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Wound Healing Potent of Lyophilized-Secretome Gel from Human Wharton's Jelly Mesenchymal Stem Cells

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Abstract

Wounds that become infected can lead to serious illness or even death if left untreated. Unfortunately, current wound healing treatments don't always work well. Achieving optimal wound healing involves the coordination of diverse cellular events facilitated by numerous growth factors, cytokines, and chemokines. One potential solution for addressing the wound healing process is using lyophilized-secretome gel from human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs-Sec). This gel, called lyophilized-hWJMSCs-Sec gel (LSG) is created by mixing carbomer gel with hWJMSCs-Sec, then freeze-dry it into a powder to maintain its quality and stability. The quality testing of the topical gel formulation is conducted by measuring. The gel is tested for pH, viscosity, consistency, and its appearance. It's also tested for antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were employed to identify the antioxidant activities of the lyophilized-gel. Proteins level of Tissue Inhibitor of Metalloproteinases-2 (TIMP-2), Epidermal Growth Factor (EGF), basic Fibroblast Growth Factor (bFGF), Interleukin-6 (IL-6), and IL-4 were also measured with the ELISA method. The LSG has a soft and non-sticky texture with clarity, a pandan-like aroma, and excellent adhesion and spreadability. It contains proteins and antioxidants that help wound healing. The lyophilized-Sec gel contained TIMP-2, EGF, bFGF, IL-6, and IL-4 as well as stronger antioxidant properties compared to un-lyophilized-hWJMSCs-Sec gel, and showed promise as an effective remedy for wound healing.

Keywords: Antioxidant, Carbomer, Secretome, Human Wharton's Jelly Mesenchymal Stem Cells, Wound healing

Introduction

A wound is a term that encapsulates a variety of injuries to the skin, ranging from minor cuts and abrasions to more severe lacerations and punctures. The human skin, being the body's biggest organ, acts as a shielding blockade against external elements [1]. When this barrier is breached, either accidentally or intentionally, a wound is formed. Wounds can result from a myriad of sources, including accidents, surgical procedures, or even underlying medical conditions [2]. Numerous types of wound dressings, including gauze, films, hydrocolloids, foam, and tissue engineered skin substitutes, aid in the wound healing process. However, some are expensive and may cause dry skin or trigger infections [2].

Comprehensive understanding of the various types of wounds is paramount for administering precise treatment and facilitating effective healing [3]. Wounds range from superficial abrasions to those penetrating deep into underlying tissues, affecting blood vessels, muscles, and occasionally, even bones. This spectrum of wound severity necessitates a nuanced approach, where the depth and extent of tissue involvement become pivotal factors [4]. Superficial wounds, confined to the skin's outer layers, often demand basic first aid measures, such as cleaning and bandaging. Conversely, wounds reaching deeper tissues necessitate heightened attention, as their impact on vital structures requires a more intricate intervention [5]. Healthcare professionals, equipped with a comprehensive understanding of wound classification, navigate the complexity of healing trajectories. The severity of a wound emerges as a guiding principle, steering the course of action in both clinical settings and the hands of individuals providing immediate first aid, ensuring that interventions align with the nuanced demands of each unique wound scenario [6]. Wound healing treatments do not always yield satisfactory results. Optimal wound healing requires the integration of various cellular events mediated by multiple growth factors, cytokines, and chemokines. The process of wound recovery involves the incorporation of intricate molecular and biological events, such as remodeling, cell growth, and inflammation [7]. One of the alternatives in treating the wound repair process is the use of lyophilized-hWJMSCs-Sec gel (LSG).

The umbilical cord, once the vital link between mother and unborn child, emerges as a remarkable source of potent healing potential. Within this unassuming structure lies a treasure trove of stem cells, the building blocks of the body's regenerative prowess [8]. The secretome from human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs-Sec), extracted from fetal tissue, possesses several distinct advantageous characteristics, such as effortless isolation, increased proliferation potential, and potent immunomodulatory effect [9]. The hWJMSCs-Sec also demonstrate the capability to accelerate the recovery process and promote tissue restoration [10]. However, the challenge lies in their optimal delivery to the wound site. This is where the intermediary role of a gel becomes crucial.

Reactive Oxygen Species (ROS) become a crucial factor in wound healing. Elevated levels of ROS can impact cell growth, inflammation, angiogenesis, granular tissue formation, and extracellular matrix formation, such as Matrix Metalloproteinases (MMPs) [11]. The existence of Tissue Inhibitor of Metalloproteinases 2 (TIMP-2) becomes vital as an inhibitor of MMPs, thereby facilitating the acceleration of granulation tissue formation [12]. With the help of Interleukin (IL), they stimulate the proliferation of fibroblasts and generate a new Extracellular Matrix (ECM), which is essential for tissue regeneration and promotes wound closure [13]. IL-4, enhanced with IL-6 [14], is proven to play an important role in skin repair by assembling collagen fibrils [15]. Other proteins such as Epidermal Growth Factor (EGF) and basic Fibroblast Growth Factor (bFGF), known as growth factor proteins, can stimulate the proliferation and migration of keratinocytes, and initiate angiogenesis [16].

A gel constitutes a partially solid structure composed of a mixture of inorganic particles or substantial organic compounds, permeated by a fluid medium. Tissue regeneration and re-epithelialization on the wound site can be promoted by the moist environment provided by the gels [17]. Gel can maintain wound

moisture, absorb a significant amount of exudate, ensure a constant wound temperature, and provide appropriate moisture levels [18]. The expected pH for the hydrogel produced ranges from 4.5 to 7.0, which is in line with the pH of normal skin [19]. Viscosity testing is conducted to determine the spreading ability to ensure even distribution of the gel when applied to the skin, with good gel spreading typically between 5 - 7 cm [20]. Organoleptic testing is performed to assess the appearance of the formulation by examining its shape, color, and odor [21]. Homogeneity testing is carried out to determine whether the formulation is homogeneous or not, as pointed out by the void of grainy particles [21].

The hWJMSCs-Sec is recognized for its efficacy in wound repair. Through the lyophilization process, the hWJMSCs-Sec gel ensures the preservation of proteins and nucleic acids, enhancing the consistency and dependability of molecular assessments [22]. This approach represents a cost-efficient and environmentally sustainable substitute for conventional frozen storage methods [22]. Furthermore, this research will investigate the potential of utilizing LSG from hWJMSCs as a potential treatment for promoting wound healing. ELISA assay conducted to measure the level of TIMP-2, EGF, bFGF, IL-6, and IL-4. In the meantime, an antioxidant assay is conducted to measure the inhibition against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and reduction of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) of lyophilized and un-lyophilized hWJMSCs-Sec gel, fresh and lyophilized- hWJMSCs-Sec.

Materials and methods

hWJ-MSCs culture

The cultivation procedure for hWJ-MSCs was delineated by Widowati *et al.* [23], and has been identified through multipotent differentiation assays and surface phenotype analysis in previous research [24]. The hWJMSCs underwent cultivation in Minimum Essential Medium-α (α-MEM) (Biowest, L0475-500), supplemented with 10 % Fetal Bovine Serum (FBS, Biowest, S1810-500) and 1 % Antibiotic and Anti-Mycotic (ABAM) Biowest, L0010-100). The cells were then incubated in a humidified environment with 5 % CO_2 at 37 °C for 72 h. Upon reaching 80 - 90 % confluence, the hWJMSCs-Sec was collected and centrifuged at 3,000×g for 4 min (MWP 260 r). The resulting supernatant, containing secreted factors, was filtered using a Durapore unit (Millipore Corporation, SLGV 033 RS).

Production of Lyophilized-hWJMSCs-Sec gel

The gel preparation involves adding fresh hWJMSCs-Sec into the carbomer gel in 3 consecutive stages, following a specific formula (1:6 g carbomer gel + 3 mL hWJMSCs-Sec, 2:6 g carbomer gel + 4.5 mL hWJMSCs-Sec, 3:6 g carbomer gel + 6 mL hWJMSCs-Sec), with each mixture stirred gently until achieving uniform consistency [25]. Then, the hWJMSCs-Sec gel is placed in a small tray and subjected to the lyophilization method using a vacuum freeze-drying machine (FD-F-CE, China) [26]. The lyophilization process is conducted for 42 h at a temperature ranging from 35 - 50 °C. Afterward, an evaluation of the gel formulation was conducted (pH, homogeneity, organoleptic properties, viscosity) [27,28].

Quality testing of the hWJMSCs-Sec gel

Organoleptic testing is conducted visually and involves the visual scrutiny of the appearance, color, and odor of the gel produced. Prior to pH measurement, the pH meter is calibrated. Then, the gel's pH is measured using the pH meter [19]. Homogeneity testing is performed by spreading a glass slide and observing the presence or absence of particles in the gel formulation [21]. Viscosity evaluation is carried out using a Brookfield viscometer, employing rotor spindle number 4 at a rotational speed of 0.3 rpm.

Spindle no. 64 is immersed in the gel formulation to be evaluated, and the measurement values are recorded when the displayed number has stabilized [21].

Proteins assay

The proteins measurement including Human Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) (Elabscience, E-EL-H0184), Epidermal Growth Factor (EGF) (Elabscience, E-EL-H0059), basic Fibroblast Growth Factor (bFGF) (Elabscience, E-EL-H6042), Interleukin 6 (IL-6) (Elabscience, E-EL-H0102), and Interleukin 4 (IL-4) (Elabscience, E-EL-H0101) levels were assayed using the Elabscience kit, based on manufacturing protocols. The ELISA Assay quantification was done to measure the protein level of lyophilized and unlyophilized-Sec gel, fresh and lyophilized hWJMSCs-Sec [29,30].

Antioxidant activities assay

DPPH scavenging assay

Into the 96 well-plate, samples (lyophilize and un-lyophilized-Sec gel, fresh and lyophilized hWJMSCs-Sec) were added as much as 50 µL. Following that, 200 µL of DPPH solution (Sigma Aldrich, D9132) was introduced and the mixture was incubated for 30 min. The solution's absorbance was measured with microplate reader (Thermo Scientific, Multiscan GO Microplate Reader) at 517 nm [31]. The samples were executed in triplicate and calculated with:

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

ABTS reducing activity assay

Potassium persulfate (4.9 mM) (Merck, EM105091) and ABTS (14 mM) (Sigma Aldrich, A1888) were combined with a 1:1 volume ration and incubated for 16 h in the dark. PBS (Phosphate Buffered Saline, pH 7.4) was added until the absorbance of the solution attained 0.70 ± 0.02 at 745 nm. The samples and ABTS•+ solutions were added, respectively of 2 and 198 µL before another incubation for 6 min [32]. ABTS reducing activity was measured at 745 nm in triplicate and calculated with:

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

Results and discussion

Production of hWJMSCs-Sec gel

The hWJMSCs characterization is already performed [24] and it has the positive immunophenotypes for hWJMSCs (CD90, CD105, CD73, and CD44). It also possesses the ability to differentiate into 3 lineages (osteocyte, chondrocyte, and adipocyte) which contributes to cell regeneration [33]. Topical formulations are an attractive choice for drug administration. They are particularly suitable for treating localized skin conditions, as they can be absorbed effectively by penetrating directly through the skin. Furthermore, topical formulations are widely recognized as one of the most effective and least toxic methods of drug delivery [33]. Among topical formulations, gel-based preparations have gained substantial popularity in both the pharmaceutical and cosmetic industries. This preference is attributed to their numerous advantages over other formulations, including excellent skin spreadability, a soothing cooling effect upon application, controlled drug release, and ease of removal [28].

Pharmaceutical formulations are deemed of high quality when they are non-toxic, efficacious, efficient, stable, and comfortable for the user. Achieving these criteria requires a rigorous optimization process during formulation development. This optimization ensures that the formulations effectively reach their intended therapeutic sites, minimize toxicity risks, maintain long-term stability, and ideally do not disrupt the user's daily routines [28].

The selection of specific ingredients in the formulation is a crucial aspect of achieving these goals. For instance, the choice of carbomer is advantageous due to its ability to disperse easily in water, even at low concentrations. Additionally, propylene glycol is incorporated to enhance carbomer properties by improving drug solubility. This improved solubility facilitates the drug's release from the base, thereby enhancing its therapeutic effectiveness. Triethanolamine is also included to create an alkaline environment within the carbomer, resulting in the formation of a dense and transparent gel [28]. This careful selection and optimization of ingredients contribute to the development of high-quality topical formulations.

Table 1 pH value of hWJMSCs-Sec gel.

	Day 1	**Day 4**	**Day 8**	**Day 10**	**Day 14**
pH	6.25	6.37	6.23	6.20	6.18

The pH range for skin typically falls within the range of 4.5 - 6.5 [34]. According to the pH test results as presented in **Table 1**, it is apparent that the carbomer-based gel complies with the recommended skin pH levels. Notably, **Table 1** illustrates a relatively consistent decline in pH during the storage period of the carbomer-based gel. This gradual pH reduction can likely be ascribed to environmental factors, including variations in temperature and storage conditions.

The quality of gel formulations is a paramount consideration within the pharmaceutical and cosmetic industries. A successful gel must possess an array of essential attributes to meet the rigorous standards expected in these fields. Chief among these attributes is the maintenance of a stable pH level. This ensures that the gel remains within the desired pH range, avoiding any potential irritation or incompatibilities when applied to the skin or mucous membranes.

Table 2 Organoleptic assessment of LSG.

Parameter	**Condition**
Form	Mild, not sticky
Color	Clear
Aroma	Pandan-like
Viscosity	+++

The organoleptic evaluation of the gel involved visual assessment of its appearance, color, and odor (**Table 2**). Typically, a well-prepared gel exhibits a transparent or clear appearance, accompanied by a semi-solid consistency, as described by Suryani [35]. Additionally, the texture of the gel plays a pivotal role, necessitating a soft and non-sticky consistency. This characteristic not only enhances user comfort but also facilitates smooth application. Exceptional clarity is another critical criterion, as it allows for the easy inspection of the gel's integrity and any potential impurities. Furthermore, the introduction of an aroma that evokes the pleasant scent of pandan can elevate the overall user experience, making the application of the gel more appealing. Uniform and consistent texture, a feature that is closely related to the gel's

homogeneity, ensures that the product is reliable and performs consistently. The attributes of excellent adhesion and spreadability enhance the gel's application and effectiveness, ensuring that it adheres well to the target area and spreads evenly, ultimately optimizing the therapeutic or cosmetic effects [36].

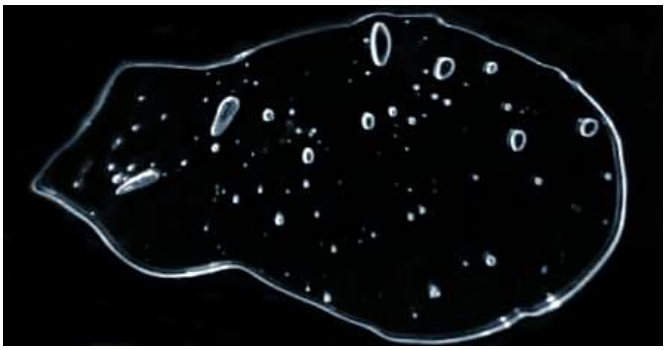

Figure 1 Gel homogeneity test.

Homogeneity testing is conducted using 2 pieces of glass slides, as depicted in **Figure 1**. A homogeneous preparation is characterized by the absence of any particles originating from the constituent materials [37]. The test results definitively confirm the homogeneity of the formulation. However, it is important to remark that the usage of a magnetic stirrer during the manufacturing process leads to the formation of bubbles.

Table 3 Viscosity Assessment of hWJMSCs-Sec gel.

Gel	Viscosity		
	1	**2**	**3**
hWJMSCs-Sec gel 50 g	880,000 cP	880,000 cP	880,000 cP
Carbomer gel base	1,560,000 cP	1,560,000 cP	1,590,000 cP

*The number on the sample indicates the formulation ratio between the gel and hWJMSCs-Sec, 1 (6 g : 3 mL), 2 (6 g : 4.5 mL) and 3 (6 g : 6 mL).

In accordance with the results of the viscosity test (**Table 3**), it was observed that the carbomer-based gel exhibited a higher viscosity compared to the hWJMSCs-Sec gel. A higher viscosity value indicates that the carbomer-based gel has a thicker consistency and is less prone to flowing compared to the hWJMSCs-Sec gel. Therefore, in this comparison, the hWJMSCs-Sec gel demonstrates superior viscosity characteristics over the carbomer-based gel. There is an inverse relationship between gel viscosity and spreadability; lower viscosity values correspond to higher spreadability [38]. Viscosity also plays a role in gel adhesion, with higher viscosity values associated with increased adhesion. The viscosity measurements for both gels revealed relatively high values. However, the hWJMSCs-Sec gel exhibited a lower viscosity compared to the carbomer-based gel, indicating better spreadability and adhesion.

Protein level

Secretome of mesenchymal stem cells is confirmed to help wound recovery with its capability to improve the growth and migratory capabilities of diverse cellular components of the dermis including dermal fibroblast, endothelial, and keratinocyte cells through p13k/Akt or FAK-ERK1/2 signaling [39]. The growth factor secreted protein present in the stem cell secretome shows a big role in mediated wound healing. EGF and bFGF are the major components in proliferation and migratory cells. In their study, Goh *et al.* [40], examined the performance of hydrogel sheets on wounded mice. The wounds in the mice were found to be 90 % closed on the 14^{th} day after treatment with hEGF stocked heparin-based hydrogel sheets, a significantly faster closure rate compared to other groups that only achieved 50 - 60 % wound closure. On the other side, bFGF has been observed to decrease the transcription of collagen I and tropoelastin mRNA transcription in periodontal ligament fibroblast, while concurrently increasing the synthesis of hyaluronic acid synthesis in skin fibroblast *in vitro* [41]. This study aligns with the findings of the LSG, which has been proven to contain EGF and bFGF proteins, indicating that the LSG has significant potential to accelerate the healing of skin wounds. In contrast, compared to FSG, there was almost no significant difference between them in EGF and bFGF concentration (**Figures 2(b) - b(c)**).

Human TIMP-2 is a suppressor of MMPs, at an excessive level, MMPs can inhibit tissue repair by stimulating the over deterioration of newly developed matrices [12]. **Figure 2(a)** illustrates the detection of TIMP-2 in the LSG sample within the LSG regimen, resulted in a significant increase in TIMP-2 level with the addition of hWJMSCs-Sec concentration. In contrast, compared to fresh hWJMSCs-Sec gel (FSG), there was almost no substantial difference between them in TIMP-2 concentration. The FS and lyophilized hWJMSCs-Sec (LS) treatment exhibited a noteworthy elevation in TIMP-2 levels compared to other treatments, notably the FS, which registered TIMP-2 levels. In a dermal wound repair process, a high level of TIMP-2 can suppress the MMP-2 mRNA level and effect the granular formation tissue and wound closure by elevated production of glycosaminoglycans and collagen [12]. The effect of TIMP for supporting wound repair and anti-apoptotic activity was also completed via the stimulations of β-catenin, NF-kB and Bcl-2 family signaling pathways [42].

Levels of (a) TIMP-2, (b) bFGF, (c) EGF, (d) IL-6, (e) IL-4 were measured. Data were presented as mean ± standard deviation, with different letters indicating significant differences at $p < 0.05$. ANOVA followed by a Dunnet T3 post hoc test was used for analysis. Samples included fresh hWJMSCs-Sec (FS), lyophilized-hWJMSCs-Sec (LS), fresh hWJMSCs-Sec gel (FSG), and lyophilizeds-hWJMSCS-Sec gel (LSG). Numbers on the sample 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. (a) TIMP-2, (b) bFGF, (c) EGF, (d) IL-6, (e) IL-4 Level. The mean ± standard deviation value was used to present the data, where the distinct letters indicated significant variations among treatments with a significance level of $p < 0.05$. (Data was analyzed using ANOVA and followed by a Dunnett T3 post hoc test). FS: fresh hWJMSCs-Sec, LS: lyophilized-hWJMSCs-Sec, FSG: fresh hWJMSCs-Sec gel, LSG: lyophilized-hWJMSCs-Sec gel. The numbers on the sample indicate the formulation ratio between the gel and hWJMSCs-Sec, 1 (6 gr : 3 mL), 2 (6 gr : 4.5 mL), and 3 (6 gr : 6 mL).

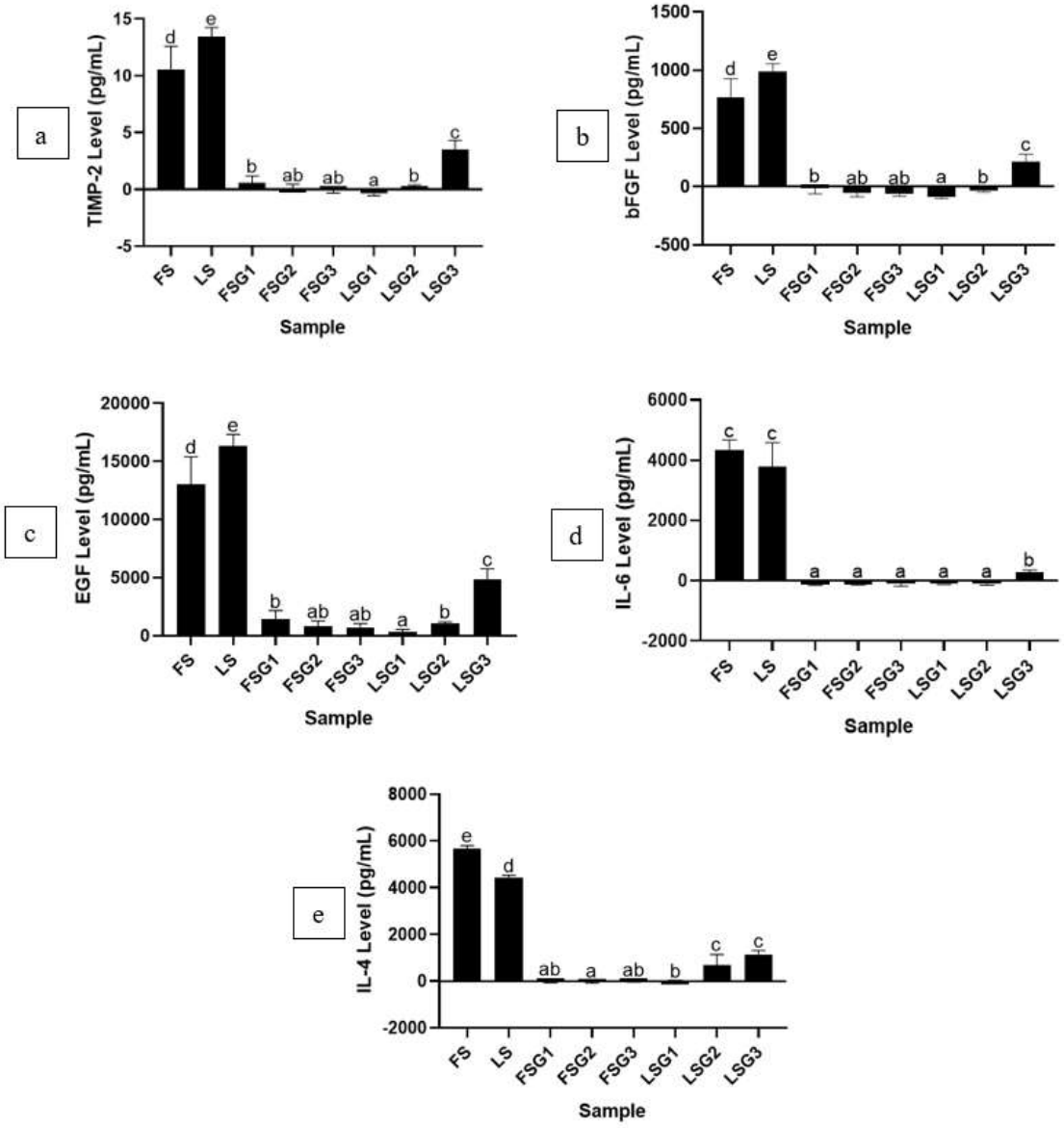

Figure 2 Effect of lyophilized-Sec gel toward proteins level.

IL-6 collaborates with IL-4 in the regulation of M2 macrophage polarization, a pivotal aspect in the reparative process during advanced stages of wound healing, leading to the expression of TGF-β and IL-10 [43]. Both IL-6 and IL-4 were found in the LSG sample (**Figures 2(d) - 2(e)**). IL-6 was identified to play an important role in the cell migration process during corneal wound healing, promoting a swifter closure of scars compared to the negative control [44].

Antioxidant assay

The scavenging activity of antioxidants from hWJMSCs-Sec gel is shown in **Tables 4 - 5**. Overall, LSG3 has the highest result compared to the other 200 μg/mL hWJMSCs-Sec gels. Even the scavenging activity of LSG3 is higher than LS without gel.

Table 5 ABTS reducing activity on various hWJMSCs-Sec gels.

Samples	Concentration (µg/mL)			
	1.56	**6.25**	**25**	**100**
FS	4.59 ± 0.13^{aB}	4.86 ± 0.14^{bB}	5.94 ± 0.04^{cB}	9.02 ± 0.67^{dA}
LS	3.21 ± 0.19^{aA}	3.90 ± 0.08^{bA}	4.86 ± 0.12^{cA}	8.73 ± 0.57^{dA}
FSG1	5.82 ± 0.16^{aD}	6.47 ± 0.18^{aD}	7.35 ± 0.32^{bD}	11.38 ± 0.08^{cA}
FSG2	5.90 ± 0.05^{aD}	6.28 ± 0.15^{bD}	7.32 ± 0.21^{cD}	11.46 ± 0.28^{dB}
FSG3	5.27 ± 0.04^{aC}	6.04 ± 0.10^{bC}	7.17 ± 0.17^{cD}	12.08 ± 0.25^{dB}
LSG1	6.12 ± 0.10^{aD}	6.82 ± 0.02^{bE}	8.31 ± 0.17^{cE}	12.73 ± 0.29^{dC}
LSG2	7.05 ± 0.06^{aF}	7.01 ± 0.11^{aF}	8.32 ± 0.06^{bE}	13.32 ± 0.07^{cD}
LSG3	6.87 ± 0.06^{aE}	7.37 ± 0.13^{bG}	8.15 ± 0.13^{cE}	13.66 ± 0.05^{dE}
Gel Base	5.63 ± 0.05^{aD}	6.00 ± 0.06^{abC}	6.38 ± 0.17^{bC}	8.22 ± 0.33^{cAF}

Data were presented as mean ± standard deviation, where the distinct letters (a, ab, b, c, d) indicated significant variations among concentration, while (A, B, C, D, E, EF, F) showed significant differences among treatments at $p < 0.05$. ANOVA followed by a Dunnet T3 post hoc test was used for analysis. Samples included fresh hWJMSCs-Sec (FS), lyophilized-hWJMSCs-Sec (LS), fresh hWJMSCs-Sec gel (FSG), and lyophilized-hWJMSCS-Sec gel (LSG). Numbers on the sample 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.

In addition to growth factor protein, the level of ROS is also recognized as a contributing factor in skin wound healing by stimulating cell migration and angiogenesis [45]. Therefore, a compound with antioxidant properties is essential for effective wound healing. The experimental results indicate that LSG has demonstrated a high level of antioxidant content, as evidenced by its ability to scavenge free radicals such as DPPH and ABTS. Cao *et al.* [46], in their study demonstrated that the antioxidant effects present in propolis can inhibit the level of ROS in L929 fibroblast cells. Additionally, they observed an increase in the mRNA level of Collagen Type I Alpha 2 Chain (COL1A2) and Collagen Type 3 Alpha 2 Chain (COL3A2), which contribute to the wound healing process.

Table 4 DPPH scavenging activity on various hWJMSCs-Sec gels.

Sample	Concentration (µg/mL)			
	3.13	**12.5**	**5**	**100**
FS	13.27 ± 1.10^{aB}	14.14 ± 0.95^{bB}	18.86 ± 0.58^{cB}	28.45 ± 1.83^{dB}
LS	19.72 ± 1.38^{aC}	21.17 ± 0.75^{aC}	24.84 ± 1.82^{bC}	36.42 ± 0.31^{cD}
FSG1	22.09 ± 0.04^{aD}	23.11 ± 0.31^{bD}	25.87 ± 0.36^{cC}	35.76 ± 0.26^{dC}
FSG2	20.64 ± 1.05^{aC}	21.98 ± 0.32^{aC}	25.58 ± 1.43^{bC}	36.05 ± 0.56^{cCD}
FSG3	21.20 ± 0.57^{aC}	23.42 ± 0.59^{bD}	25.83 ± 0.52^{cC}	38.90 ± 0.31^{dEF}
LSG1	20.30 ± 0.98^{aC}	21.99 ± 0.75^{aCD}	26.24 ± 0.08^{bC}	38.20 ± 0.45^{cE}
LSG2	22.41 ± 0.22^{aD}	24.08 ± 1.66^{bD}	27.25 ± 0.24^{bD}	38.38 ± 0.86^{cEF}
LSG3	26.57 ± 0.19^{aE}	28.26 ± 0.90^{abE}	31.45 ± 1.83^{bE}	40.83 ± 1.46^{cF}
Gel Base	0.57 ± 0.16^{aA}	1.47 ± 0.14^{bA}	2.72 ± 0.45^{bA}	7.47 ± 0.12^{cA}

Data were presented as mean ± standard deviation, where the distinct letters (a, ab, b, c, d) indicated significant variations among concentration, while (A, B, C, D, E, EF, F) showed significant differences among treatments at $p < 0.05$ ANOVA followed by Dunnet T3 post hoc test was used for analysis. Samples included fresh hWJMSCs-Sec (FS), lyophilized hWJMSCs-Sec (LS), fresh hWJMSCs-Sec gel (FSG), and lyophilizeds-hWJMSCS-Sec gel (LSG). Numbers on the sample 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.

Conclusions

In this study, the gel was assessed for various quality attributes, including stable pH, soft and non-sticky texture, clarity, pandan-like aroma, consistent and homogeneous texture, as well as good adhesion and spreadability. Additionally, the incorporation of hWJMSCs-Sec into the carbomer gel led to increased level of growth factor (bFGF and EGF), TIMP-2, IL-6, and IL-4. Due to its high antioxidant content, the hWJMSCs-Sec gel demonstrates the ability to neutralize free radicals and accelerate wound recovery. Overall, the delivery of lyophilized hWJ-MSCs-Sec through the carbomer gel holds promise for wound healing treatment.

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