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TNF-α and IL-10 as paracrine effect of encapsulated mesenchymal stem cells coating by platelet lysate

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ABSTRACT

Mesenchymal stem cells (MSCs) have been used as a cellular therapy for infectious and degenerative diseases due to their paracrine effect, immunomodulatory capability, high ability differentiation, and high plasticity. The paracrine effect of MSCs releases many growth factors and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10), enabling them to modulate the immune system. Nevertheless, there are many obstacles to maintaining paracrine effects in cellular therapy due to a shortage of cellular retention. MSC encapsulation provides a favourable environment for the enhanced viability of MSCs. Platelet lysate is comprised of many growth factors that support the paracrine effect of mesenchymal stem cells (MSCs). In this study, MSCs were encapsulated within alginate, crosslinked using calcium chloride (CaCl2), and subsequently coated with platelet lysate. Encapsulated MSCs coated by platelet lysate were cultured for 21 days and analyzed for IL-10 and TNF-α levels. The findings of our study performed that TNF-α in encapsulated mesenchymal stem cells (MSCs) coated with platelet lysate increased until day 21. IL-10 was retained within the capsule and detected very in day 14. This study showed that encapsulated MSCs coated with platelet lysate affected paracrine effect TNF-α of MSC and retained IL-10 inside the capsule.

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INTRODUCTION

MSCs are utilized in clinical for their immunomodulatory and therapeutic properties in ischemic tissue repair (Liang et al., 2014). Besides their ability to differentiate into several cell types, MSCs have been recognized for their substantial paracrine capacity, the primary mechanism responsible for tissue repair and regeneration(Driscoll & Patel, 2019).

MSCs possess the ability to modulate the innate and adaptive immune systems through the production of anti-inflammatory cytokines and compounds. Paracrine produced by mesenchymal stem cells also contains growth factors and cytokines which are important in tissue regeneration, immunoregulatory and anti-inflammatory (Yani & Pawitan, 2023). MSCs may be responsible as immunomodulatory by an increased concentration of cytokines, including TNF-α, IL-1, IL-6, and IL-10 (Driscoll & Patel, 2019). The immunomodulatory capacity of MSCs was enhanced by factors secreted by both pro-inflammatory and anti-inflammatory macrophages (Saldaña et al., 2019). The immunosuppressive ability of MSCs is associated with the production of many cytokines, including TGF-α, IDO, NO, PGE2, IL-10, and TSG-6. TNF-α and IL-10 regulate inflammatory responses(R. Chen et al., 2022)(Z. Chen et al., 2023). The anti-inflammatory IL-10 are attributed to its ability to inhibit the synthesis of pro-inflammatory cytokines, such as IFN- α, IL-2, and TNF-α (Kandilarov et al., 2023)(Markovics et al., 2021)(Porro et al., 2020). According to a prior study, it was observed that TNF-activated MSCs exhibit anti-inflammatory effects by stimulating the production of IL-10 in macrophage cells in septic mice. Ultimately, this intervention proved efficacious in prolonging the survival of mice (Tsuji et al., 2014). IL-10 has been shown to potentially mitigate the inflammatory response and attenuate the adverse effects associated with natural killer T cells (Driscoll & Patel, 2019).

Previous research showed that mesenchymal stem cells stimulated with TNF-α affected IL-10 secretion (et al., 2023). IL-10 is an important cytokine in immunological regulation and plays a role in eliminating bacteria as an anti-inflammatory (Hati et al., 2023)(Wang et al., 2022). This paracrine IL-10 has been developed in previous studies as a secretion that can act as an immunoregulator and regenerate damaged tissue (Hati et al., 2023)(Wang et al., 2022).

Nevertheless, despite their favourable attributes, MSCs as cellular therapy exhibit many limitations. The low persistence of mesenchymal stem cells when migrating to damaged tissue makes it difficult to maintain the ability for paracrine effects. This presents a challenge in the development of cellular therapy, to be addressed effectively sustain the paracrine effects and optimize cell retention (Nurhayati et al., 2019).

Many studies have been conducted to answer these challenges. The approach of encapsulating mesenchymal stem cells provides a microenvironment that supports cell viability. The encapsulating MSCs enhance the resistance against immunological attacks, improving their therapeutic efficacy in disease treatment (Meftahpour et al., 2021). Furthermore, this process ensures the preservation of cells within the capsules and effectively restricts the infiltration of host immune cells. It also enables the transmission of metabolic signals from injured tissue to the encapsulated cells, allowing them to sense and respond to their environment. Moreover, it facilitates the paracrine repair mechanisms by enabling the diffusion of small molecules secreted by the encapsulated cells outside the capsules. The favourable physical dimensions of the capsules, along with their semi-permeability, hinder the removal of transplanted cells by mechanisms such as phagocytosis, migration, and venous and lymphatic drainage (Landázuri et al., 2016).

Platelet lysate enhances the adhesion, migration, and differentiation of encapsulated MSCs. Furthermore, previous studies have seen enhanced vascularization and the emergence of new cells in vivo when comparing coated MSCs to uncoated MSCs (Altaie et al., 2016). Nevertheless, the impact of platelet lysate on the immunoregulatory capacity of MSCs remains uncertain. The objective of this study is to examine the paracrine effects of TNF-α and Il-10 on MSCs that are both encapsulated and coated with platelet lysate.

RESEARCH METHOD

This in-vitro research was carried out in the SCTE IMERI FK UI with ethical clearance KET-732/UN2.F1/ETIK/PPM.00.02/2022.

Culture Mesenchymal Stem Cells

The cryopreserved umbilical cord mesenchymal stem cells from previous study, that were isolated by the procedure developed previously (Liem, 2015), thawed and cultivated in T flasks.

Umbilical cord mesenchymal stem cells were cultured in αMEM medium supplemented with platelet lysate obtained from the local Indonesian Blood Bank, Glutamax (Gibco), and heparin (Sibuea et al., 2020). The purity of mesenchymal stem cells was evaluated by flow cytometry, using criteria established by the International Society of Cell and Gene Therapy (ISCT) for the CD105, CD90, and CD73 markers. The mesenchymal stem cells were incubated and harvested when confluence.

Encapsulated Mesenchymal Stem Cells

The alginate was dissolved in phosphate-buffered saline (PBS, Gibco) to prepare a 1.8% alginate solution. Encapsulated was prepared by mixing a suspension of 2,5 x 10^5 mesenchymal stem cells with alginate in ratio 1:4. The mixture was dropped into 0.2M CaCl2 solution using an insulin syringe, as adapted from the previous research (Sibuea et al., 2020). Encapsulated mesenchymal stem cells were washed 3 times using PBS.

Coating Encapsulated Mesenchymal Stem Cells

Coating solution was prepared by mixing 2 ml solution of a platelet lysate with 200 µL of heparin. Subsequently, the encapsulated mesenchymal stem cells were suspended in platelet lysate mixture and incubated for 10 minutes. The encapsulation was subjected to three washes with phosphate-buffered saline (PBS) and transferred into a six-well plate containing a culture medium. The microencapsulation process involved the direct suspension of platelet lysate-uncoated microcapsules in alginate solution, which has been previously utilized, followed by an incubation period of 10 minutes and underwent three washes in phosphate-buffered saline (PBS) before being transferred into a six-well plate containing the appropriate culture medium.

Analyzed TNF and IL-10

The culture medium encapsulated mesencymal stem cells was collected and centrifuged at 15,000 rpm. Subsequently, the supernatant of the encapsulated culture medium was examined for levels of TNF-α and IL-10 on the 2nd, 7th, 14th, and 21st days. TNF-α analysis was conducted using Human TNF-α ELISA Kit (Quantikine) CAT: DTA00D, and IL-10 analysis using Human IL-10 ELISA Kit (Quantikine) CAT: D1000D, following the protocol provided by the manufacturer of the kits.

RESULTS AND DISCUSSIONS

The level of TNF-α and IL-10 performed low level. TNF-α was greater than IL-10. TNF-α increased until day 21, meanwhile IL-10 was not detected until day 14 and showed very low level (Table 1, Figure 1 and 2).

Table 1. TNF-α and IL-10 Level in Coated Encapsulated MSCs

Duration	TNF-α (pg/dL)	IL-10 (pg/dL)
Day 2	0,026	0,00
Day 7	0,033	0,00
Day 14	0,038	0,03
Day 21	0,086	0,00

The upregulation of TNF-α and the downregulation of IL-10 production were performed as MSCs paracrine in this noninflammatory condition, whereas the coating encapsulated MSCs cultured in medium culture. Mesenchymal stem cells secrete many paracrines, such as TNF-a and IL-10. TNF-α can regulate IL-10 secretion. Cell death increases the secretion of IL-10, and this stimulates the secretion of TNF-α. The secretion of TNF-α by coating encapsulated mesenchymal stem cells will suppress IL-10 secretion so that IL-10 levels decrease. Previous research showed upregulated TNF-α levels and downregulated IL-10 (Putra et al., 2018). Study conducted by Mead et al. showed that there was an upregulation of rat IL-10 expression at spinal cord injury sites in rats, one week following the administration of intrathecal lumbar injection of MSC encapsulated in alginate (Mead et al., 2020). Ecapsulated MSC may upregulate IL-10 and TNF-α downregulate it. This study showed an upregulated of TNF-α and downregulated of IL-10. It may indicate an inflammatory response to cell death or apoptosis. IL-10 might occur initially and subsequently

trigger TNF-a secretion. Increased TNF-a suppressed IL-10, so it performed a low level of IL-10 until day 14.

IL-10 is an anti-inflammatory mediator, where increased secretion occurs due to damaged tissue or cells (Liu et al., 2022)(de Los Reyes Jiménez et al., 2020). The disappearance of IL-10 on day 21 could be caused by a decrease in mesenchymal stem cell viability. The viability of mesenchymal stem cells decreases with the duration of culture

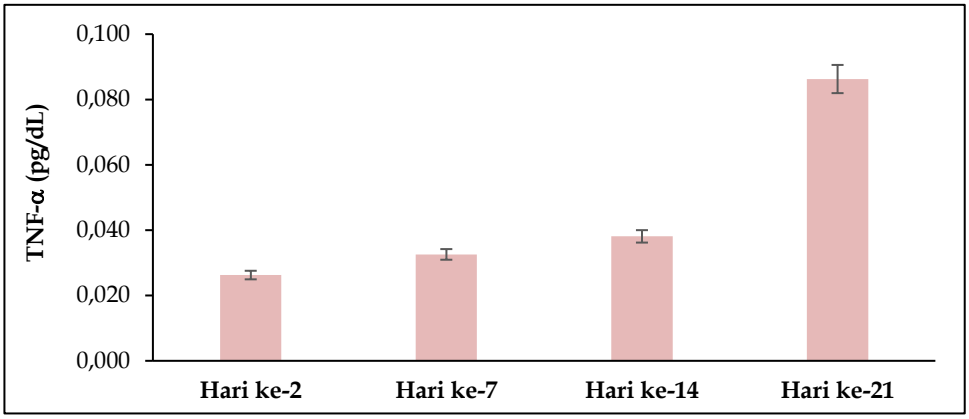

Figure 1. TNF-α Level in Coated Encapsulated MSCs

Encapsulation protects the mesenchymal stem cells inside the capsule from stimuli from the environment outside the capsule. IL-10 secretion only came from mesenchymal stem cells inside the capsule and was not influenced by stimulation of inflammatory mediators from outside the capsule. Kumar et al also showed that encapsulation of mesenchymal stem cells reduced the expression of anti-inflammatory cytokines (Kumar et al., 2022).

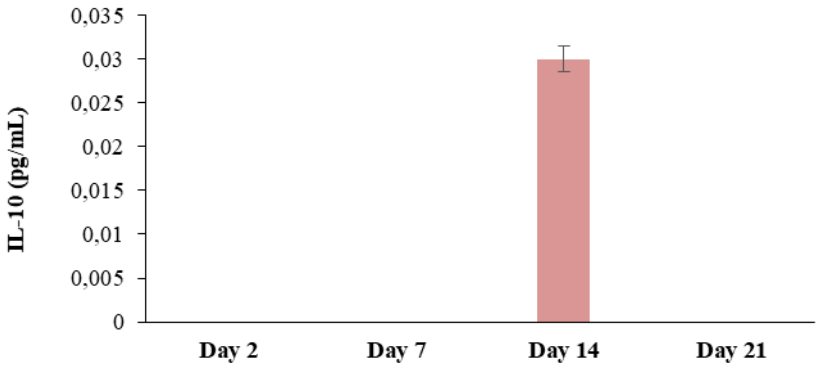

Figure 2. IL-10 Level in Coated Encapsulated MSCs

Platelet lysate induces an inflammatory reaction in MSCs, leading to the synthesis of certain compounds that sustain the pro-inflammatory state of macrophages (Ulivi et al., 2014). In this study, encapsulated MSCs coating by platelet lysate activated TNF-α and had not performed IL-10 level yet until day 14. Basal in-vitro coating encapsulated MSCs did not induce inflammatory reaction in MSCs, so IL-10 had not increased due to normal condition. Platelet lysate retained the encapsulated MSCs and protected from outside trigger.

CONCLUSION

The findings of this study demonstrated that the encapsulated MSCs coated with platelet lysate had an impact on the paracrine action of MSCs in terms of TNF-α levels, while also effectively retaining IL-10 within the encapsulation.

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References

Altaie, A., Owston, H., & Jones, E. (2016). Use of platelet lysate for bone regeneration-are we ready for clinical translation? *World Journal of Stem Cells*, *8*(2), 47.

Chen, R., Hao, Z., Wang, Y., Zhu, H., Hu, Y., Chen, T., Zhang, P., & Li, J. (2022). Mesenchymal stem cell–immune cell interaction and related modulations for bone tissue engineering. *Stem Cells International*, *2022*.

Chen, Z., Jin, M., He, H., Dong, J., Li, J., Nie, J., Wang, Z., Xu, J., & Wu, F. (2023). Mesenchymal stem cells and macrophages and their interactions in tendon-bone healing. *Journal of Orthopaedic Translation*, *39*, 63–73.

de Los Reyes Jiménez, M., Lechner, A., Alessandrini, F., Bohnacker, S., Schindela, S., Trompette, A., Haimerl, P., Thomas, D., Henkel, F., & Mourão, A. (2020). An anti-inflammatory eicosanoid switch mediates the suppression of type-2 inflammation by helminth larval products. *Science Translational Medicine*, *12*(540), eaay0605.

Driscoll, J., & Patel, T. (2019). The mesenchymal stem cell secretome as an acellular regenerative therapy for liver disease. *Journal of Gastroenterology*, *54*, 763–773.

Hati, I. P., Putra, A., & Sumarawati, T. (2023). Effect of Topical Gel Administration of Secretome Hypoxia Mesenchymal Stem Cells (SH-MSCs) on IL-10 and TNF-α Gene Expression (In Vivo Experimental Study in Male Rats of Wistar Strains Model of Fluconazole-Induced Alopecia). *INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND ANALYSIS*, *6*(12), 5600–5610.

Kandilarov, I., Gardjeva, P., Georgieva-Kotetarova, M., Zlatanova, H., Vilmosh, N., Kostadinova, I., Katsarova, M., Atliev, K., & Dimitrova, S. (2023). Effect of Plant Extracts Combinations on TNF-α, IL-6 and IL-10 Levels in Serum of Rats Exposed to Acute and Chronic Stress. *Plants*, *12*(17), 3049.

Kumar, S., Kabat, M., Basak, S., Babiarz, J., Berthiaume, F., & Grumet, M. (2022). Anti-inflammatory effects of encapsulated human mesenchymal stromal/stem cells and a method to scale-up cell encapsulation. *Biomolecules*, *12*(12), 1803.

Kusuma, Y. T., & Subchan, P. (2023). Effect of Gel Secretome Hypoxia Mesenchymal Stem Cell on Expression of TGF- β and IL-6 (In Vivo Experimental Study in Male Rats of Wistar Strains Model Hyperglycemic Wounds). *International Journal of Multidisciplinary Research and Analysis*, *06*(12). https://doi.org/10.47191/ijmra/v6-i12-23

Landázuri, N., Levit, R. D., Joseph, G., Ortega-Legaspi, J. M., Flores, C. A., Weiss, D., Sambanis, A., Weber, C. J., Safley, S. A., & Taylor, W. R. (2016). Alginate microencapsulation of human mesenchymal stem cells as a strategy to enhance paracrine-mediated vascular recovery after hindlimb ischaemia. *Journal of Tissue Engineering and Regenerative Medicine*, *10*(3), 222–232.

Liang, X., Ding, Y., Zhang, Y., Tse, H.-F., & Lian, Q. (2014). Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. *Cell Transplantation*, *23*(9), 1045–1059.

Liem, I. K. (2015). Simple production method of umbilical cord derived mesenchymal stem cell using xeno-free materials for translational research. In *Journal of Chemical and Pharmaceutical Research* (p. 8). JOCPR.

Liu, D., Huang, S.-Y., Sun, J.-H., Zhang, H.-C., Cai, Q.-L., Gao, C., Li, L., Cao, J., Xu, F., & Zhou, Y. (2022). Sepsis-induced immunosuppression: mechanisms, diagnosis and current treatment options. *Military Medical Research*, *9*(1), 1–19.

Markovics, A., Rosenthal, K. S., Mikecz, K., Carambula, R. E., Ciemielewski, J. C., & Zimmerman, D. H. (2021). Restoring the balance between pro-inflammatory and anti-inflammatory cytokines in the treatment of rheumatoid arthritis: New insights from animal models. *Biomedicines*, *10*(1), 44.

Mead, B., Chamling, X., Zack, D. J., Ahmed, Z., & Tomarev, S. (2020). TNFα-mediated priming of mesenchymal stem cells enhances their neuroprotective effect on retinal ganglion cells. *Investigative Ophthalmology & Visual Science*, *61*(2), 6.

Meftahpour, V., Malekghasemi, S., Baghbanzadeh, A., Aghebati-Maleki, A., Pourakbari, R., Fotouhi, A., & Aghebati-Maleki, L. (2021). Platelet lysate: a promising candidate in regenerative medicine. *Regenerative Medicine*, *16*(01), 71–85.

Nurhayati, R. W., Antarianto, R. D., Pratama, G., Rahayu, D., Mubarok, W., Kobayashi, M., & Hutabarat, M. (2019). Encapsulation of human hematopoietic stem cells with a biocompatible polymer. *AIP Conference Proceedings*, *2092*(1).

Porro, C., Cianciulli, A., & Panaro, M. A. (2020). The regulatory role of IL-10 in neurodegenerative diseases. *Biomolecules*, *10*(7), 1017.

Putra, A., Ridwan, F. B., Putridewi, A. I., Kustiyah, A. R., Wirastuti, K., Sadyah, N. A. C., Rosdiana, I., & Munir, D. (2018). The role of TNF-α induced MSCs on suppressive inflammation by increasing TGF-β and IL-10. *Open Access Macedonian Journal of Medical Sciences*, *6*(10), 1779.

Saldaña, L., Bensiamar, F., Vallés, G., Mancebo, F. J., García-Rey, E., & Vilaboa, N. (2019). Immunoregulatory potential of mesenchymal stem cells following activation by macrophage-derived soluble factors. *Stem Cell Research & Therapy*, *10*(1), 1–15.

Sibuea, C. V., Pawitan, J. A., Antarianto, R., Jasirwan, C. O. M., Sianipar, I. R., Luviah, E., Nurhayati, R. W., Mubarok, W., & Mazfufah, N. F. (2020). 3D Co-Culture of Hepatocyte, a Hepatic Stellate Cell Line, and Stem Cells for Developing a Bioartificial Liver Prototype. *International Journal of Technology 11(5)*, *11*(5), 95–962.

Tsuji, W., Rubin, J. P., & Marra, K. G. (2014). Adipose-derived stem cells: Implications in tissue regeneration. *World Journal of Stem Cells*, *6*(3), 312.

Ulivi, V., Tasso, R., Cancedda, R., & Descalzi, F. (2014). Mesenchymal stem cell paracrine activity is modulated by platelet lysate: induction of an inflammatory response and secretion of factors maintaining macrophages in a proinflammatory phenotype. *Stem Cells and Development*, *23*(16), 1858–1869.

Wang, J., Chen, Z., Li, M., Song, Y., Xu, W., Wang, L., & Chen, S. (2022). Genome-wide identification, immune response profile and functional characterization of IL-10 from spotted knifejaw (Oplegnathus punctatus) during host defense against bacterial and viral infection. *Fish & Shellfish Immunology*, *124*, 513–524.

Yani, S., & Pawitan, J. A. (2023). Stem cell mechanism of action in neuroplasticity after stroke. *Egyptian Pharmaceutical Journal*, *22*(3), 344–352.