VOLUME 10 NOMOR 2 DESEMBER 2023

ISSN 2548 – 611X

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejournal.brin.go.id/JBBI/index

ANALYSIS OF INTERLEUKIN-10 LEVELS IN MESENCHYMAL STEM CELL SECRETOME CREAM WITH ELISA METHOD

Analisis Kadar Interleukin-10 pada Krim *Secretome Mesenchymal Stem Cell* dengan Metode ELISA

Vika Amelia[1], Marlina[2], Ikhwan Resmala Sudji[3]

[1)]Postgraduate Biotechnology Study Program, Andalas University, Unand Limau Manih Campus, Padang 25163, West Sumatra, Indonesia

[2)]Faculty of Pharmacy, Andalas University, Unand Limau Manih Campus, Padang 25163, West Sumatra, Indonesia

[3)]Faculty of Health Sciences, Indonesian Perintis University, Padang, West Sumatra, Indonesia

*E-mail: vikaa9341@gmail.com

ABSTRACT

Mesenchymal Stem Cells (MSC) are cells that are able to differentiate into other cells and have immunomodulatory properties that are used to treat inflammatory diseases. MSCs have the ability to repair damaged tissue by regenerating damaged tissue. MSCs produce a secretome, where the MSC secretome has various cytokines, chemokines, growth factors, anti-inflammatory factors and proteins that are produced in molecular form. One of the molecules secreted by the secretome is Interleukin-10.IL-10 is an anti-inflammatory cytokine which functions to inhibit the production of several other types of cytokines such as TNF, IL-1, chemokine and IL-12). Apart from that, IL-10 is able to inhibit the function of macrophages in assisting T cell activity. One of the products from Secretome MSC is a cream preparation. To find out whether the MSC cream contains IL-10 protein, Interleukin-10 levels were analyzed in the MSC secretome cream with 3 different MSC cream concentration formulations (2%, 3% and 5%). Analysis of IL-10 levels was carried out using ELISA to detect IL-10 antigen. The results of the examination of 9 samples showed that all samples were positive for IL-10 with a concentration of 2% dilution 1:2 showing the best results with an Optical Density (OD) of 0.069 and followed by a concentration of 5% dilution 1:2 showing an OD value of 0.065.

Keywords: *Cream, Inflammation, Interleukin-10, Mesenchymal Stem Cell, secretome*

ABSTRAK

Mesenchymal Stem Cell (MSC) merupakan sel yang mampu berdiferensiasi menjadi sel lain dan memiliki sifat imunodulator yang digunakan untuk mengatasi penyakit inflamasi. MSC memiliki kemampuan memperbaiki jaringan rusak dengan melakukan regenerasi pada jaringan rusak. MSC menghasilkan *secretome*, dimana *secretome* MSC memiliki berbagai sitokin, kemokin, faktor pertumbuhan, faktor antiinflamasi dan protein yang diproduksi dalam bentuk molekul. Salah satu molekul yang disekresikan *secretome* adalah Interleukin-10. IL-10 merupakan salah satu sitokin antiinflamasi yang berfungsi menghambat produksi beberapa jenis sitokin lain seperti TNF, IL-1, *chemokine* dan IL-12). Selain itu IL-10 mampu menghambat fungsi makrofag dalam membantu aktivitas sel T. Salah satu produk hasil dari *Secretome* MSC adalah sediaan krim. Untuk mengetahui apakah krim MSC tersebut terdapat protein IL-10 maka dilakukan analisis kadar Interleukin-10 pada krim *secretome* MSC dengan 3

***Received**: 22 November 2023* ***Accepted**: 19 December 2023* ***Published**: 20 December 2023*

formulasi konsentrasi krim MSC yang berbeda (2%, 3% dan 5%). Analisis kadar IL-10 dilakukan menggunakan ELISA untuk mendeteksi antigen IL-10. Hasil pemeriksaan dari 9 sampel, didapatkan hasil semua sampel positif terdapat IL-10 dengan konsentrasi 2% pengenceran 1:2 menunjukkan hasil terbaik dengan *Optical Density* (OD) 0,069 dan diikuti konsentrasi 5% pengenceran 1:2 menunjukkan nilai OD 0,065.

Kata Kunci: Inflamasi, Interleukin-10, krim, *Mesenchymal Stem Cell*, *secretome*

INTRODUCTION

Stem cells are cells originating from embryos, fetuses or adult cells that have the ability to reproduce themselves over a long period of time and are able to differentiate into certain cell types. According to Choi et al., (2017) Mesenchymal Stem Cells (MSC) are adult marrow pluripotent progenitor cells that have the ability to become several mesenchymal lineages such as chondrocytes, osteocytes, adipocytes and myocytes. It is known based on research conducted according to Caplan et al., (2019) and Paterson et al., (2014) that Mesenchymal Stem Cells after transplantation can repair damaged tissue via paracrine.

MSCs have immunomodulatory properties that can be used to treat inflammatory diseases and repair tissue damage by regenerating the damaged tissue. According to Willms et al., (2016) mesenchymal stem cells (MSCs) are very influential and beneficial in accelerating wound healing, increasing angiogenesis, regulating extracellular matrix repair, increasing recoilization and assisting in fibroblast repair. According to Paterson et al., (2014) MSCs can repair damaged tissue through paracrine effects. Not only that, according to Motegi et al., (2016) and Oh et al., (2020) MSCs have a good ability to modulate the inflammatory response, accelerate remodeling and increase the migration of fibroblasts and keratinocytes in accelerating the wound closure process.

MSCs are known to produce secrets in the form of a secretome. The secretome has various regenerative factors, namely cytokines and growth factors, which are able to modulate the formation of new tissue. The MSC secretome has various cytokines, chemokines, growth factors, anti-inflammatory factors and proteins produced in molecular form. Protein molecules have the form of exosomes with a size of 40-120 nm and in the form of microvesicles around 50-1000 nm. According to Eleuteri & Fierabracci (2019), the molecules secreted by the MSC secretome are TGF-β, IL-1, IL-10, HGF-1, VEGF, LIF, PGE2, TSG6, mpCCL2, Gal-1 and Gal-9. According to Lee et al., (2016) and Pawitan (2014) the secretome is able to accelerate the wound closure process, increase the resolution of wound inflammation, increase angiogenesis, regulate extracellular matrix remodeling and encourage faster skin regeneration. The MSC secretome has an expression pattern that changes cytokines from pro-inflammatory (TNF-α and IL-2) to anti-inflammatory in T cells (Ezquer et al., (2012).

One of the molecules secreted by the secretome is Interleukin-10 (IL-10). IL-10 is a protein consisting of 3 polypeptides of 178 amino acids with molecular weights of 17kD, 18kD, 19kD and 20 kD (Kresno, 2013). IL-10 is an anti-inflammatory cytokine which functions to inhibit the production of several other types of cytokines such as TNF, IL-1, chemokine and IL-12). Apart from that, IL-10 is able to inhibit the function of macrophages in assisting T cell activity. Interleukin-10 activity provides obstacles to non-specific inflammatory reactions mediated by T cells. Interleukin-10 functions as an antifibrotic cytokine in regulating extracellular matrix remodeling activity by controlling fibroblast activity.

One of the products from Secretome MSC is a cream preparation. According to the Indonesian Pharmacopoeia IV, cream is a semi-solid dosage form containing one or more dissolved ingredients dispersed into a suitable base ingredient. Research conducted by Marlina (2021), succeeded in making a cosmetic cream preparation containing 5% MSC-CM with additional

ingredients Olivem® 1000 and orange oil with an average pH of 7.06 and an average globule size of 0.205 µm and has thixotropic flow properties. MSC cream is known to have proteins that are observed based on their molecular weight using SDS-PAGE. Where proteins detected at 38 kDa - 98 kDa consist of VEGF at 45 kDa (Stefanini et al., 2008), EGF Containing Fibulinka at 55 kDa, ECM protein 1 at 61 kDa and TGFβ at 75 kDa (Simper et al., 2010) .

Based on the description above, it is known that Mesenchymal Stem Cell Secretome cream has proteins that have been detected, but it is not yet known whether Mesenchymal Stem Cell (MSC) Secretome cream has Interleukin-10 protein as an anti-inflammatory or anti-inflammatory. For this reason, research was carried out by measuring and analyzing interleukin-10 levels in MSC secretome cream using the ELISA technique.

MATERIALS AND METHODS

Location and time

The research was conducted in August 2023 at the Biomedical Laboratory, Faculty of Medicine, Andalas University.

Materials and tools

The tools used in this research were ice box, ELISA, well wash, microplate reader, micropipette and ELISA reader. Meanwhile, the materials used in this research are secretome cream which is made from MSC, Elabscience Human IL-10 ELISA Kit consisting of; Micro ELISA plate, Concentrated Biotinylated Detection, Concentrated HRP Conjugate, Biotinylated Detection Ab Diluent, HRP Conjugate Diluent, Concentrated Wash Buffer, Stop Solution, sterile distilled water, tips, sterile 1.5 microtube, and tissue.

Experimental design

This type of research was experimental, to measure IL-10 expression levels in secretome cream. MSCs were divided into three groups, namely group P1 (2% dose of secretome cream), group P2 (3% dose of secretome cream), and group P3 (5% dose of secretome cream).) which is then measured using ELISA. The results of the research data were analyzed using One Way Anova and continued with the Tukey test. For the measurement treatment of IL-10 levels in the MSC secretome cream, see Table 1.

Table 1. Identification treatment of IL-10 in MSC secretome cream using ELISA

Dilution	Cream Dosage Concentration		
	2%	**3%**	**5%**
1:2	P1	P2	P3
1:4	P1	P2	P3
1:8	P1	P2	P3

Cream formulation

In this research, the cream formulation used was based on study by Pradifta *et al*., (2019), with modification of concentrations of MSC. The cream formulation can be seen in table 2.

Table 2. Cream formula (Hallstar, 2022; Fox *et al*., 2011; Das & Ahmed, 2017; Sohn *et al*., 2018; H. J. Kim *et al*., 2020)

Material name	Function	Concentration (2%)	Concentration (3%)	Concentration (5%)
MSC-CM/Secretome	Nutritious ingredients	2	3	5
Olivem® 1000	Emulsifier/ cream base	5	5	5
Orange oil	Penetration enhancer	4	4	4
Methyl paraben	Preservative	0.1	0.1	0.1
Aquadest	Solvent	Ad 100	Ad 100	Ad 100

ELISA analysis

Determine wells for diluted standard, blank and sample. Add 100 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Immediately add 100 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C. Add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C (Elabscience Human IL-10 ELISA Kit protocol).

Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3. Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement. Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution. Determine the optical density (OD value) of each well at once with a microplate reader set to 450 nm (Elabscience Human IL-10 ELISA Kit protocol).

Analysis data

The obtained data were statistically analyzed using the One-Way ANOVA method in IBMSPSS® Statistics 25 software. Normality and homogeneity tests were performed using the Shapiro Wilk. If a significant difference was found, the Tukey test with a confidence level of 95% was used.

RESULTS AND DISCUSSION

Research conducted Motegi et al., (2016) and Oh et al., (2018) stated that MSCs have several growth factors and extracellular matrix proteins that are able to modulate inflammatory responses in the skin. According to Sapudom et al., (2017), it is known that MSCs have IL-10 andTGF-β is capable of acting as an anti-inflammatory by activating fibroblasts to become myofibroblasts which leads to skin repair. This proves that IL-10 is a potential biomarker in treating inflammation in the skin.

Saeedi et al., (2019) revealed that the protective effect of Mesenchymal Stem Cells in sepsis conditions includes antimicrobial activity against harmful agents which is associated with the release of the antimicrobial peptide human cathelicidin (hCAP-18/LL-37). According to Olievera et al., (2016) peptides as host defense are able to increase the expression of the TLR3, IDO, IL-10, TGF-β, IL-6 and IL-1β genes to cause an immunosuppressive environment in a setting where the immune system response is inappropriately enhanced. normal.

To test the protein content of the MSC cream used in this study, it was carried out using ELISA (Enzyme-Linked Immunosorbent Assay). ELISA is a molecular technique used to detect and measure interactions between antigens and antibodies. The basic principle of ELISA is the binding of antibodies to the mobilized antigen and forming an antigen-antibody complex. After the antigen-antibody reaction, a color change will occur. The color intensity is directly proportional to the amount of antigen present in the sample. So it is necessary to read the results with an ELISA Reader in the form of Optical Density (OD).

Table 3. Data standards

Std#	Conc (pg/mL)	Well	OD
1	0	H1	0.064
2	1.56	G1	0.070
3	3.13	F1	0.099
4	6.25	E1	0.166
5	12.5	D1	0.351

Std#	Conc (pg/mL)	Well	OD
6	25	C1	0.822
7	50	B1	1,712
8	100	A1	2,769

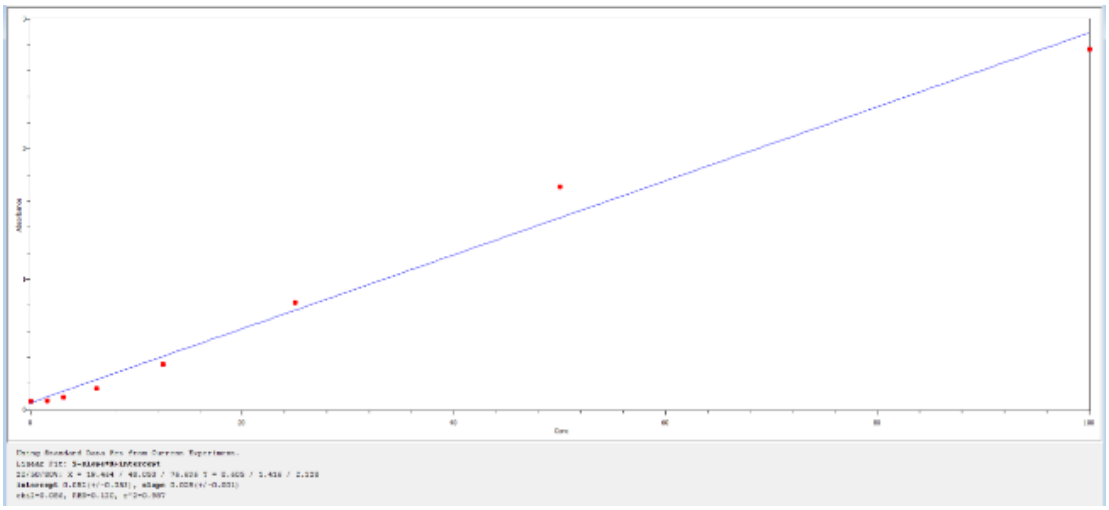

Figure 1. Standard curve

Before the MSC secretome cream samples were tested by elisa, samples with concentrations of 2%, 3% and 5% were first diluted (1:2, 1:4 and 1:8) (Kit Elabscience protocol). MSC secretome cream is diluted using distilled water because distilled water has a neutral pH (pH=7), is able to dissolve and only a dilution reaction occurs. The protein profile in MSC cream was observed using the OD (Optical Density) value. The absorbance results obtained in the research show that the greater the concentration, the greater the OD value or is directly proportional. The table of standard measurement results can be seen in table 3 with the standard curve can be seen in figure 1. where the x axis is concentration (pg/ml) and y is the absorbance value. From the standard curve it is known that the regression value is r= 0.987. This equation will be used to calculate the sample concentration.

Table 4. ELISA results

Dilution	Cream Dosage Concentration		
	2%	3%	5%
1:02	0.583	0.160	0.442
1:04	0.195	0.125	0.160
1:08	0.266	0.301	0.054

In the 2% sample (1:2 dilution) the absorbance value was 0.069, after entering the standard curve equation the IL-10 level value was 0.583 pg/ml. the 2% sample (1:4 dilution) had an absorbance value of 0.058 and the IL-10 level was 0.195 pg/ml. the 2% sample (1:8 dilution) had an absorbance value of 0.060 and the IL-10 level was 0.266 pg/ml. in samples with a concentration of 3% (dilution 1:2, 1:4, 1:8) the absorbance values were respectively: 0.057, 0.056 and 0.061 with IL-10 levels respectively: 0.160 pg/ml, 0.125 pg/ml and 0.301 pg /ml. whereas at the 5% cream dose (dilution 1:2, 1:4, 1:8) the absorbance values were respectively 0.065, 0.057 and 0.054 with the IL-10 levels being 0.442 pg/ml, 0.160 pg/ml and 0.054 pg/ml . The ELISA results can be seen in table 4. From the ELISA results, it is proven that MSC secretome cream with cream doses of 2% and 5% has the highest IL-10 levels, namely 0.583 pg/ml and 0.442 pg/ml. The conclusion of the ELISA results can be seen in Figure 2.

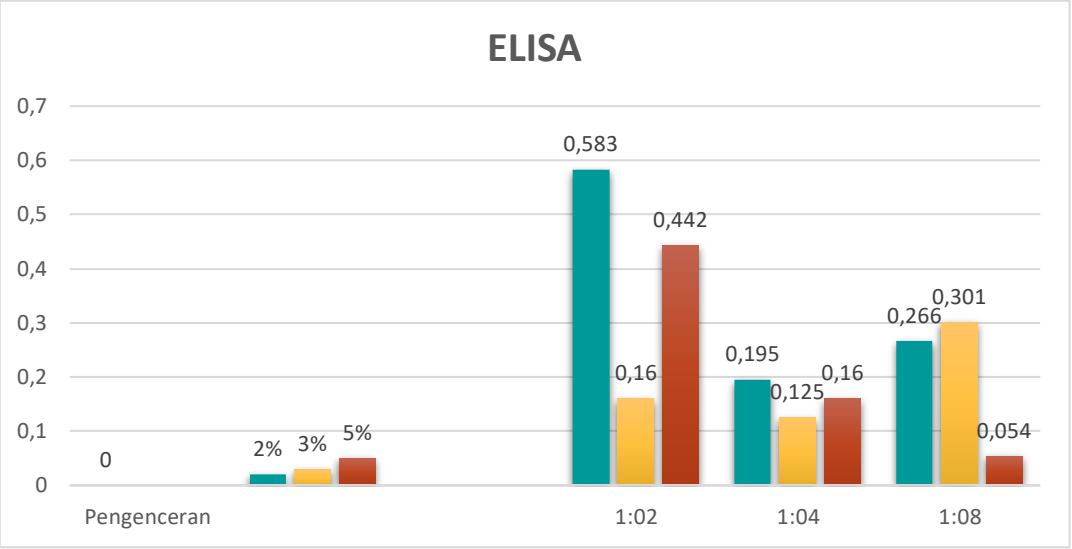

Figure 2. ELISA results diagram

To see the comparison of the results of MSC secretome cream analysis with ELISA, statistical analysis was carried out using the Tukey advanced test with a confidence level of 95%. Based on the results of further test output, the sig value is known. amounting to 0.565 > 0.05 so it can be concluded that the average IL-10 levels at concentrations of 2%, 3% and 5% are the same. The results of statistical analysis can be seen in table 5.

Table 5. Statistic analysis

Concentration	N	Subset for alpha = 0.05 1
3% concentration	3	.19533
5% concentration	3	.21867
2% concentration	3	.34800
Sig.		,565

Based on the results of the examination, it was discovered that there was IL-10 protein in the MSC secretome cream. Research conducted by Ho et al., (2018), MSCs which consist of many IL-10 molecules can trigger regenerative tissue repair through regulating anti-inflammatory pathways to encourage healing of skin excision wounds. Research was also carried out by Sapudom et al., (2017), IL-10 and TGF-β are important molecules in the wound healing process by activating fibroblasts to become myofibroblasts and increasing the production of extracellular matrix which leads to wound closure without scar tissue. This proves that IL-10 is a potential biomarker in the healing process of skin tissue excision wounds.

CONCLUSION

From the research results it can be concluded that there is Interleukin-10 protein in the Mesenchymal Stem cell secretome cream. Where at each cream concentration of 2%, 3% and 5% there are different levels of Interleukin-10. Where IL-10 levels were highest at a secretome cream concentration of 2% and followed by a concentration of 5%. From the statistical results it can be concluded that there is no significant difference in the amount of Interleukin-10 at each concentration.

THANK-YOU NOTE

We would like to express our gratitude to the Graduate School of Andalas University (Research Grant) as well as all other in-

dividuals and organizations who have contributed to completion of this research.

REFERENCES

Caliari-Oliveira, C; Yaochite, J. N. U., *et al.,* 2016. Xenogeneic Mesenchymal Stromal Cells improve wound Healing and Modulate the Immune response in an Extensive Burn Model. *Cell Transplant*. 25.

Caplan, H., Olson, S. D., Kumar, A., George, M., Prabhakara, K. S., Wenzel, P., et al. (2019). Mesenchymal stromal cell therapeutic delivery: translational challenges to clinical application. *Front Immunol*. 10:1645.

Choi, Y. J., Lee, K. S., Yeom, S. H., and Cho, Y. W. (2017). Exosomes secreted by human adipose-derived stem cells regulate the expression of collagen synthesis related genes in human dermal fibroblasts. *J. Extracell*. Vesicles 6:141.

Das, A. Ahmed, A. B. 2017. Formulation and evaluation of transdermal patch of infomethachin containing patchouli oil as natural penetration enchancer. *Asian Journal of Pharmaceutical and Clinical Research*. 10(11).

Eleuteri, S., Fierabracci, A. 2019. Insight into the secretome of Mesenchymal Stem Cells and its potential applications. *International Journal of Molecular*. 20(18).

Ezquer, F., *et al*., 2012. The antidiabetic effect of Mesenchymal Stem Cells is unrelated to their transdifferentiation potential but to their capability to restore th1/th2 balance and to modify th pancreatic microenvironment STEM CELL. 30(8).

Fox, L. T. *et al*. 2011. Transdermal drug delivery enchancement by compounds of natural origin. *Molecules*. 16(12).

Hallstar. 2002. Olivem® The First Emulsifying Active Ingredient. *Product Literature*. Chicago: The Hallstar Company.

Kim, K. H. *et al*., 2020. The effect of three-dimensional cultured adipose tissue derived mesenchymal stem cell-conditioned and the antiaging effect of cosmetic product containing the medium. *Biomedical Dermeatology*. 4(1).

Kresno, S. B. 2013. Imunologi: Diagnosis dan Prosedur laboratorium. Edisi Kelima. Jakarta: Badan Penerbit FK Universitas Indonesia.

Lee D.E, A. N., A. D. K., (2016). Mesenchymal stem cells and cutaneous wound healing: novel methods to increase cell delivery and therapeutic efficacy. *Stem Cell Research & Therapy*, *7*(37).

Marlina., Pradifta R., Lucida H., 2021. Analisis Protein pada Medium Terkondisi Sel Punca Mesenkimal. *Jurnal Medika Kesehatan*. 14(2).

Motegi, S., Ishikawa. 2016. Mesenchymal Stem Cells: The roles and functions in cutaneous wound healing and tumor growth. *Journal of Dermatological Scinece*. http://doi.org/10.1016/j.jdermsci.2016.11.005

Oh, H. A., Kwak, J., Kim, B. J., Jin, H. J., Park, W. S., Choi, S. J., et al. (2020). Migration inhibitory factor in conditioned medium from human umbilical cord blood-derived mesenchymal stromal cells stimulates hair growth.

Paterson Y. Z., Rash N., Garvican E.R., Paillot R., Guest D.J. 2014. Equine Mesenchymal Stromal Cells and Embryo-derived Stem Cells are Immune Privileged in Vitro. *Stem Cell Research and Therapy*. 5(4): 90.

Pawitan J. A. (2014). Prospect of Stem Cell Conditioned Medium in Regenerative Medicine. *BioMed Research International*.

Saeedi P, Halabian R, Imani Fooladi AA. 2019. A revealing review of mesenchymal stem cells therapy, clinical perspectives and Modification strategies. *Stem Cell Investig*. 6(34).

Sapudom, J., W. X., C. M. , A. M. , A. U. , & P. T. (2017). Fibroblast fate regulation by time dependent TGF-β1 and IL-10 stimulation in biomimetic 3D matrices. *Biomaterials Science*, *5*(9), 1858–1867.

Simper, D. *et al*., 2010. Comparative proteomics profiling reveals role of smooth muscle progenitors in extracellular matrix production. *Arteriosclerosis, Thrombosis and Vascular Biology*. 30(7).

Sohn, S. J. *et al*. 2018. Anti-aging Properties of Conditioned Media of Epidermal Progenitor cells Derved from Mesenchymal Stem Cell. *Dermatology and Therapy*. 8(2).

Stefanini, M. O. *et al*., 2008. A compartment model of VEGF distribution in blood, healthy and diseased tissues. *BMC System Biology*.

Willms E, Johansson HJ, Mäger I, Lee Y, Blomberg KE, Sadik M, Alaarg A, Smith CI, Lehtiö J, El Andaloussi S, Wood MJ, Vader P. 2016. Cells release subpopulations of exosomes with distinct molecular and biological properties. 6: 22519