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# The Potential of Human Wharton's Jelly Mesenchymal Stem Cells Secretome Based Topical Gel for Therapeutic Application

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## Abstract

**Background:** Diabetic Foot Ulcer (DFU) might be worsened by neuropathy and vascular issues. This condition can cause 14.3% fatality, stressing the need for effective wound healing therapy. Wound healing is a complex biological process, and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) may help manage DFU treatment issues. This research focuses on utilizing a gel carrier to deliver bioactive substances from Wharton's Jelly Mesenchymal Stem Cells secretome (hWJ-MSCs-Sec) as a possible treatment for DFU.

**Methods:** To maintain quality, hWJMSCs-Sec is thoroughly mixed with carbomer gel and freeze-dried. ELISA test is performed to determine the characterization of the gel of hWJMSCs-Sec such as Keratinocyte Growth Factor (KGF), Platelet-Derived Growth Factor (PDGF), Hepatocyte Growth Factor (HGF), Epidermal Growth Factor (EGF), and Heparin-Binding EGF-Like Growth Factor (HB-EGF). The antioxidant activity was also measured with Hydrogen peroxide ($H_2O_2$), Nitric oxide (NO), and Ferric Reducing Antioxidant Power (FRAP) assay. Proliferation assay was utilized using WST-8 and the wound healing potential was assessed *via* the migration cell ability of scratched-human skin fibroblast (BJ cells).

**Results:** The freeze-dried hWJ-MSCs-Sec showed higher levels of KGF, HGF, PDGF, EGF, HB-EGF, and the antioxidant activities compared to fresh hWJ-MSCs-Sec. Additionally, the gel of freeze-dried hWJ-MSCs-Sec exhibited higher levels compared to the gel of fresh hWJMSCs-Sec. This was evidenced by faster closure of scratched wounds on BJ cells treated with hWJMSCs-Sec and freeze-dried hWJ-MSCs-Sec gel.

**Conclusion:** The freeze-dried hWJ-MSCs-Sec gel exhibits superior quality compared to the non-freeze-dried hWJ-MSCs-Sec gel. This demonstrates that the freeze-drying procedure can maintain the bioactive chemicals found in hWJMSCs-Sec, potentially enhancing the efficacy of this gel in promoting cell regeneration for wound healing.

**Keywords:** Antioxidants, Carbomer gel, Freeze dried secretome gel, hWJ-MSCs, Wound healing
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## Introduction

Diabetic Foot Ulcer (DFU) is an open sore or wound that is caused by complications of diabetes mellitus. DFU affects the feet of diabetes patients and is characterized by sensory, motor, and autonomic neu-

ropathy, as well as macrovascular and microvascular disturbances. DFU can lead to ulcers, infections, gangrene, amputations, and even death. Amputation, a serious outcome of DFU, results in 14.3% of individuals passing away within a year after the procedure [1]. Effective strategies for healing DFU wounds must be established to avoid the amputation [2]. Efficient wound healing necessitates the coordination of several cellular processes facilitated by multiple growth factors, cytokines, and chemokines. There are four distinct phases involved in the process of wound healing, namely hemostasis, inflammation, proliferation, and remodeling [3]. The inflammatory response is triggered to help the body and initiate the healing process by widening the blood vessels and releasing certain signaling molecules such as nitric oxide, bradykinin, histamine, and prostaglandins [4]. But in cases where subsequent damage occurs, the inflammation doesn't resolve as expected, instead, it persists and intensifies, characterized by an abundance of neutrophils releasing Reactive Oxygen Species (ROS) [5]. The excessive generation of ROS has been linked to the onset of numerous persistent and degenerative disorders [6]. The antioxidant agents are needed to mitigate the harmful effect of ROS and enhance the body's ability to withstand diverse environmental stress conditions [7].

The process of wound repair involves various growth factors, including Epidermal Growth Factor (EGF), which promotes cell proliferation and cytoprotection [8]. Other growth factors such Platelet-Derived Growth Factor (PDGF), Heparin Binding EGF Like Growth Factor (HB-EGF), and Keratinocyte Growth Factor/Fibroblast Growth Factor-7 (KGF/FGF-7) play a significant role in angiogenesis, inflammation regulation, collagen deposition [9], keratinocyte migration, keratinocyte proliferation [10] and reepithelization in wound healing [11]. Currently, ongoing research is being conducted in the field of wound therapy with the aim of identifying appropriate treatment agents that can facilitate rapid wound healing while minimizing adverse effects. This pertains specifically to agents that possess elevated concentrations of antioxidants and growth factor proteins. One potential solution that could effectively tackle this matter is the implementation of stem cell treatment.

In the study by Park *et al* [12], it was discovered that various growth factors, including EGF, PDGF, bFGF, HB-EGF, FGF7/KGF, and HGF, are actively produced within stem cells and released through the secretome. The significant presence of these growth factors suggests their potential in wound healing, particularly in the case of DFU. Wharton's Jelly-derived MSCs (WJMSCs) are a type of stem cell derived from the umbilical cord. WJMSCs have been found to enhance the proliferation and migration of fibroblasts, speed up re-epithelialization, and facilitate wound repair through paracrine signaling [13]. The MSCs secretome, which consists of various bioactive substances released into the surrounding cellular environment, exerts a strong impact on nearby tissues [14]. The Wharton's Jelly Mesenchymal Stem Cells Secretome (hWJ-MSCs-Sec) has been commonly used to enhance wound healing after severe injuries [15]. Therefore, the hWJ-MSCs-Sec can be developed into a gel product for wound healing or DFU treatment. Gel products create a moist environment at the wound site, fostering tissue renewal through granulation and re-epithelialization [16]. The excellent adhesive and spreading properties of the gel enhance its application and therapeutic effectiveness, ensuring robust adherence to the target area, uniform spreading. and ultimately optimizing the therapeutic or cosmetic effects [17]. To safeguard the active compounds within the secretome, the freeze-drying process is employed. This technique ensures the preservation of proteins within the secretome, enhancing the reliability and consistency of molecular assessments [18].

This study aims to explore the efficacy of topical gel containing hWJMSCs-Sec for the treatment of DFU. This will be achieved through an examination of the gel's antioxidant properties by its scavenging activity towards Hydrogen peroxide ($H_2O_2$), Nitric Oxide (NO), and Ferric Reducing Antioxidant Power (FRAP). The analysis of the proteins content of freeze dried hWJ-MSCs-Sec gel involved the implementation of the Enzyme-Linked Immunosorbent Assay (ELISA) method, focusing on the evaluation of Keratinocyte Growth Factor (KGF), Platelet-Derived Growth Factor (PDGF), Hepatocyte Growth Factor (HGF), EGF, and hHB-EGF. Finally, the *in vitro* potential of hWJMSCs-Sec gel both freeze dried and fresh for wound healing was assessed by evaluating the cells proliferation and the cells migration of scratched-human skin fibroblast cells (BJ cells).

## Materials and Methods

### *Formulation of carbomer gel*

The carbomer gel formulation was conducted based on the study of Zhang *et al* [19], with certain alterations. Disperse 7.5 *g* carbomer 940 slowly into 250 *ml* aquadest with a continuous stirring using a Stirring Hotplates (Fisher Scientific, 1110217SH). After the carbomer swells in distilled water, add 7.5 *ml* triethanolamine, 5 *ml* propylene glycol, and 5 *ml* glycerin drop by drop while stirring gently until the mixture forms a homogeneous gel. Lastly, add distilled water gently until it reaches 500 *ml*.

### *Production of hWJ-MSCs Freeze-Dried hWJ-MSCs-Sec Gel (FDSG)*

***Preparation of hWJMSCs Secretome:*** The hWJMSCs passage 5 were obtained from Aretha Medika Utama Bandung, Indonesia. The cultivation protocol for Human Wharton's Jelly Stem Cells (hWJ-MSCs) was elucidated by Widowati, *et al* [20] and has been characterized using multipotent differentiation experiments and surface phenotypic analysis in prior studies [21]. Cells were cultured in Dulbecco's Modified Eagle Me-

dium (DMEM) High Glucose (Biowest, L0475-500) with 10% FBS, 1% Antibiotic and Antimycotic (ABAM) (Biowest, L0010-100), 0.1% Gentamicin (Gibco, 15750060), 1% Amphotericin B (Biowest, L0009-100), and 1% Nanomycopulitin (Biowest, L-X16-100) and incubated with $CO_2$ Incubator (Thermo IH3543) at 37°*C* with 5% $CO_2$. After the hWJMSCs reached 80-90% confluence, the culture medium was collected and centrifuged with Refrigerated Centrifuge (MWP 260r) at 3000×*g* for 4 *min* at 37°*C*. The resulting supernatant, containing secreted factors, was purified using a Durapore (Millipore Corporation, SLGV 033 RS) filter unit [22].

***Production of Freeze-Dried hWJ-MSCs-Sec gel***

The preparation of the gel involves incorporating fresh and fresh hWJMSCs-Sec into the carbomer gel in three sequential stages, following a specific formula (P1: 6 *g* carbomer gel+3 *ml* hWJ-MSCs-Sec, P2: 6 *g* carbomer gel+4.5 *ml* hWJ-MSCs-Sec, P3: 6 *g* carbomer gel+*6 ml* hWJ-MSCs-Sec), each mixture is stirred gently until it reaches a homogeneous consistency [23]. Subsequently, the hWJ-MSCs-Sec gel is placed in a small tray and placed inside the vacuum freeze-drying machine used for the lyophilization method with a freeze dryer (FD-F-CE, China) tool [24]. The lyophilization process using a freeze dryer was carried out for 42 *hr* at a temperature of 35-50°*C*.

***Proteins assay***

The Human KGF (E-EL-H0092), HGF (E-EL-H0084), PDGF (E-EL-H2211), EGF (E-EL-H0059), and HB-EGF (E-EL-H2667) level were assayed using the Elabscience kit, based on manufacturing protocols. The ELISA Assay quantification was done to measure the hWJ-MSCs-Sec gel characteristics and the protein level of hWJ-MSCs-Sec gel [25].

***Antioxidant assay***

***$H_2O_2$ Scavenging Activity Assay:*** hWJ-MSCs-Sec gel was added 60 $\mu l$ to the 96-well microplate. In the control and blank wells, 12 $\mu l$ of 1 *mM* ferrous ammonium sulfate (Sigma Aldrich, 215406) were introduced. Following this, dimethyl sulfoxide (DMSO) (Supelco, 1.02952.1000) was added to the control (63 $\mu l$) and blank (90 $\mu l$) wells. Then, 3 $\mu l$ of 5 *mM* $H_2O_2$ (Merck, 1.08597.1000) was added to each well of the microplate. After incubating time in a dark room for 5 min, 1,10-phenanthroline (Sigma Aldrich, 131377) was added (75 $\mu l$) into each well. Following another 10-minutes incubation, absorbance at 510 *nm* was measured using a spectrophotometer [26]. $H_2O_2$ scavenging activity was determined using the formula:

$$\%\ \text{scavenging activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

***NO scavenging activity assay***

Sodium nitroprusside 10 *mM* (Merck, 106541) in PBS (Gibco, 1740576) as much as 40 $\mu l$ was mixed with 10 $\mu l$ of the hWJ-MSCs-Sec gel sample. After incubating the mixture at room temperature for 2 *hr*, 100 $\mu l$ of Griess reagent containing 1% sulfanilamide (Merck, 111799), 2% $H_3PO_4$ (Merck, 100573), and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (Sigma Aldrich, 222488) was added to each well of 96-well plate and the absorbance was measured at 546 *nm* [27]. NO scavenging activity was determined using the formula:

$$\%\ \text{scavenging activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

***FRAP assay***

To start preparing the reagent, 10 *ml* of 300 *mM* acetate buffer (pH=3.6) was combined with 1 *ml* of 20 mM ferric chloride hexahydrate (Merck 1.03943.0250) and 1 *ml* of 10 *mM* 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma- Aldrich, T1253). Following that, 7.5 $\mu L$ of hWJ-MSCs-Sec gel sample and 142.5 $\mu l$ of the FRAP reagent were introduced into the wells and incubated at 37°*C* for 6 *min* before it got measured at 593 *nm* [28]. FRAP scavenging activity was determined using the formula:

$$\%\ \text{scavenging activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

***Proliferation assay***

The human skin fibroblast cell line (BJ cells) (ATCC, CRL-2522) was cultured based on previous study [29]. As much as $5\times10^3$ cells were grown in a 96 well plate. After reaching confluence, the cells were treated with various samples, while untreated cells act as a negative control (NC). WST-8 were introduced after 24 *hr* of treatment and the absorbance was read at 595 *nm* [30].

***Cells migration***

To determine the ability of hWJ-MSCs-Sec gel in wound healing, cells migration assays were conducted on scratched-human skin fibroblast cells as wounded cells model. The fibroblast cells were plated on a 24 well plate at a density of $7\times10^3$. After reaching 90% confluence, the cells were scratched across the well to create a wound model by using a blue tip. Cells were then treated with the hWJ-MSCs-Sec gel samples, and cell without treatment act as a NC. After 24 *hr*, the scratched area was measured under the microscope using Image J software [31].

***Statistical analysis***

The resulting data represents the mean ± standard deviation from tree replicate measurements of each sample. Comparisons of treatments were undertaken using ANOVA, with a significance level of $p<0.05$. The post hoc test, namely Tukey Honestly Significant Difference, along with the Dunnett T3 test with a 95% confidence interval, was utilized. The statistical analy-

sis was conducted using SPSS software (version 20.0), and the findings were visualized using GraphPad Prism program (version 9.0).

## Results

### *Protein content of Freeze-Dried hWJMSCs-Sec gel*

The influence of hWJMSCs-Sec treatments on the levels of Growth Factor Proteins are elucidated in figure 1. As shown in figure 1, the hWJMSCs-Sec treatment in the FDSG regimen demonstrated an increasing trend in protein levels alongside the addition of hWJMSCs-Sec volume to the carbomer gel. On the other hand, the Freeze-Dried hWJ-MSCs-Sec (FDS) treatment significantly increased the levels of KGF, HGF, PDGF, EGF, and HB-EGF compared to other treatments, especially fresh hWJ-MSCs-Sec (S). Additionally, Freeze-Dried Gel Based (FDGB) and Freeze-Dried Medium Basal (FDMB) were used as negative control groups ($p<0.05$).

### *Antioxidant activity of Freeze-Dried hWJMSCs-Sec gel*

To determine its potential as a wound healing treatment, antioxidant testing was conducted by evaluating its ability to scavenge $H_2O_2$, NO, and FRAP (Figures 2-4). The antioxidant activity of FDSG exhibits a significant superiority in all antioxidant tests compared to the Secretome (S) and hWJMSCs-Sec Gel (SG). The results of antioxidant activity tend to change with different hWJMSCs-Sec Gel formulation. FDSG itself exhibits the highest antioxidant activity in the formulation of most concentrated hWJMSCs-Sec Gel, with the highest concentration being 400 *µg/ml*.

### *Proliferation effect of Freeze-Dried hWJMSCs-Sec gel*

Proliferation assays were conducted to assess the cells viability of freeze-dried hWJMSCs-Sec and freeze-dried hWJMSCs-Sec gel on human skin fibro-

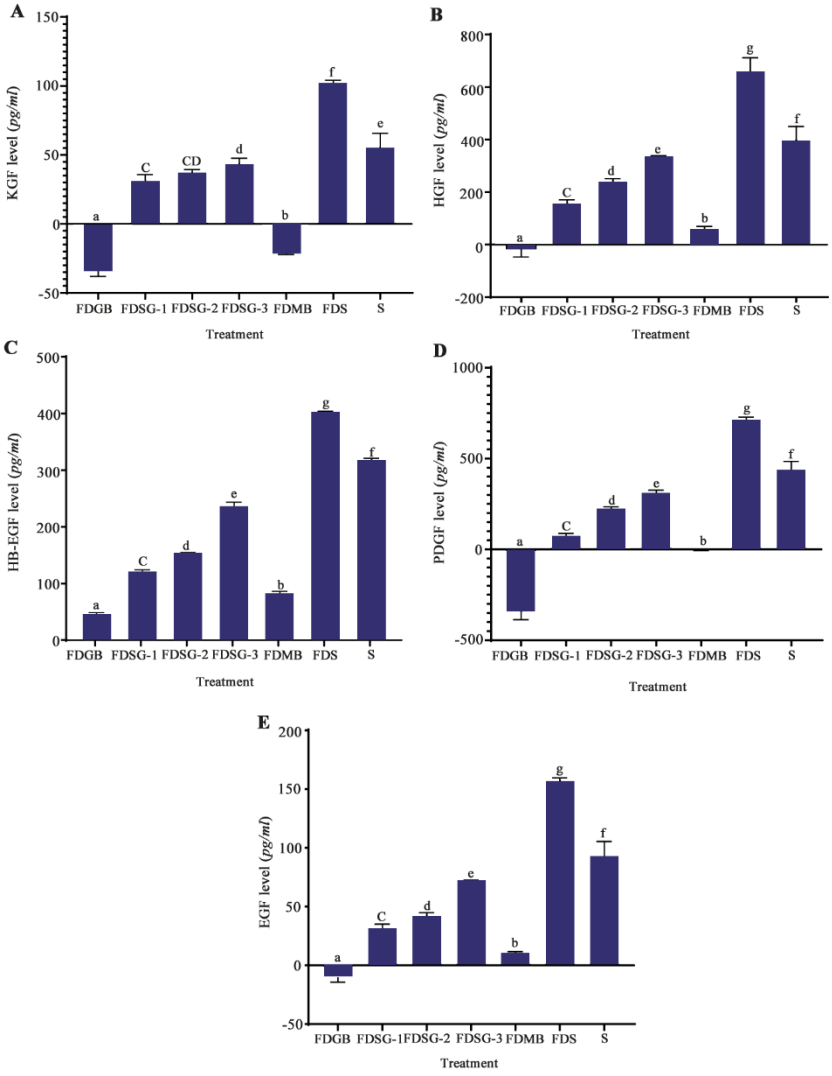


Figure 1. Effect of various treatments on hWJMSCs-Sec toward growth factor proteins level.
A) KGF, B) HGF, C) HB-EGF, D) PDGF, E) EGF. The data were presented as mean ± standard deviation. Different superscript letters on Figure A (a, b, c, cd, d, e, f), and B, C, D, E (a, b, c, d, e, f, g) showed significant differences among treatments at $p<0.05$ (Data was analyzed using ANOVA followed by Tukey HSD post hoc test). Samples included hWJMSCs-Sec (S), Freeze-dried hWJMSCs-Sec (FDS), Freeze-dried Medium Basal (FDMB), Freeze-Dried hWJMSCs-Sec Gel (FDSG), and Freeze-dried Gel Based (FDGB). Number on samples indicate the ratio of gel to hWJMSCs-Sec, 1) 6 *g*: 3 *ml*, 2) 6 *g*: 4.5 *ml*, and 3) 6 *g*: 6 *ml*.

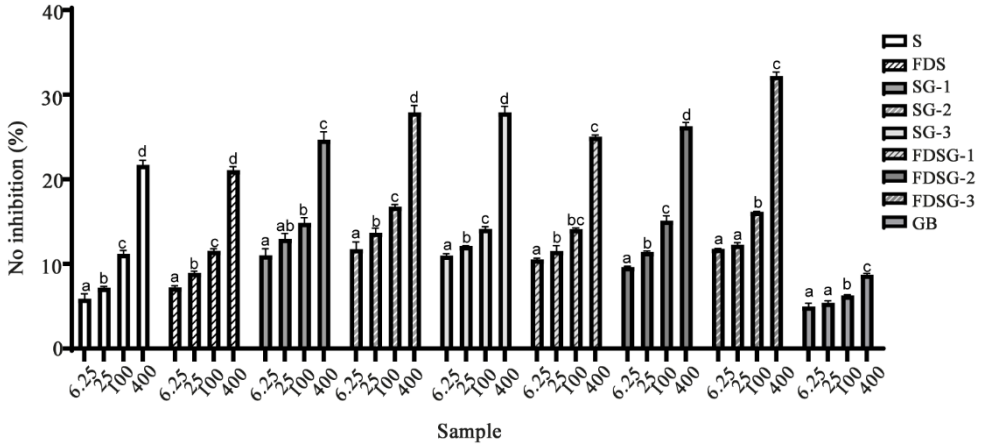


Figure 2. Antioxidant level of hWJ-MSCs-Sec gels in various formulations by its ability to scavenge $H_2O_2$.
*The data were presented as mean ± standard deviation. Different letters (a, ab, b, bc, c, d) showed significant variation among concentration at $p<0.05$. Data was analyzed using ANOVA and followed by Tukey HSD & Dunnett T3 *post hoc* test. Samples included hWJ-MSCs-Sec (S), Freeze-dried hWJ-MSCs-Sec (FSD), hWJ-MSCs-Sec Gel (SG), Freeze dried hWJ-MSCs-Sec gel (FDSG), and Carbomer gel base (GB). Number on samples indicate the ratio of gel to hWJMSCs-Sec, 1) 6 *g*: 3 *ml*, 2) 6 *g*: 4.5 *ml*, and 3) 6 *g*: 6 *ml*.

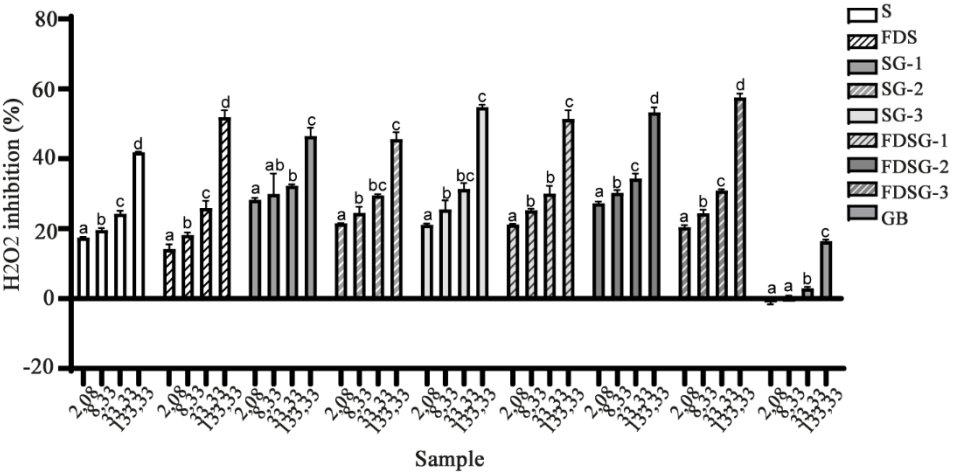


Figure 3. Antioxidant level of hWJMSCs-Sec gels in various formulations by its ability to scavenge NO.
*The data were presented as mean ± standard deviation. Different letters (a, ab, b, bc, c, d) showed significant variation among concentration at $p<0.05$. Data was analyzed using ANOVA and followed by Tukey HSD & Dunnett T3 *post hoc* test. Samples included hWJ-MSCs-Sec (S), Freeze-dried hWJ-MSCs-Sec (FDS), hWJ-MSCs-Sec Gel (SG), Freeze dried hWJ-MSCs-Sec gel (FDSG), and Carbomer gel base (GB). Number on samples indicate the ratio of gel to hWJMSCs-Sec, 1) 6 *g*: 3 *ml*, 2) 6 *g*: 4.5 *ml*, and 3) 6 *g*: 6 *ml*.

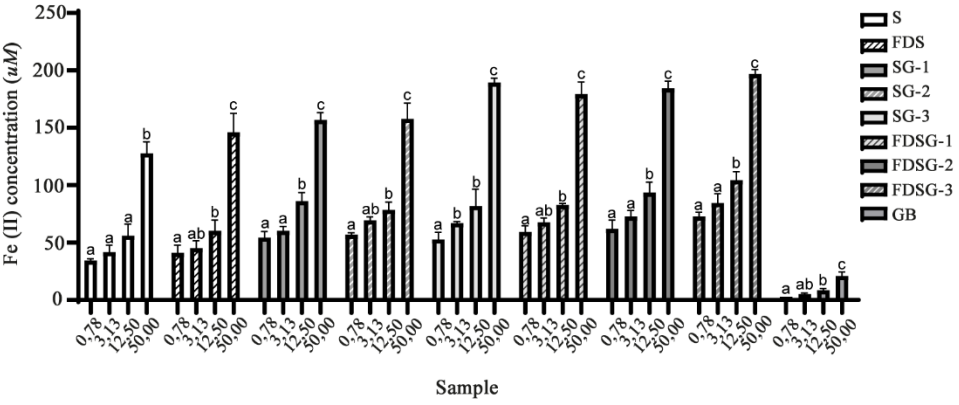


Figure 4. Antioxidant level of hWJ-MSCs-Sec gels in various formulations by its ability to scavenge FRAP.
*The data were presented as mean ± standard deviation. Different letters (a, ab, b, c) showed significant variaton among concentration at $p<0.05$. Data was analyzed using ANOVA and followed by Tukey HSD & Dunnett T3 *post hoc* test. Samples included hWJ-MSCs-Sec (S), Freeze-dried hWJ-MSCs-Sec (FDS), hWJ-MSCs-Sec Gel (SG), Freeze dried hWJ-MSCs-Sec gel (FDSG), and Carbomer gel base (GB). Number on samples indicate the ratio of gel to hWJMSCs-Sec, 1) 6 *g*: 3 *ml*, 2) 6 *g*: 4.5 *ml*, and 3) 6 *g*: 6 *ml*.

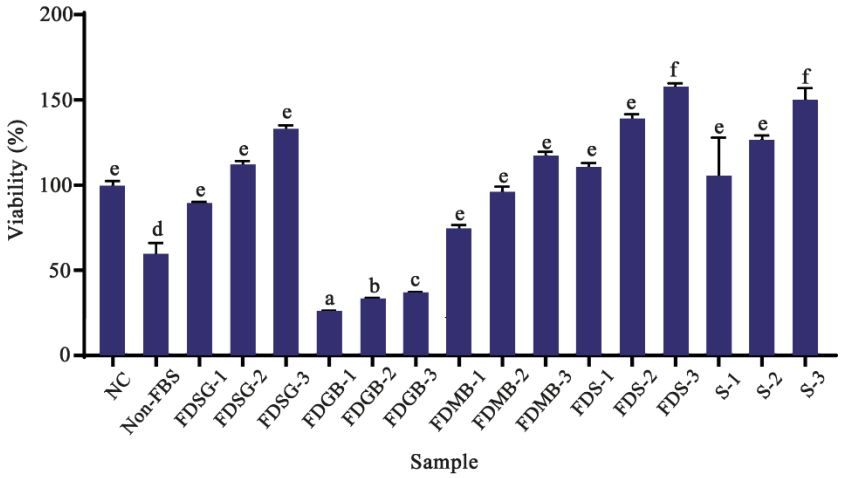


Figure 5. Proliferation effect of hWJMSCs-Sec gel towards human fibroblast cells.
*The data were presented as mean ± standard deviation. Different letters (a, b, c, d, e, f) showed significant variation among concentration at P<0.05. Data was analyzed using T-Test. Samples included: Negative Control: untreated-cells (NC), Cells without FBS (Non-FBS), Cells+hWJMSCs-Sec (S), Cells+Freeze-dried hWJMSCs-Sec (FDS), Cells+Freeze-dried Medium Basal (FDMB), Cells+ Freeze-Dried hWJMSCs-Sec Gel (FDSG), and Cells + Freeze-dried Gel Based (FDGB). Number on samples indicates the concentration used for treatment, 1) 1.3 *µg/ml*, 2) 12.5 *µg/ml*, and 3) 100 *µg/ml*. While for Secretome (S), the number indicates the concentration of 1) 3.25%, 2) 12.5%, and 3) 50%.

blast cells. The results of these assays are presented in figure 5. The addition of hWJMSCs-Sec at various concentrations did not exhibit any toxic activity towards fibroblast cells. Conversely, cells treated with hWJMSCs-Sec demonstrated enhanced proliferation compared to the Negative Control (NC). The freeze-dried secretome showed superior cell viability compared to the S samples. These findings indicate that the freeze-drying process preserves important bioactive components in hWJMSCs-Sec and is non-toxic to fibroblast cells.

***Migration of fibroblast cells after the treatment with Freeze-Dried hWJ-MSCs-Sec gel***

The proliferation and migration of fibroblast are crucial for the process of skin repair/regeneration. Therefore, migration assays were conducted on fibroblast cells that were subjected to scratching to assess the wound healing process by evaluating the effects of hWJMSCs-Sec gel on cell proliferation and migration abilities. The migration data are shown in figure 6. The results demonstrate increased cell migration values with higher addition of hWJMSCs-Sec. FDS exhibits the highest efficacy in promoting cell migration, surpassing even that of S itself. Similarly, FDSG demonstrates superior efficacy compared to S, closely resembling the outcome of FDS. This substantiates that the growth factors within the secretome are preserved through the freeze-drying process.

## Discussion

Wharton's Jelly is widely regarded as a highly reliable and abundant source of mesenchymal stem cells. In the preceding investigation, a comprehensive analysis of hWJMSCs was undertaken. It has been demonstrated that hWJMSCs exhibit a high expression of CD90, CD105, CD44, and CD73, while displaying a negative expression of CD11b, CD19, CD34, CD45, and HLA-DR [21]. In this experiment, passage 5 of the hWJMSCs cells was selected due to its notable and consistent proliferation [32]. Carbomer is utilized as the primary component to formulate the secretome from WJMSCs into a gel form. Freeze-drying, also known as lyophilization, is a crucial technique utilized in this study to safeguard the integrity of hWJMSCs-Sec, particularly due to their susceptibility to heat-induced damage, as emphasized by previous research findings [33]. This method involves the removal of moisture under low temperatures and reduced pressure, mitigating the potential harm caused by heat to the sensitive cellular structure of hWJMSCs-Sec. Moreover, freeze-drying serves as a promising approach for the preservation of biomaterial and natural cells in a desiccated state, a process that enhances their stability during storage and transportation. The significance of this technique lies in its ability to prolong the shelf life of hWJMSCs-Sec by maintaining their structural and functional characteristics, even at room temperature. Recent studies, such as the work by Merivaara *et al* [34], underscore the efficacy of freeze-drying in providing optimal storage conditions, making it a valuable method for the long-term preservation of biological materials.

ELISA results have illuminated a significant disparity between freeze-dried hWJ-MSCs-Sec and its conventional counterpart, particularly concerning the protein content of critical growth factors. This disparity is marked by notably higher concentrations of several growth factors, specifically KGF, HGF, PDGF, EGF, and HB-EGF, within the freeze-dried hWJ-MSCs-Sec.

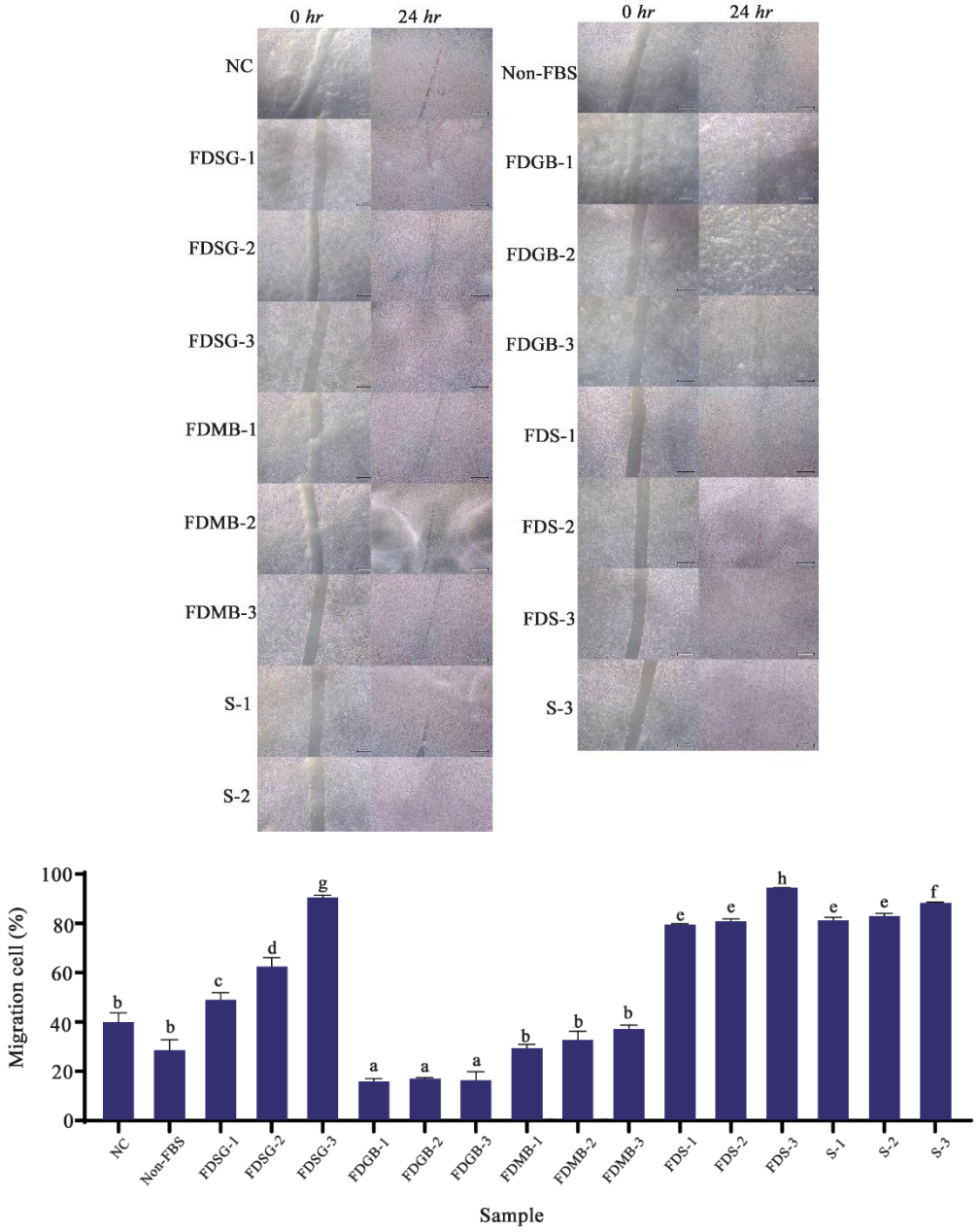


Figure 6. Effect of various treatments on hWJMSCs-Sec toward the migration of human skin fibroblast cells.
*The data were presented as mean±standard deviation. Different letters (a, b, c, d, e, f, g, h) showed significant variation among samples at $p<0.05$. Data was analyzed using T-Test. Samples included: Scratched-cells (NC), Scratched-cells without FBS (Non-FBS), Scratched-cells+hWJMSCs-Sec (S), Scratched-cells+Freeze-dried hWJMSCs-Sec (FDS), Scratched-cells + Freeze-dried Medium Basal (FDMB), Scratched-cells+Freeze-Dried hWJMSCs-Sec Gel (FDSG), and Scratched-cells + Freeze-dried Gel Based (FDGB). Number on samples indicate the concentration used for treatment, 1) 1.3 *µg/ml*, 2) 12.5 *µg/ml*, and 3) 100 *µg/ml*. While for Secretome (S), the number indicates the concentration of 1) 3.25%, 2) 12.5%, and 3) 50%.

These findings bear profound implications for the applications of hWJ-MSCs-Sec in regenerative medicine and tissue engineering. The elevated content in KGF, HGF, PDGF, EGF, and HB-EGF in freeze-dried hWJ-MSCs-Sec underscores its potential in accelerating wound healing and tissue repair, demonstrating a promising avenue for therapeutic interventions [35].

Growth factors are signaling molecules that play a critical role in cell growth, differentiation, and survival [36]. The five growth factors discussed in this article are all involved in different aspects of wound healing, from inflammation and angiogenesis to cell proliferation and extracellular matrix deposition. KGF is a growth factor that is primarily produced by keratinocytes, the cells that make up the epidermis [37]. KGF is a potent stimulant of keratinocyte proliferation and differentiation. While HGF is a potent stimulant of cell proliferation, migration, and differentiation. Together,

they play a role in angiogenesis and inflammation [38,39]. The present investigation observed a positive correlation between the formulation of hWJ-MSC-Sec and the levels of KGF and HGF in FDSG. Previous research has elucidated that the levels of HGF exhibit an elevation in rats afflicted with DFU following treatment with secretome derived from umbilical cord mesenchymal stem cells (UCMSCs) [40].

PDGF is an additional growth factor that can be specifically targeted for the therapy of DFU. So far, the Food and Drug Administration (FDA) has granted permission to PDGF for its capacity to expedite the process of wound healing [41] because of its capacity for attracting fibroblast [42]. PDGF was detected in FDSG samples as well in this analysis. This finding suggests that FDSG exhibits promise in the treatment of DFU. EGF and HB-EGF was also found in FDSG, as a growth factor that is produced by a variety of cells, including keratinocytes, fibroblasts, and macrophages [43]. Kuo *et al* [44] observed that the incorporation of stem cells resulted in a significant increase in the concentration of EGF on the accelerated healing of wounds in individuals with diabetes. Another study stated that the addition of HB-EGF resulted in an increase in the formation of granulation tissue in mice with diabetic wounds [45]. In all protein tests performed, FDS exhibited the highest efficacy among the samples, including FDSG. This could be influenced by the addition of carbomer gel, resulting in a lower concentration of active ingredients in the secretome gel compared to the secretome itself. As seen in figure 1, freeze-dried carbomer gel (FDGB) did not show any presence of growth factor proteins.

In addition to growth factors, the presence of oxidative stress within the wound will also exert an influence on the process of wound healing. High levels of $H_2O_2$ at a wound site serve as a signal for initiating chemotactic and inflammatory response [46]. The inflammatory response can also be exacerbated by the presence of NO. Nitric oxide plays a pivotal role in regulating three crucial aspects of wound healing: vascular homeostasis, inflammation, and antimicrobial activity [47]. These radical compounds can be identified as potential targets for wound healing interventions. The gel hWJ-MSCs-Sec exhibits a high antioxidant level (Figures 2-4). After the freeze-drying process, FDSG still exhibited remarkably high antioxidant results. This data provides evidence that the freeze-drying process effectively preserves the active compounds within the hWJ-MSCs-Sec gel. Based on the results, it was proven that FDSG comprises growth factor protein and high antioxidant levels, rendering it a promising DFU agent.

To further explore the potential of gel secretome as a wound healer, cell proliferation and migration assays on human skin fibroblast cells were conducted. FDSG shows no toxic effect towards the fibroblast cells. Instead, the cell's viability increased after the treatment

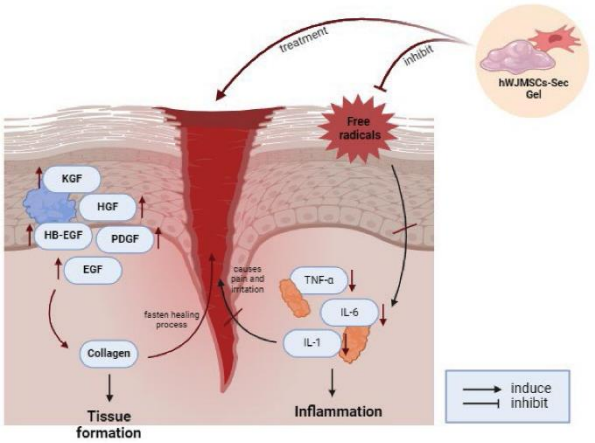


Figure 7. Proposed mechanism of hWJMSCs-Sec gel as a wound healing agent.
* The treatment of hWJMSCs-Sec gel into the wounded skin can regulate some growth factors such as KGF, HGF, HB-EGF, PDGF, and EGF. The upregulation of these growth factors can induce the production of collagen, which plays a crucial role to regenerate tissue formation. The high antioxidant hWJMSCs-Sec gel also has the ability to scavenge free radicals, and inhibit the excessive inflammation that can cause irritation, redness, and pain in the wound.

with FDSG, FDS, and S itself (Figure 5). Cell migration is pivotal in developmental processes within cells. MSCs exhibits multipotent characteristic and reparative abilities, rendering them promising sources of stem cells for degenerative therapy. MSCs possess various chemical factors such as chemokines, cytokines, and other growth factors, which support MSC migration to wound sites [48]. Growth factors like EGF, FGF, TGFs, VEGF, and PDGF play a crucial role in regulating cell division, differentiation, and remodeling, [49] which facilitate cell proliferation and cell migration [50]. The proposed mechanism of hWJMSCs-Sec as a wound healing agent was summarized in figure 7. The study results indicate that hWJMSCs-Sec contains several growth factors, thus supporting fibroblast cells to close faster after treatment with hWJMSCs-Sec. FDS-100 *µg/ml* and FDSG-100 *µg/ml* exhibited superior abilities with wound closure of 95.62% and 90.40%, when compared to NC. This result also further proves that the freeze-drying process on hWJMSCs-Sec gel can maintain quality by dehydrating the product into powder form. The dehydrated product also supports longer storages with good quality [51]. However, this study was hindered by a lack of verification assays. Further in vitro as well as in vivo approaches are needed to support this study.

## Conclusion

In this study, hWJ-MSCs-Sec is thoroughly mixed with the carbomer gel and freeze-dried to maintain quality stability. This is evidenced by the fact that after undergoing the freeze-drying process, the protein levels of growth factors and antioxidant activity from hWJ-MSCs-Sec still yielded excellent results, even

surpassing those that did not undergo the freeze-drying process. The application of freeze-dried hWJ-MSCs-Sec *via* carbomer gel has demonstrated the preservation of growth factors and antioxidant properties. This endows it with exceptional migratory capabilities towards the wound site, indicating potential for wound healing therapy.

## Ethical Issue

This research obtained ethical approval from the Research Ethics Committee of the Faculty of Medicine at Maranatha Christian University (Approval No: 097/KEP/VII/2020, dated July 25th, 2020).

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## Conflict of Interest

The authors declare no conflict of interest.

## References

1. Miller TA, Campbell JH, Bloom N, Wurdeman SR. Racial disparities in health care with timing to amputation following diabetic foot ulcer. Diabetes Care 2022 Oct 1;45(10):2336-41.
2. Kavitha KV, Tiwari S, Purandare VB, Khedkar S, Bhosale SS, Unnikrishnan AG. Choice of wound care in diabetic foot ulcer: A practical approach. World J Diabetes 2014 Aug 8;5(4):546.
3. Morales-González M, Díaz LE, Dominguez-Paz C, Valero MF. Insights into the design of polyurethane dressings suitable for the stages of skin wound-healing: A systematic review. Polymers (Basel) 2022 Jul 24;14(15):2990.
4. Serra MB, Barroso WA, Silva NN, Silva SD, Borges AC, Abreu IC, Borges MO. From inflammation to current and alternative therapies involved in wound healing. Int J Inflam 2017;2017(1):3406215.
5. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. Int J Mol Sci 2016 Dec 11;17(12):2085.
6. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science 2014 Dec 2;2:53.
7. Liu Z, Ren Z, Zhang J, Chuang CC, Kandaswamy E, Zhou T, Zuo L. Role of ROS and nutritional antioxidants in human diseases. Front Physiol 2018 May 17;9:360203.
8. Dao DT, Anez-Bustillos L, Adam RM, Puder M, Bielenberg DR. Heparin-binding epidermal growth factor–like growth factor as a critical mediator of tissue repair and regeneration. Am J Pathol 2018 Nov 1;188(11):2446-56.
9. Jian K, Yang C, Li T, Wu X, Shen J, Wei J, et al. PDGF-BB-derived supramolecular hydrogel for promoting skin wound healing. J Nanobiotechnology 2022 Apr 26;20 (1):201.
10. Woodby B, Scott M, Bodily J. The interaction between human papillomaviruses and the stromal microenvironment. Prog Mol Biol Transl Sci 2016 Jan 1;144:169-238.
11. Velardi E, Tsai JJ, van den Brink MR. T cell regeneration after immunological injury. Nat Rev Immunol 2021 May;21(5):277-91.
12. Park SR, Kim JW, Jun HS, Roh JY, Lee HY, Hong IS. Stem cell secretome and its effect on cellular mechanisms relevant to wound healing. Mol Ther 2018 Feb 7;26(2):606-17.
13. Kamolz LP, Keck M, Kasper C. Wharton's jelly mesenchymal stem cells promote wound healing and tissue regeneration. Stem Cell Res Ther 2014 Sep;5:1-2.
14. Arifka M, Wilar G, Elamin KM, Wathoni N. Polymeric hydrogels as mesenchymal stem cell secretome delivery system in biomedical applications. Polymers (Basel) 2022 Mar 17;14(6):1218.
15. Zhao G, Liu F, Lan S, Li P, Wang L, Kou J, et al. Large-scale expansion of Wharton's jelly-derived mesenchymal stem cells on gelatin microbeads, with retention of self-renewal and multipotency characteristics and the capacity for enhancing skin wound healing. Stem Cell Res Ther 2015 Dec;6:1-6.
16. Firlar I, Altunbek M, McCarthy C, Ramalingam M, Camci-Unal G. Functional hydrogels for treatment of chronic wounds. Gels 2022 Feb 17;8(2):127.
17. Ho AY, Wright JL, Blitzblau RC, Mutter RW, Duda DG, Norton L, et al. Optimizing radiation therapy to boost systemic immune responses in breast cancer: a critical review for breast radiation oncologists. Int J Radiat Oncol Biol Phys 2020 Sep 1;108(1):227-41.
18. Molnar A, Lakat T, Hosszu A, Szebeni B, Balogh A, Orfi L, et al. Lyophilization and homogenization of biological samples improves reproducibility and reduces standard deviation in molecular biology techniques. Amino Acids 2021 Jun;53(6):917-28.
19. Zhang Y, Ng W, Hu J, Mussa SS, Ge Y, Xu H. Formulation and in vitro stability evaluation of ethosomal carbomer hydrogel for transdermal vaccine delivery. Colloids Surf B Biointerfaces 2018 Mar 1;163:184-91.

20. Widowati W, Widyastuti H, Murti H, Laksmitawati DR, Kusuma HS, Rizal R, et al. Interleukins and VEGF secretome of human Wharton's jelly mesenchymal stem cells-conditioned medium (hWJMSCs-CM) in different passages and oxygen tensions. Biosci Res 2017 Oct 1;14 (4):776-87.
21. Widowati W, Gunanegara RF, Rizal R, Widodo WS, Amalia A, Wibowo SH, Handono K, Marlina M, Lister IN, Chiuman L. Comparative analysis of Wharton's Jelly mesenchymal stem cell (WJ-MSCs) isolated using explant and enzymatic methods. InJournal of Physics: Conference Series 2019 Nov 1 (Vol. 1374, No. 1, p. 012024). IOP Publishing.
22. Kusuma HS, Widowati W, Gunanegara RF, Juliandi B, Lister NE, Arumwardana S, et al. Effect of conditioned medium from IGF1-induced human Wharton's jelly mesenchymal stem cells (IGF1-hWJMSCs-CM) on osteoarthritis. Avicenna J Med Biotechnol 2020 Jul;12(3): 172.
23. Zukhiroh Z, Putra A, Chodidjah C, Sumarawati T, Subchan P, Trisnadi S, Hidayah N, et al. Effect of secretome-hypoxia mesenchymal stem cells on regulating SOD and MMP-1 mRNA expressions in skin hyperpigmentation rats. Open Access Macedonian Journal of Medical Sciences 2022 Dec 15;10(A):1-7.
24. Nowak D, Jakubczyk E. The freeze-drying of foods—The characteristic of the process course and the effect of its parameters on the physical properties of food materials. Foods 2020 Oct 18;9(10):1488.
25. Widowati W, Widyastuti H, Murti H, Laksmitawati DR, Kusuma HS, Rizal R, et al. Interleukins and VEGF secretome of human Wharton's jelly mesenchymal stem cells-conditioned medium (hWJMSCs-CM) in different passages and oxygen tensions. Biosci Res 2017 Oct 1;14 (4):776-87.
26. Priyandoko D, Widowati W, Kusuma HS, Afifah E, Wijayanti CR, Wahyuni CD, et al. Antioxidant Activity of Green Tea Extract and Myricetin. In2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce) 2021 Jul 14 (pp. 1-5). IEEE.
27. Widowati W, Prahastuti S, Hidayat M, Hasianna ST, Wahyudianingsih R, Eltania TF, et al. Detam 1 black soybean against cisplatin-induced acute ren failure on rat model via antioxidant, antiinflammatory and antiapoptosis potential. J Tradit Complement Med 2022 Jul 1; 12(4):426-35.
28. Widowati W, Rani AP, Hamzah RA, Arumwardana S, Afifah E, Kusuma HS, et al. Antioxidant and antiaging assays of Hibiscus sabdariffa extract and its compounds. Natural Product Sciences 2017 Sep 1;23(3):192-200.
29. Girsang E, Lister IN, Ginting CN, Widowati W, Arumwardana S, Marthania M, et al. Chlorogenic acid in preventing and curing ultraviolet-induced damage in human skin fibroblast as an antiaging cell model. Pharmaciana 2023;13(2):159-65.
30. Widowati W, Priyandoko D, Lenny L, Revika R, Novianti S, Kusuma HS, et al. Camellia sinensis L. Extract Suppresses Inflammation on Acute Respiratory Distress Syndrome Cells Models via Decreasing IL-1ß, IL-6 and COX-2 Expressions. Trends in Sciences 2024;21(1): 7010.
31. Kruse CR, Singh M, Targosinski S, Sinha I, Sørensen JA, Eriksson E, et al. The effect of pH on cell viability, cell migration, cell proliferation, wound closure, and wound reepithelialization: In vitro and in vivo study. Wound Repair Regen 2017 Apr;25(2):260-9.
32. Widowati W, Noverina R, Ayuningtyas W, Kurniawan D, Kusuma HS, Arumwardana S, et al. Proliferation, characterization and differentiation potency of adipose tissue-derived mesenchymal stem cells (AT-MSCs) cultured in fresh frozen and non-fresh frozen plasma. Int J Mol Cell Med 2019;8(4):283.
33. Wallace EW, Kear-Scott JL, Pilipenko EV, Schwartz MH, Laskowski PR, Rojek AE, et al. Reversible, specific, active aggregates of endogenous proteins assemble upon heat stress. Cell 2015 Sep 10;162(6):1286-98.
34. Merivaara A, Zini J, Koivunotko E, Valkonen S, Korhonen O, Fernandes FM, Yliperttula M. Preservation of biomaterials and cells by freeze-drying: Change of paradigm. J Control Release 2021 Aug 10;336:480-98.
35. Kolimi P, Narala S, Nyavanandi D, Youssef AA, Dudhipala N. Innovative treatment strategies to accelerate wound healing: trajectory and recent advancements. Cells 2022 Aug 6;11(15):2439.
36. Tarnawski AS, Ahluwalia A. The critical role of growth factors in gastric ulcer healing: the cellular and molecular mechanisms and potential clinical implications. Cells 2021 Aug 2;10(8):1964.
37. Rezvanian M, Ng SF, Alavi T, Ahmad W. In-vivo evaluation of Alginate-Pectin hydrogel film loaded with Simvastatin for diabetic wound healing in Streptozotocin-induced diabetic rats. Int J Biol Macromol 2021 Feb 28;171:308-19.
38. Ruttanapattanakul J, Wikan N, Okonogi S, Takuathung MN, Buacheen P, Pitchakarn P, et al. Boesenbergia rotunda extract accelerates human keratinocyte proliferation through activating ERK1/2 and PI3K/Akt kinases. Biomed Pharmacother 2021 Jan 1;133:111002.
39. Gallo S, Sala V, Gatti S, Crepaldi T. Cellular and molecular mechanisms of HGF/Met in the cardiovascular system. Clin Sci 2015 Dec 1;129(12):1173-93.
40. Shi R, Lian W, Jin Y, Cao C, Han S, Yang X, et al. Role and effect of vein-transplanted human umbilical cord mesenchymal stem cells in the repair of diabetic foot ulcers in rats. Acta Biochimica et Biophysica Sinica 2020 Jun;52(6):620-30.
41. Ahangar P, Mills SJ, Cowin AJ. Mesenchymal stem cell secretome as an emerging cell-free alternative for improving wound repair. Int J Mol Sci 2020 Sep 24;21 (19):7038.
42. Martino MM, Briquez PS, Maruyama K, Hubbell JA. Extracellular matrix-inspired growth factor delivery systems for bone regeneration. Adv Drug Deliv Rev 2015 Nov 1;94:41-52.
43. Stunova A, Vistejnova L. Dermal fibroblasts—A heterogeneous population with regulatory function in wound healing. Cytokine Growth Factor Rev 2018 Feb 1;39: 137-50.

44. Kuo YR, Wang CT, Cheng JT, Kao GS, Chiang YC, Wang CJ. Adipose-derived stem cells accelerate diabetic wound healing through the induction of autocrine and paracrine effects. Cell Transplantation 2016 Jan;25(1): 71-81.
45. White MJ, Briquez PS, White DA, Hubbell JA. VEGF-A, PDGF-BB and HB-EGF engineered for promiscuous super affinity to the extracellular matrix improve wound healing in a model of type 1 diabetes. NPJ Regen Med 2021 Nov 18;6(1):76.
46. Zhu G, Wang Q, Lu S, Niu Y. Hydrogen peroxide: a potential wound therapeutic target. Medical Principles and Practice 2017 Apr 5;26(4):301-8.
47. Malone-Povolny MJ, Maloney SE, Schoenfisch MH. Nitric oxide therapy for diabetic wound healing. Advanced Healthcare Materials 2019 Jun;8(12):1801210.
48. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song G. Mesenchymal stem cell migration and tissue repair. Cells 2019 Jul 28;8(8):784.
49. Kardas G, Daszyńska-Kardas A, Marynowski M, Brząkalska O, Kuna P, Panek M. Role of platelet-derived growth factor (PDGF) in asthma as an immunoregulatory factor mediating airway remodeling and possible pharmacological target. Frontiers in Pharmacology 2020 Feb 14;11:47.
50. Augustine R, Hasan A, Dalvi YB, Rehman SR, Varghese R, Unni RN, et al. Growth factor loaded in situ photocrosslinkable poly (3-hydroxybutyrate-co-3-hydroxyvalerate)/gelatin methacryloyl hybrid patch for diabetic wound healing. Materials Science and Engineering: C 2021 Jan 1;118:111519.
51. Oyinloye TM, Yoon WB. Effect of freeze-drying on quality and grinding process of food produce: A review. Processes 2020 Mar 20;8(3):354.