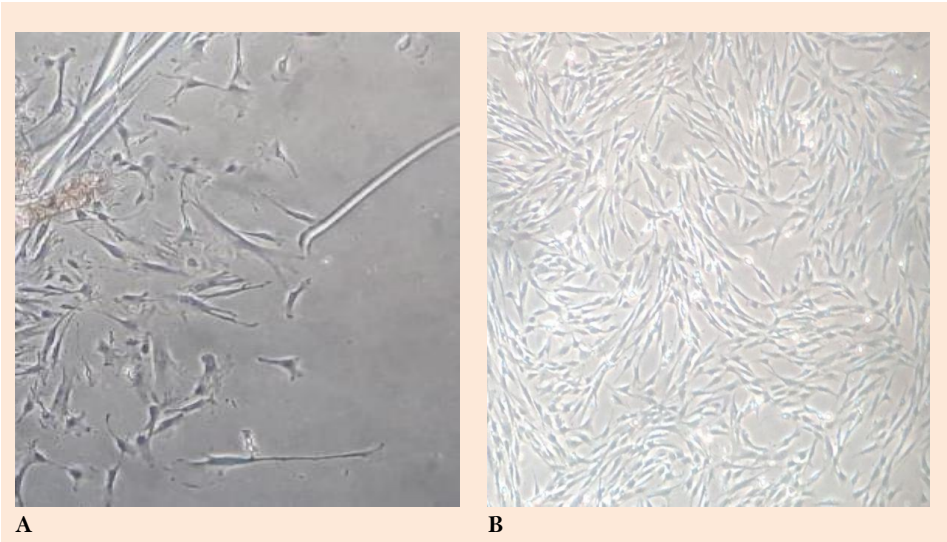

Figure 1. Morphology DPSCs culture. (A) Morphology DPSCs fibroblastic-like at passage 1; (B) Morphology DPSCs fibroblastic-like until confluence at day 14.

Using the flow cytometry (FCM) test, other characteristics were observed by analyzing DPSCs surface expression markers. The FCM test revealed that CD105 was expressed but not CD35. Antibodies that are

specific for CD105 are markers of the mesenchymal stem cell lineage. The specific antibodies CD35, on the other hand, are indicators of the hematopoietic stem cell lineage. This study's findings are consistent with previous hypotheses (Figure 2).

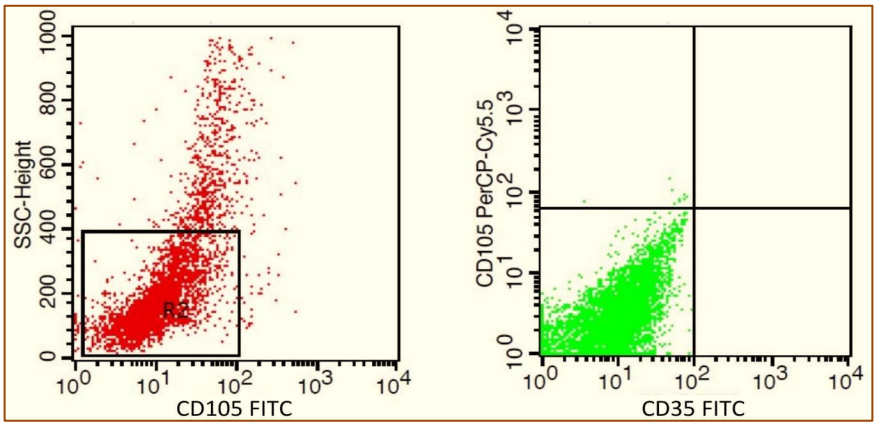

Figure 2. Flow cytometry examination showed that the majority of the DPSCs subpopulation expressed approximately 99.63% CD105 FITC and a minority of subpopulations expressed approximately 3% CD35 FITC.

TNF-α, IFN-γ, VEGF, and IGF-1 levels in the control treatment and administration of robusta coffee bean extract with concentrations of 0.0625%, 0.125%, 0.25%, and 0.5% with immersion times of 24 hours, 48 hours and 72 hours show that VEGF and IGF-1 levels at a concentration of 0.125% and 72 hours showed very high results compared to the control and other treatment groups ($p \leq 0.05$). Meanwhile, TNF-α and IFN-γ at concentrations of 0.125% and 72 hours showed low results compared to control and other treatment groups (Figure 3).

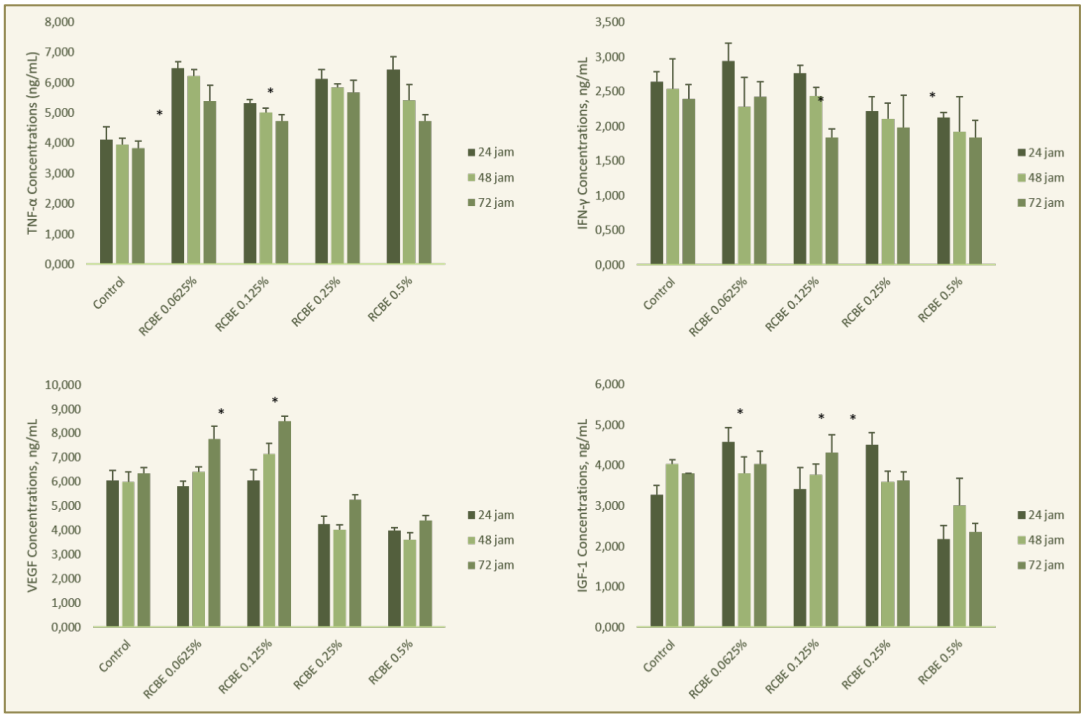

Figure 3. The results of TNF-α, IFN-γ, VEGF, and IGF-1 levels in each control group and the robusta coffee bean extract group from various concentrations and times showed a significant difference ($p \leq 0.05$)

SOX2, Oct4, and Wnt levels in the control treatment and administration of robusta coffee bean extract with concentrations of 0.0625%, 0.125%, 0.25%, and 0.5% with immersion times of 24 hours, 48 hours, and 72 hours show that SOX2, Oct4, and Wnt levels at a concentration of 0.125% and 72 hours showed very high results compared to the control and other treatment groups ($p \leq 0.05$) (Figure 4).

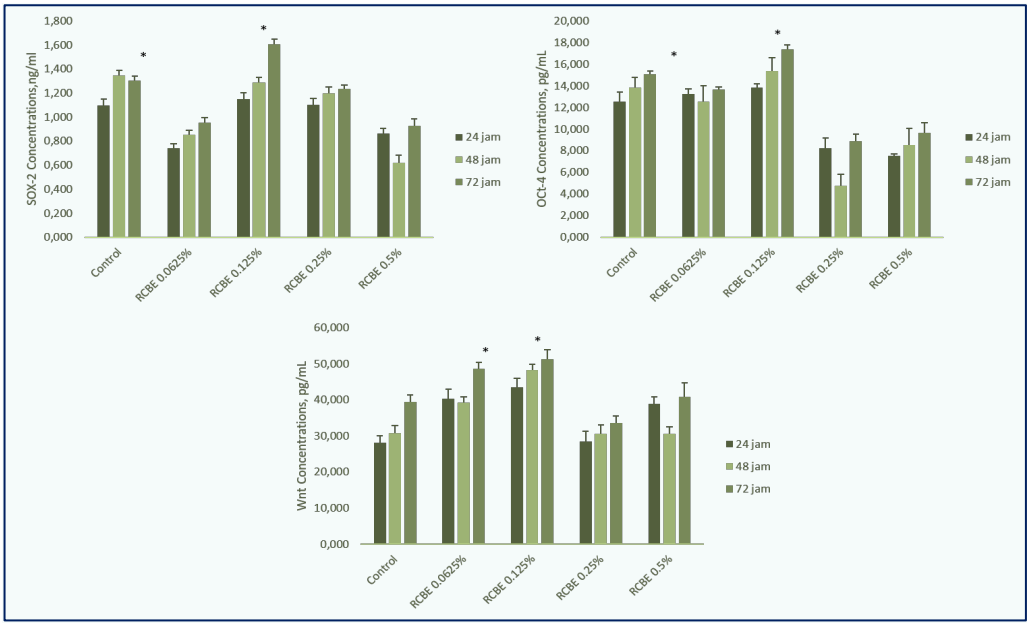

Figure 4. The results of SOX2, Oct4, and Wnt levels in each control group and the robusta coffee bean extract group from various concentrations and times showed a significant difference ($p<0.05$).

Discussion

MSCs' therapeutic effects are mediated by a variety of mechanisms. The most significant mechanisms are the immunoregulator and secretory properties [15]. Inflammatory cells produce cytokines such as (TNF-α, and IFN-γ) [6,20-22]. MSCs secrete growth factors such as (VEGF, and IGF-1) as well as markers of cell differentiation (SOX2, Oct4, and Wnt) [23-26]. Recently, medicinal plants have been considered as therapeutic triggers stimulus for the proliferation and differentiation of DPSCs in vitro and in vivo [27-29]. The coffee extract can be widely accepted by the public where there are bioactive compounds that have the potential to activate MCSc cells to secrete TNF-α, IFN-γ, VEGF, IGF-1, SOX2, Oct4, and Wnt [1].

TNF-α plays an important role in acute and chronic inflammation with cell infiltration through neutrophil and lymphocyte adhesion and stimulates neutrophils to release cytokines and chemokines [30]. Meanwhile, IFN-γ induces autophagy to remove pathogens within the cell and increases pro-inflammatory cytokine secretion, as well as enhanced antigen processing and presentation through upregulation of class II MHC by activating macrophages and improving their ability to mount an effective immune response [31,32]. Through enhanced phagocytosis, pro-inflammatory responses, and lymphocyte recruitment, enhanced immune activation ultimately leads to effective pathogen clearance [33,34]. Over-action of IFN-γ has been accounted for to cause inordinate tissue harm, putrefaction, and aggravation and may add to infection pathology [35,36].

Numerous biological functions, including cell adhesion, chemotaxis, blood vessel development regulation, hematopoietic stem cell development, extracellular matrix remodeling, and inflammatory cytokine regeneration, are thought to involve VEGF [37,38]. Whereas IGF-1 is a growth factor produced by fibroblasts and other epithelial cells, this has an important role in the process of epithelization and granulation of tissue

during the wound healing process [39]. One of the roles of vascular endothelial insulin/IGF-1 is to provide vascular homeostasis to the neovascularization during the wound healing process [40].

The differentiation of DPSCs is regulated by multiple signaling pathways, such as enhancing osteoblast function and regulating bone mass [43,44]. Meanwhile, SOX2 is associated with Beta-catenin in osteoblasts and can inhibit Wnt activity [45,46]. SOX2 and Oct4 genes regulate stem cell function in regulating differentiation and interact with Wnt to maintain pluripotency [47,48]. Giving robusta coffee bean extract to MSCs culture can increase differentiation markers [1,13].

Based on the attained data, TNF-α and IFN-γ levels decreased after induction of RCBE 0.125% in DPSCs cultured for 72 hours compared to the control group. Whereas VEGF and IGF-1 levels at a concentration of 0.125% and 72 hours showed very high results compared to the control and other treatment groups. The role of robusta coffee bean extract in cytokine production is to reduce TNF-α and IFN-γ and increase VEGF and IGF-1 production after 72 hours. Thus, inhibition of TNF-α and IFN-γ and enhancement of VEGF, IGF-1, SOX2, Oct4, and Wnt can reduce the severity of inflammation and accelerate the regeneration in the healing process.

This study showed that the right concentration of RCBE is 0.125%. It is based on previous studies that stated RCBE at 0.1 mM increased osteoprotegerin gene expression and could inhibit osteoclast differentiation, had pro-osteogenic and anti-adipogenic properties, whereas, at 0.3 mM dose, it had anti-osteogenic properties and couldn't regulate DPSCs cell differentiation [13,49].

Conclusion

Administration of robusta coffee bean extract can reduce inflammation and increase growth factors and differentiation markers in dental pulp mesenchymal stem cell cultures.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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References

[1] Martin M, Sari DS, Mantika RA, Praharani D. Combination of dental pulp stem-cell secretome and robusta coffee bean extract (Coffea canephora) in enhancing osteocalcin and alkaline phosphatase expression in periodontitis-induced Wistar rats. J Orofac Sci 2021; 13(2):136-141. https://doi.org/10.4103/jofs.jofs_157_21

[2] Massieh CM, El Zainy MA, Amin RMM, Fathy IA. Systemic versus local injection of bone marrow mesenchymal stem cells on 5-fluorouracil treated parotid glands of albino rats. Braz Dent Sci 2021; 24(4 suppl 1):1-13. https://doi.org/10.4322/bds.2021.e3104

[3] Costa FH, Dutra MJ, Vasconcellos LMR, Vegian MRC, Silva CD, Santos HFS, et al. Effect of radiotherapy on the differentiation and osteogenic activity of mesenchymal stem cells on dental implants. Braz Dent Sci 2023; 26 (1):e3660. https://doi.org/10.4322/bds.2023.e3660

[4] Ogata K, Moriyama M, Matsumura-Kawashima M, Kawado T, Yano A, Nakamura S. The therapeutic potential of secreted factors from dental pulp stem cells for various diseases. Biomedicines 2022; 10(5):1049. https://doi.org/10.3390/biomedicines10051049.

[5] Greene CJ, Anderson S, Barthels D, Howlader MSI, Kanji S, Sarkar J, et al. DPSC products accelerate wound healing in diabetic mice through induction of SMAD molecules. Cells 2022; 11(15):2409. https://doi.org/10.3390/cells11152409

[6] Hernawati S, Sutjiati R, Sulistiyani, Martin M, Fadiyah SN. The effect of cacao bean extract on the number of osteoblasts on orthodontic tooth movement. J Int Dent Med Res 2023; 16(2)(22):594-599.

[7] Sandra Sari D, Martin M, Maduratna E, Basuki Notobroto H, Mahyudin F, Sudiana K, et al. Combination adipose-derived mesenchymal stem cells-demineralized dentin matrix increase bone marker expression in periodontitis rats. Aaudi Dent J 2023; 35(8):960-968. https://doi.org/10.1016/j.sdentj.2023.07.019

[8] Abdelgawad LM, Hasan MN, Sabry D, Abdelgwad M. Influence of photobiomodulation and tricalcium silicate-based cement on viability and dentinogenic activity of cultured human dental pulp stem cells. Braz Dent Sci 2022; 25(1):e2497. https://doi.org/10.4322/bds.2022.e2497

[9] L. PK, Kandoi S, Misra R, S. V, K. R, Verma RS. The mesenchymal stem cell secretome: A new paradigm towards cell-free therapeutic mode in regenerative medicine. Cytokine Growth Factor Rev 2019; 46:1-9. https://doi.org/10.1016/j.cytogfr.2019.04.002.

[10] Pankajakshan D, Agrawal DK. Mesenchymal stem cell paracrine factors in vascular repair and regeneration. J Biomed Technol Res 2014; 1(1):1-21. https://doi.org/10.19104/jbtr.2014.107

[11] Shah D, Lynd T, Ho D, Chen J, Vines J, Jung HD, et al. Pulp-dentin tissue healing response: A discussion of current biomedical approaches. J Clin Med. 2020; 9(2):434. https://doi.org/10.3390/jcm9020434.

[12] Chow L, Johnson V, Impastato R, Coy J, Strumpf A, Dow S. Antibacterial activity of human mesenchymal stem cells mediated directly by constitutively secreted factors and indirectly by activation of innate immune effector cells. Stem Cells Transl Med 2020; 9(2):235-249. https://doi.org/10.1002/sctm.19-0092

[13] Sari DS, Sakinah N, Nuri, Suswati E, Widyowati R, Maduratna E. Chlorogenic acid fractionation in robusta green bean extract as a combination agent of dental pulp stem cells in periodontal tissue engineering. Res J Pharm Technol 2022; 15(11):5005-5010. https://doi.org/10.52711/0974-360X.2022.00841

[14] Pérez-Jimenez VR, Chumpitaz-Cerrate VM, Chávez-Rimache LK, Moreno-Villalobos DE, Rodríguez-Flores AA, Franco-Quino CI, et al. Synergistic anti-inflammatory effect of NSAIDs plus B vitamins administered pre and postoperatively in third molar surgery: Randomized clinical trial. Braz Dent Sci 2022; 25(4):e3441 https://doi.org/10.4322/bds.2022.e3441

[15] Hernández-Monjaraz B, Santiago-Osorio E, Monroy-García A, Ledesma-Martínez E, Mendoza-Núñez VM. Mesenchymal stem cells of dental origin for inducing tissue regeneration in periodontitis: A mini-review. Int J Mol Sci 2018; 19(4):944. https://doi.org/10.3390/ijms19040944.

[16] Sudarmono, Widyaputra S, Sitam S, Suherna I, Fitri AD, Rachman A. Comparison of bone regeneration in hADMSC versus hUCBMSC with hBMMSC as a reference: A literature review of potential bone regeneration. Res J Pharm Technol 2021; 14(4):1993-1998. https://doi.org/10.52711/0974-360X.2021.00353

[17] Nagata M, Iwasaki K, Akazawa K, Komaki M, Yokoyama N, Izumi Y, et al. Conditioned medium from periodontal ligament stem cells enhances periodontal regeneration. Tissue Eng Part A 2017; 23(9-10):367-377. https://doi.org/10.1089/ten.TEA.2016.0274

[18] Yulianti Y, Ibrahim K, Kurniawan T. Effect of wound care using robusta coffee powders on diabetic ulcer healing in Sekarwangi Hospital Sukabumi. Padjadjaran Nurs J 2018; 6(1). https://doi.org/10.24198/jkp.v6i1.412

[19] Shear M, Speight P. Cysts of the salivary glands. In: Shear M, Speight P. Cysts of the Oral and Maxillofacial Regions. Hoboken: John Wiley & Sons; 2007. pp.171-180.

[20] Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomol Concepts 2018; 9(1):64-79. https://doi.org/10.1515/bmc-2018-0007.

[21] Neto ND, Porpino MTM, Antunes HDS, Rodrigues RCV, Perez AR, Pires FR, et al. Pro-inflammatory and anti-inflammatory cytokine expression in post-treatment apical periodontitis. J Appl Oral Sci 2018; 26:e20170455. https://doi.org/10.1590/1678-7757-2017-0455.

[22] Akcalı A, Kahraman Çenelı S, Meriç P, Nalbantsoy A, Özçaka Ö, Buduneli N. Altered levels of inhibitory cytokines in patients with thalassemia major and gingival inflammation. Braz Dent Sci 2019; 22(3):349-357.
[23] Ren B, Feng Q, He S, Li Y, Fan J, Chai G, et al. VEGF as a potential molecular target in periodontitis: a meta-analysis and microarray data validation. J Inflamm 2021; 18(1):18. https://doi.org/10.1186/s12950-021-00281-9
[24] Shahsavari A, Weeratunga P, Ovchinnikov DA, Whitworth DJ. Pluripotency and immunomodulatory signatures of canine induced pluripotent stem cell-derived mesenchymal stromal cells are similar to harvested mesenchymal stromal cells. Sci Rep 2021; 11:3486. https://doi.org/10.1038/s41598-021-82856-3 11(1)
[25] Schizas NP, Zafeiris C, Neri AA, Anastasopoulos PP, Papaioannou NA, Dontas IA. Inhibition versus activation of canonical Wnt-signaling, to promote chondrogenic differentiation of mesenchymal stem cells. A review. Orthop Rev 2021; 13(2):27098. https://doi.org/10.52965/001c.27098
[26] Zhang Z, Nör F, Oh M, Cucco C, Shi S, Nör JE. Wnt/β-catenin signaling determines the vasculogenic fate of postnatal mesenchymal stem cells. Stem Cells 2016; 34(6):1576-1587. https://doi.org/10.1002/stem.2334
[27] Bouhtit F, Najar M, Agha DM, Melki R, Najimi M, Sadki K, et al. The biological response of mesenchymal stromal cells to thymol and carvacrol in comparison to their essential oil: An innovative new study. Food Chem Toxicol 2019; 134:110844. https://doi.org/10.1016/j.fct.2019.110844
[28] Bouhtit F, Najar M, Rahmani S, Melki R, Najimi M, Sadki K, et al. Bioscreening and pre-clinical evaluation of the impact of bioactive molecules from Ptychotis verticillata on the multilineage potential of mesenchymal stromal cells towards immune- and inflammation-mediated diseases. Inflamm Res 2022; 71(7-8):887-898. https://doi.org/10.1007/s00011-022-01573-3
[29] Maeda A. Recruitment of mesenchymal stem cells to damaged sites by plant-derived components. Front Cell Dev Biol 2020: 8:437. https://doi.org/10.3389/fcell.2020.00437
[30] Ogawa S, Kitaura H, Kishikawa A, Qi J, Shen WR, Ohori F, et al. TNF-α is responsible for the contribution of stromal cells to osteoclast and odontoclast formation during orthodontic tooth movement. PLoS One 2019; 14(10):e0223989. https://doi.org/10.1371/journal.pone.0223989
[31] Zhang F, Si M, Wang H, Mekhemar MK, Dörfer CE, Fawzy El-Sayed KM. IL-1/TNFα inflammatory and anti-inflammatory synchronization affects gingival stem/progenitor cells' regenerative attributes. Stem Cells Int 2017; 2017:1349481. https://doi.org/10.1155/2017/1349481
[32] Xu C, Evensen Ø, Munang'andu HM. Transcriptome analysis shows that IFN-I treatment and concurrent SAV3 infection enriches MHC-I antigen processing and presentation pathways in Atlantic salmon-derived macrophage/dendritic cells. Viruses 2019; 11(5):464. https://doi.org/10.3390/v11050464
[33] Kornstädt L, Pierre S, Weigert A, Ebersberger S, Schäufele TJ, Kolbinger A, et al. Bacterial and fungal toll-like receptor activation elicits type I IFN responses in mast cells. Front Immunol 2021; 11:607048. https://doi.org/10.3389/fimmu.2020.607048
[34] Yang SL, Tan HX, Niu TT, Liu YK, Gu CJ, Li DJ, et al. The IFN-γ-IDO1-kynureine pathway-induced autophagy in cervical cancer cell promotes phagocytosis of macrophage. Int J Biol Sci 2021; 17(1):339-352. https://doi.org/10.7150/ijbs.51241
[35] Tan AHM, Sanny A, Ng SW, Ho YS, Basri N, Lee AP, et al. Excessive interferon-α signaling in autoimmunity alters glycosphingolipid processing in B cells. J Autoimmun 2018; 89:53-62. https://doi.org/10.1016/j.jaut.2017.11.004
[36] Cheng X, Chi L, Lin T, Liang F, Pei Z, Sun J, et al. Exogenous monocyte myeloidderived suppressor cells ameliorate immune imbalance, neuroinflammation and cognitive impairment in 5xFAD mice infected with Porphyromonas gingivalis. J Neuroinflammation 2023; 20(1):55. https://doi.org/10.1186/s12974-023-02743-8
[37] Kulikova N V., Litvinova LS, Shperling N V., Ivanov A V. Association of VEGF cytokine levels and single nucleotide polymorphisms of the VEGF-Agene with the genital endometriosis in the female population of the Northwestern Federal District of Russia. Gynecology 2022; 24(4):289-293. https://doi.org/10.26442/20795696.2022.4.201771
[38] Swidan SA, Elazab S, Hassan MMA. Effect of thymoquinone on skeletal muscle regeneration via assessment of Pax-7 and Myo-D expression in the DMBA-treated hamster pouch. Braz Dent Sci 2023; 26(2):e3620 https://doi.org/10.4322/bds.2023.e3620
[39] Seyed Ahmadi SG, Farahpour MR, Hamishehkar H. Topical application of Cinnamon verum essential oil accelerates infected wound healing process by increasing tissue antioxidant capacity and keratin biosynthesis. Kaohsiung J Med Sci 2019; 35(11):686-694. https://doi.org/10.1002/kjm2.12120
[40] Yang Y, Zhang Y, Zhu Y, Xia F. The effect of hyperbaric oxygen therapy on serum HMGB-1, GSN, IGF-1, vascular endothelial function and immune function in patients with hypertensive cerebral hemorrhage. Acta Med Mediterr 2021; 37(1):281-285. https://doi.org/10.19193/0393-6384_2021_1_43
[41] Jiang W, Wang D, Alraies A, Liu Q, Zhu B, Sloan AJ, et al. Wnt-GSK3β/β-catenin regulates the differentiation of dental pulp stem cells into bladder smooth muscle cells. Stem Cells Int 2019; 2019:8907570. https://doi.org/10.1155/2019/8907570
[42] Vallée A, Lecarpentier Y, Vallée JN. Cannabidiol and the canonical WNT/β-catenin pathway in glaucoma. Int J Mol Sci 2021; 22(7):3798. https://doi.org/10.3390/ijms22073798.
[43] Shen L, Sharma D, Yu Y, Long F, Karner CM. Biphasic regulation of glutamine consumption by WNT during osteoblast differentiation. J Cell Sci 2021; 134(1):jcs251645. https://doi.org/10.1242/jcs.251645

[44] Sari DS, Pujiastuti P, Fatmawati DWA, Mardiyana MA, Wulandari AT, Arina YMD. Inhibiting the growth of periopathogenic bacteria and accelerating bone repair processes by using robusta coffee bean extract. Saudi Dent J 2023; 35(4):322-329. https://doi.org/10.1016/j.sdentj.2023.03.007

[45] Lefebvre V. Roles and regulation of SOX transcription factors in skeletogenesis. Curr Top Dev Biol 2019; 133:171-193. https://doi.org/10.1016/bs.ctdb.2019.01.007.

[46] Gagliardi CF, Vasconcellos LMR, Cairo CAA, Rabelo SB, Prado RF. Expression of BMP ii by human osteoblasts cultivated on dense or porous titanium. Braz Dent Sci 2018; 21(3):275-281.

[47] Singh N, Padi SKR, Bearss JJ, Pandey R, Okumura K, Beltran H, et al. PIM protein kinases regulate the level of the long noncoding RNA H19 to control stem cell gene transcription and modulate tumor growth. Mol Oncol 2020; 14(5):974-990. https://doi.org/10.1002/1878-0261.12662

[48] Pecori F, Akimoto Y, Hanamatsu H, Furukawa JI, Shinohara Y, Ikehara Y, et al. Mucin-type O-glycosylation controls pluripotency in mouse embryonic stem cells via Wnt receptor endocytosis. J Cell Sci 2021; 133(20):jcs245845. https://doi.org/10.1242/jcs.245845

[49] Araújo JCR, Espírito Santo LL, Schneider SG, Santana Melo GF, Chaves I, Franzini DR, et al. Influence of titanium nanotubular surfaces, produced by anodization, on the behavior of osteogenic cells: In vitro evaluation. Braz Dent Sci 2022; 25(1):e2528. https://doi.org/10.4322/bds.2022.e2528