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Efficacy of Freeze-Dried Human Wharton's Jelly Mesenchymal Stem Cell Secretome Gel Toward Wound Healing Rat Model

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Abstract:

Background: Burns are a major global health problem, often leading to prolonged inflammation and impaired wound healing. Current treatments face limitations, and human Wharton's Jelly-derived mesenchymal stem cell (hWJ-MSCs) secretome offers a promising non-cellular alternative. Freeze-dried secretome gel (FDSG) preserves bioactive molecules that promote tissue repair. This study evaluated the potential of FDSG for burn wound healing in a rat model. **Methods:** The secretome was obtained as a conditioned medium from hWJ-MSCs. FDSG was made by mixing 6 g of carbomer gel with 6 mL hWJ-MSCs secretome, then followed by lyophilization. An in vivo test was conducted on six Sprague-Dawley rats with a third-degree burn model (BM) with various treatments. Burn healing was evaluated on days 3, 10, and 24 based on wound reduction, IL-1β protein levels (Immunohistochemistry), and collagen density (Masson's trichrome staining). **Results:** FDSG increased the percentage of wound reduction and collagen density from day 3 to 24. Our findings also indicate a regulatory pattern of IL-1β protein levels throughout the biological mechanism of tissue repair, which is strongly associated with the distinct phases of healing. The treatment group that applied FDSG twice daily demonstrated the highest effectiveness. **Conclusion:** FDSG shows promising results as an effective wound healing agent.

Keywords: Burns, Freeze-dried, hWJ-MSCs, Secretome, Wound healing.

1. Introduction

Burns are a complex type of traumatic injury that can have long-term effects, both locally and systemically (Nielson et al., 2017; Jeschke et al., 2020). Every year, almost 9 million new cases of burns occur globally, with 120,000 deaths, mainly in developing countries (Hebron et al., 2022). In many Asian countries, it remains high due to socioeconomic factors. The severity of burns is classified based on burn depth, ranging from first- to fourth-degree burns, which can cause complications such as prolonged inflammation, impaired wound healing, and systemic inflammatory response syndrome (Rowan et al., 2015; Schaefer & Tannan, 2021). One of the main complications is prolonged inflammation, which can interfere with the normal healing process and cause further tissue damage (Rowan et al., 2015). This uncontrolled inflammation can trigger the potentially life-threatening systemic inflammatory response syndrome (Sikora et al., 2023). Impaired wound healing also represents another serious complication associated with burn injuries.

Interleukin (IL)-1β is an inflammatory cytokine that plays a role in both initiating inflammation and supporting tissue regeneration during the healing process. Previous research indicates that IL-1β stimulates fibroblasts, increasing collagen (Shagdarova et al., 2021). This finding also aligns with Mozzati et al. (2010), who stated that IL-1β promotes fibroblast proliferation and stimulates collagen synthesis and collagenase production, which are important for collagen remodeling during wound healing. On the other hand, collagen density is a key factor in determining tissue strength and integrity. The wound healing process usually involves the initial production of collagen type III, which is later replaced by collagen type I, the main structural component of the dermis (Alsarayreh et al., 2022). In addition, the reconstruction stage of wound healing involves the coordinated alignment and restructuring of collagen fibers, which are crucial for effective wound contraction and tissue restoration

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Given the complexity and severity of burns, developing effective treatment methods is essential. Anti-inflammatory drugs, including corticosteroids, are commonly used to address excessive inflammation in chronic wounds. Although these drugs can effectively reduce inflammation, long-term use can impair wound healing by inhibiting fibroblast function and collagen synthesis (Casado-Santos, 2024). Moreover, nonsteroidal anti-inflammatory drugs (NSAIDs) may lead to gastrointestinal complications and renal impairment, particularly in individuals with underlying health conditions (Lanci et al., 2019).

Contemporary wound management emphasizes rapid wound closure, restoration of function, reduction of scarring, and improvement in overall quality of life (Salibian et al., 2016). Recent advances in burn treatment, such as the application of engineered skin grafts, regenerative stem cell treatments, and therapeutic growth factors modulation, have shown promising potential in enhancing patient outcomes (Srinivasan et al., 2021).

The use of mesenchymal stem cells (MSCs) is gaining increasing attention in wound healing, including burns (Gentile & Garcovich, 2019). MSCs have differentiation, immunomodulatory, anti-inflammatory, and proangiogenic properties that support tissue repair (Wang et al., 2014). Wharton's Jelly (WJ) is a promising source of MSCs due to its high proliferation rate, high differentiation potential, and strong immunomodulatory profile (El Omar et al., 2014). Harvesting of WJ-MSCs is noninvasive and ethical, utilizing umbilical cords that are usually discarded (Ding et al., 2015). However, live cell therapy faces challenges in storage, transportation, regulation, and the risk of malignant transformation (Moll et al., 2014).

Alternatively, the WJ-MSC secretome (WS), an assemblage of biologically active compounds such as extracellular vesicles and growth factors, offers a non-cellular approach to accelerate wound healing through mechanisms of immunomodulation, angiogenesis, and fibroblast activation (Deshpande et al., 2018; Tan et al., 2023). This secretome can be integrated into a secretome gel (SG) for sustained release of molecules, enhancing therapeutic efficacy (Alapure et al., 2018; Rong et al., 2019). The freeze-drying process of secretome has been shown to preserve active compounds and improve stability, as shown in the study by Widowati et al. (2024a, 2024b), where freeze-dried secretome gel (FDSG) had higher levels of growth factors, cytokines, and antioxidants compared to non-freeze-dried secretome (NFDS). This approach offers great potential in overcoming conventional therapies' limitations and improving burn patients' quality of life.

Thus, this study offers important evidence supporting the potential of FDSG as an effective and safe wound healing agent through in vivo testing as a further stage. The potential of FDSG as a wound healing agent was analyzed based on IL-1β expression (Immunohistochemistry), collagen density (Masson's trichrome staining), and wound reduction in rats given third-degree burns.

2. Material and Methods

2.1. Culture and Collection of hWJ-MSCs Secretome

The hWJ-MSCs were identified through multipotent differentiation assay and surface phenotype analysis (Widowati et al., 2019). hWJ-MSCs were cultured based on the protocol described in previous studies by Widowati et al. (2024a, 2024b) using Minimum Essential Medium-α (α-MEM) (Biowest, L0475500) supplemented with 10% Fetal Bovine Serum. Then the cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Upon reaching 80–90% confluence, the cells were harvested and centrifuged at 3,000×g for 4 minutes (MWP 260 r). The obtained supernatant, which contained the secretome, was subsequently filtered through a Durapore unit (Millipore Corporation, SLGV 033 RS).

2.2. Carbomer Gel Formulation

The formulation of carbomer gel (CG) was done according to Widowati et al. (2024a). CG was made by dissolving 7.5 g of carbomer 940 in 250 mL of distilled water while stirring continuously. After swelling, 7.5 mL of triethanolamine, 5 mL of propylene glycol, and 5 mL of glycerin were added dropwise while mixing thoroughly until a homogeneous gel was obtained. The volume was adjusted to 500 mL with distilled water. The quality of CG has been analyzed in previous studies based on organoleptic testing, pH measurements, homogeneity tests, and viscosity evaluations (Widowati et al., 2024b).

2.3. Production of FDSG

FDSG was prepared by mixing 6 g of carbomer gel with 6 mL of WS, which is the best formula from previous studies (Widowati et al., 2024a; 2024b). The mixture was stirred slowly until it reached a homogeneous consistency (Zukhiroh et al., 2022). The mixture was placed in a small tray and subjected to lyophilization using a vacuum freeze dryer (FD-F-CE, China), as described by Nowak and Jakubczyk (2020). The freeze-drying process was performed over 42 hours at a controlled temperature ranging from −50 to −35 °C.

2.4. Burn-Induced Rats

In vivo tests were conducted on male Sprague-Dawley rats from iRATco Veterinary Laboratory, Bogor, Indonesia. The protocol was carried out following the approval of the Research Ethics Committee of the Faculty of Medicine, Maranatha Christian University (No. 046/KEP/V/2024). The burn wound model was conducted following the procedures of Tran-Nguyen et al. (2020) and Laksmitawati et al. (2022) with modifications. The burn model (BM) was performed by inducing third-degree burns using a 1 cm diameter metal rod that had been heated beforehand and applied to the back of the test animal for 10 seconds. A total of 36 rats were divided into 6 groups (Table 1), including acclimatization for 7 days. The test material was administered until it completely encompassed the entire wound area.

Table 1. Treatment groups in burn rat model

Group	Treatment	Total Rats
I: NC (Negative Control)	Normal rats, untreated rats	6
II: PC (Positive Control)	Burn model	6
III: VC (Vehicle Control)	PC + CG freeze dried 1x/day, 08:00 am	6
IV: Treatment 1 (FDSG 1)	PC + FDSG, 1x/day, 08:00 am	6
V: Treatment 2 (FDSG 2)	PC + FDSG, 2x/day, 08:00 am and 04:00 pm	6
VI: CpG (Comparison Group)	PC + Bioplacenton 1x/day, 08:00 am	6

2.5. Wound Healing Scoring: Wound Reduction, IHC, and MT

The wound anatomy, including wound diameter, was observed on days 3, 10, and 24 by measuring the mean measurements of the wound's vertical and horizontal dimensions macroscopically. Termination was carried out on days 3, 10, and 24, with 2 rats from each group. Dorsal skin tissue was fixed in 10% Bouin's fixative (BNF) solution (Merck, MFCD00146169) for IHC and MT analysis. IHC was performed to analyze IL-1β protein levels using primary antibodies (Elabscience, E-AB-70048), which was incubated at ambient temperature overnight. The Rabbit-Specific HRP/DAB (ABC) Detection IHC Kit (Elabscience, E-IR-R213) was utilized for detection, while MT was performed to analyze collagen density. Color changes indicating protein levels and collagen density were measured using ImageJ software. These parameters were examined under a light microscope (Olympus BX 31, Japan) equipped with a USB CCD10 Camera with 40× magnification (Laksmitawati et al., 2022; Widowati et al., 2024c).

2.6. Statistical Analysis

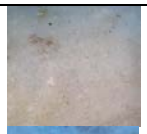
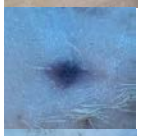
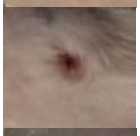
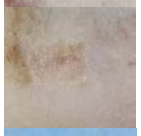
Data are presented as mean ± standard deviation. Statistical analysis was performed using ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$). All analyses were conducted with SPSS software (version 20.0), and visualizations were created using GraphPad Prism (version 9.0) (Widowati 2024a; Widowati et al., 2024c).

3. Results

3.1. Effect of FDSG on wound reduction in burn rat model

Clinical development and wound reduction analysis results can be seen in Table 2 and Figure 1. The results demonstrated that the FDSG treatment group increased the percentage of wound reduction compared to PC, with FDSG 2 being the most effective group. Figure 1 also shows that FDSG 2 can effectively reduce wounds on day 24 compared to CpG.

Table 2. Effect of FDSG toward wound reduction in burn rat model

Group	D3	D10	D24
I			
II			
III			
IV			
V			
VI			

*(I) NC (untreated rats); (II) PC (burn model); (III) VC (PC + CG freeze-dried 1×/day); (IV) FDSG 1 (PC + FDSG 1×/day); (V) FDSG 2 (PC + FDSG 2×/day); (VI) CpG (PC + Bioplacenton 1×/day).

Figure 1. Effect of FDSG on wound reduction in burn rat model

*Data presented as mean ± SD. Different superscripts indicate significance between treatment groups based on Tukey's test ($p < 0.05$). (I) NC (untreated rats); (II) PC (burn model); (III) VC (PC + CG freeze-dried 1×/day); (IV) FDSG 1 (PC + FDSG 1×/day); (V) FDSG 2 (PC + FDSG 2×/day); (VI) CpG (PC + Bioplacenton 1×/day).

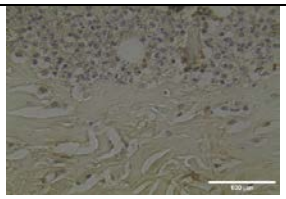
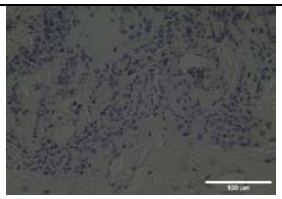
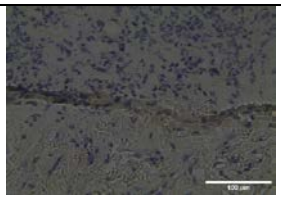
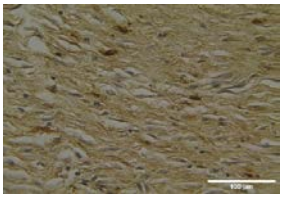
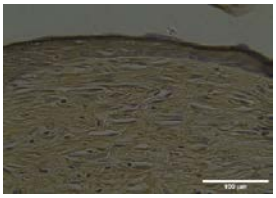
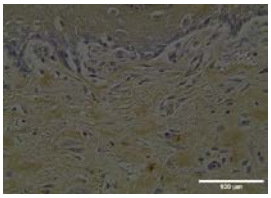
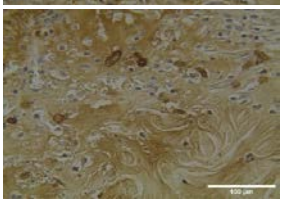
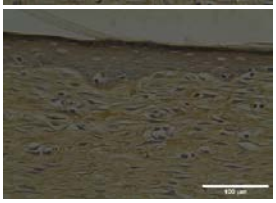
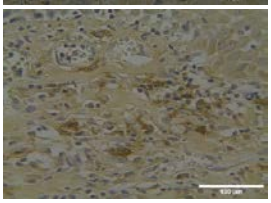
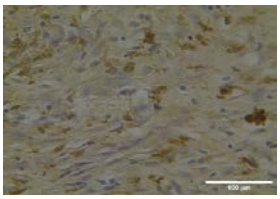
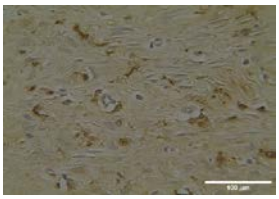
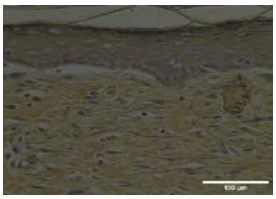
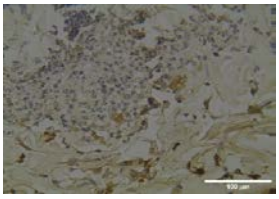
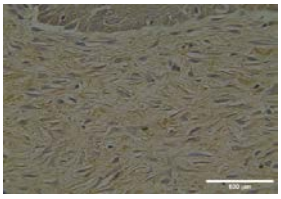
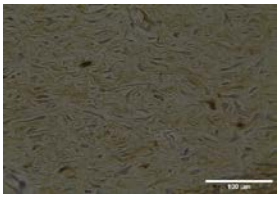
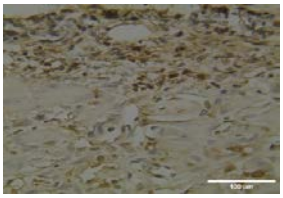
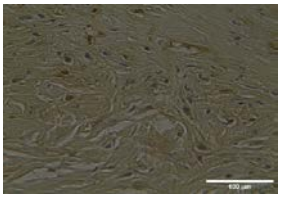
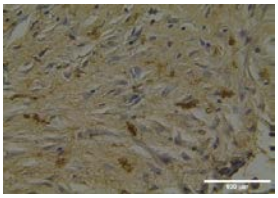
3.2. Effect of FDSG on IL-1β protein levels in burn rat model

The IHC results of IL-1β protein in test animals are presented in Table 3, while the quantitative analysis of protein levels is shown in Figure 2. In the IHC staining, IL-1β protein is indicated by a brown color; the more intense and widely distributed the brown staining, the higher the protein expression. FDSG 2 is the most pronounced effect. On day 3, IL-1β protein levels were high because the inflammatory process was still actively taking place in the early phase of wound healing. On day 10, IL-1β protein levels began to decrease because inflammation subsided and the proliferation phase began, and on day 24 IL-1β levels decreased further because inflammation was minimal and the tissue repair process was dominant.

Figure 2. Effect of FDSG on IL-1β levels in burn rat model

*Data presented as mean ± SD. Different superscripts indicate significance between treatment groups based on Tukey's test ($p < 0.05$). (I) NC (untreated rats); (II) PC (burn model); (III) VC (PC + CG freeze-dried 1×/day); (IV) FDSG 1 (PC + FDSG 1×/day); (V) FDSG 2 (PC + FDSG 2×/day); (VI) CpG (PC + Bioplacenton 1×/day).

Table 3. IHC of IL-1β protein in dorsal skin tissue of burn rat model

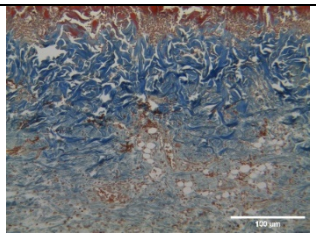
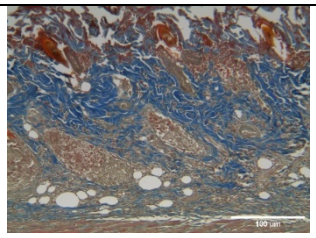
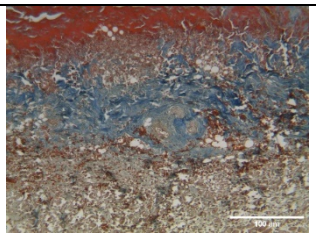
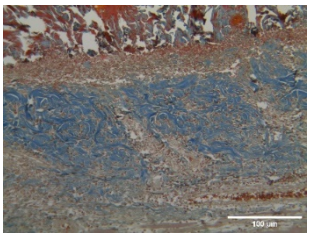
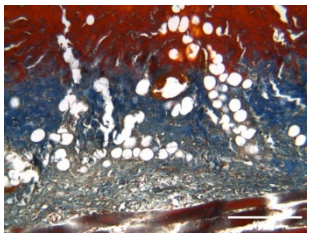
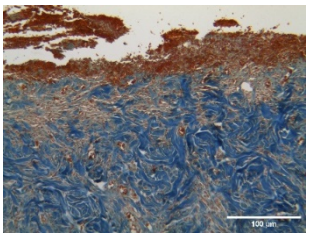
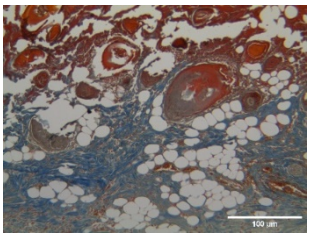
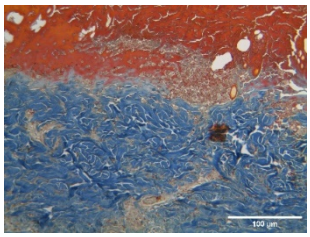
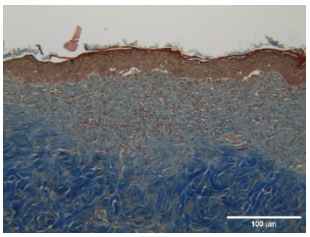
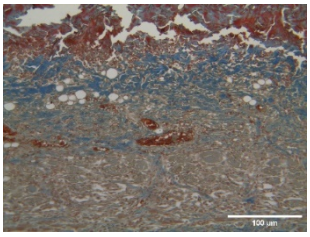
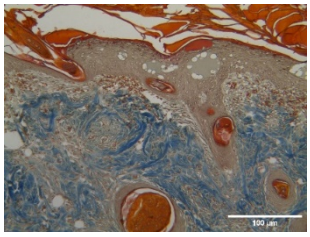
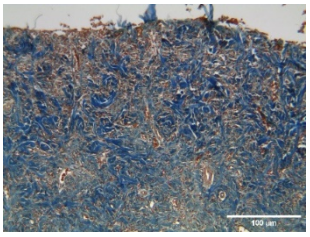
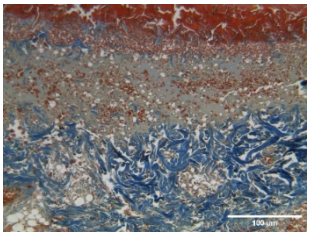
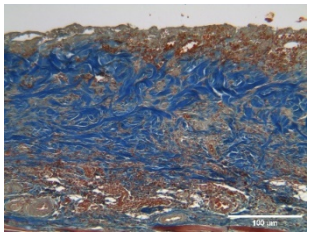
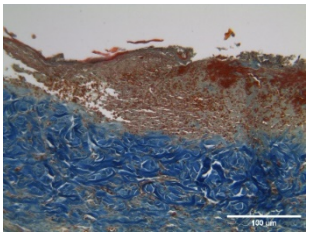
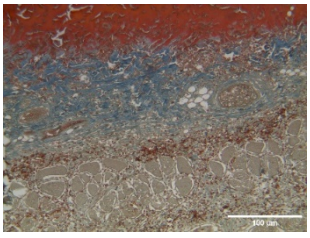
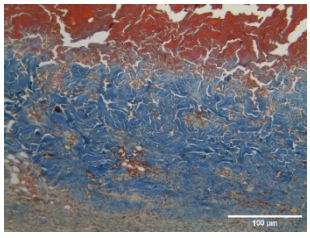
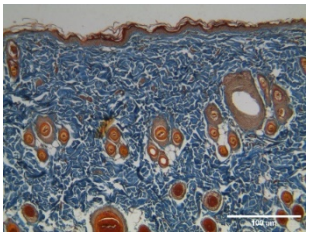
Group	D3	D10	D24
I			
II			
III			
IV			
V			
VI			

*(I) NC (untreated rats); (II) PC (burn model); (III) VC (PC + CG freeze dried 1×/day); (IV) FDSG 1 (PC + FDSG 1×/day); (V) FDSG 2 (PC + FDSG 2×/day); (VI) CpG (PC + Bioplacenton 1×/day).
*IL-1β protein levels are indicated by brown color.

3.3. Effect of FDSG toward Collagen Density in burn rat model

Collagen density from the FDSG effect can be seen in Table 4 and Figure 3. Collagen density based on MT staining is marked in blue (Table 4). The results showed that FDSG treatment significantly increased collagen density compared to PC from day 3 to day 24 (Figure 3).

Table 4. Effect FDSG toward collagen density in burn rat model

Group	D3	D10	D24
I			
II			
III			
IV			
V			
VI			

*(I) NC (untreated rats); (II) PC (burn model); (III) VC (PC + CG freeze dried 1×/day); (IV) FDSG 1 (PC + FDSG 1×/day); (V) FDSG 2 (PC + FDSG 2×/day); (VI) CpG (PC + Bioplacenton 1×/day).
*Collagen density is indicated by blue color.

Figure 3. Effect of FDSG toward collagen density in burn rat model

*Data are presented as mean ± SD. Different superscripts indicate significance between treatment groups based on Tukey's test ($p < 0.05$). (I) NC (untreated rats); (II) PC (burn model); (III) VC (PC + CG freeze-dried 1×/day); (IV) FDSG 1 (PC + FDSG 1×/day); (V) FDSG 2 (PC + FDSG 2×/day); (VI) CpG (PC + Bioplacenton 1× /day).

4. Discussion

Wound healing is known as a multifaceted and evolving biological process that generally unfolds in distinct phases: hemostasis, inflammation, proliferation, and remodelling (Tofiq et al., 2021). This process involves a cellular series and molecular events, such as cell migration, inflammation, extracellular matrix (ECM) synthesis, and angiogenesis (Li et al., 2017). In burn injuries, these processes are often disrupted, which may result in a slower recovery process, hypertrophic scarring, contractures, or chronic wounds (Finnerty et al., 2016). Wound secretome (WS) is known to contain key factors such as Tissue Inhibitor of Metalloproteinases-2 (TIMP-2), Epidermal Growth Factor (EGF), basic Fibroblast Growth Factor (bFGF), IL-6, IL-4, Keratinocyte Growth Factor (KGF), Hepatocyte Growth Factor (HGF), Platelet-Derived Growth Factor (PDGF), Heparin-Binding EGF-like Growth Factor (HB-EGF), and antioxidants, all of which play important roles in the healing process (Widowati et al., 2024a; Widowati et al., 2024b); these components may be able to act as burn wound therapy agents.

During the hemostasis phase, the initial response to injury involves the formation of a fibrin clot, which serves as a temporary matrix for cell migration and a scaffold for subsequent healing. This phase plays a crucial role in minimizing blood loss and kickstarting the body's inflammatory response. The initial step involves the activation of hemostatic mechanisms, followed by the inflammatory cells recruitment, notably neutrophils and macrophages, which signal the onset of the inflammatory phase. Among the key players in this process is IL-1β, a pro-inflammatory cytokine whose levels surge significantly right after a burn injury. This elevation of IL-1β helps to recruit and activate immune cells, such as macrophages and neutrophils, which are essential for clearing debris and pathogens from the wound site, thereby promoting the early stages of healing (Kimball et al., 2017; Schneider et al., 2021; Uchiyama et al., 2021). During the inflammatory phase, increased IL-1β levels activate various signaling pathways, particularly the NF-κB pathway, which amplifies the inflammatory response by inducing the expression of additional pro-inflammatory mediators including IL-6 and IL-8 (Kartika et al., 2021). This inflammatory response also facilitates the recruitment of additional fibroblasts to the wound site, where they contribute to collagen formation and help promote the repair process. The initial collagen formed during this phase is predominantly type III collagen, which is essential for providing temporary structural support to the wound (Gugliandolo et al., 2021; Hakim, 2023). The density of collagen fibers during this phase is critical as it influences the wound's ability to withstand mechanical stress and facilitate granulation tissue formation. Increased levels of inflammatory cytokines typically occur within the first week, with significant activity seen around day 7 (Shi et al., 2020).

The results showed that in burn rat model, there was an increase in IL-1β protein levels (Table 3) (Figure 2) and collagen density (Table 4) (Figure 3) after 3 days of FDSG treatment. Following injury, WS stimulates IL-1β secretion, a key factor in triggering inflammation during early wound repair. Increased IL-1β facilitates immune cell migration to the injury site, which promotes the clearance of debris and pathogens (Gonçalves et al., 2022; Tilotta et al., 2023). The presence of IL-1β in WS also activates fibroblasts, increasing their proliferation and collagen synthesis, particularly type III collagen (Lee et al., 2021). This early collagen deposition is vital for granulation tissue formation and provides structural support to wounds during the healing process. Other studies have shown that IL-6 in WS can increase IL-1β levels in various cell types, including fibroblasts and keratinocytes, during the inflammatory phase (Yin et al., 2019). Additionally, WS is known to contain EGF and HB-EGF, which can modulate the inflammatory response (Serra et al., 2018).

As the wound healing process moves into the proliferative phase, IL-1β levels begin to decline. This decrease is important in preventing chronic inflammation and initiating tissue regeneration. The downregulation of IL-1β is associated with a shift from a pro-inflammatory to an anti-inflammatory environment that is necessary for fibroblast activation and collagen formation (Sugioka et al., 2017). During this phase, the focus shifts toward production of extracellular matrix elements such as type I and type III collagen, which are essential for restoring tissue integrity and strength. Decreased IL-1β levels also correlate with reduced MMP activity, a balance that is essential for proper tissue remodeling (Bian et al., 2015). The balance between collagen synthesis and degradation is critical, as MMPs are involved in the breakdown of the ECM and ensure that collagen fibers are well organized and interconnected (Gugliandolo et al., 2021). The increased collagen density during this phase is critical to support the wound closure and reestablishment of tissue structure. This phase typically occurs around days 3 to day 10. By day 10, the proliferation process is usually well underway, with increased collagen density and vascularization observed in the wound area.

In the remodeling phase, IL-1β levels continue to decline, allowing maturation and reorganization of the ECM. This phase is characterized by the transition from collagen types III to I, providing greater strength to the healed tissue, which is important for preventing fibrosis

(Zhang, 2011). Regulation of IL-1β protein levels is important for ensuring a balanced inflammatory response, promoting effective tissue regeneration, and preventing pathological scarring. In the remodeling phase, collagen density also continues to play an important role. The transition from collagen type III to I is a critical event, as collagen type I contributes to long-term strength and stability, withstands normal physiological stresses, and promotes functional tissue recovery (Tombultürk et al., 2023). By day 24, the remodeling phase is usually well established, with ongoing adjustments to the structure and density of collagen in the tissue continuing to increase (El-Sayed, 2016).

These findings are consistent with the study's results, which indicate that FDSG can reduce IL-1β protein levels (Table 3) (Figure 2) and increase collagen density (Table 4) (Figure 3) after 10 days and 24 days of treatment. When the healing process continues to the proliferative phase, WS provides an anti-inflammatory effect. WS contains inflammation-regulating molecules such as IL-10 that can inhibit the synthesis of pro-inflammatory agents, including IL-1β (Sari et al., 2021; Jiao et al., 2021). This role occurs in the proliferation and remodeling phases. This shift in cytokine balance is essential to overcome inflammation and promote tissue repair and facilitate the transition to the remodeling phase (Cassano et al., 2018). The modulation of IL-1β protein levels and collagen density throughout the wound healing process can be impacted by inflammatory triggers (Ganesan et al., 2014) and cellular interactions, including the regulation of macrophage activity (Ferreira et al., 2020) and the presence of hypoxic conditions in the wound microenvironment which can stimulate WS to produce cytokines and growth factors that promote wound healing (Bundgaard et al., 2020).

IL-1β is crucial in tissue repair due to its role in stimulating collagen production and fibroblast activity. Collagen density in the wound area is essential for structural integrity and functional tissue recovery, thus promoting increased wound reduction. This is supported by the results of a study showing a wound reduction process from day 3 to 24 in FDSG compared to PC (Table 2) (Figure 1). On day 3, a reduction in the necrotic area can be seen. The tissue around the wound appears more hydrated and shows early signs of granulation tissue formation (El-Sayed et al., 2023). On day 10, it shows clearer granulation tissue growth, with a significantly reduced necrotic area. The wound becomes flatter, indicating the process of keratinocyte and fibroblast migration to repair damaged tissue (Vaidyanathan, 2021; Kumar et al., 2021). In the FDSG 2 and CpG groups, wound morphology showed faster improvement with smoother tissue and color closer to normal tissue. Furthermore, on day 24, the wound was almost completely healed, with epithelial regeneration approaching normal. In the FDSG and CpG groups, scars were almost invisible, with skin color and texture resembling healthy tissue. Epithelialization and tissue remodeling took place better, indicating the effectiveness of therapy in accelerating regeneration (Lee et al., 2021).

FDSG has several advantages that make it a potential burn wound therapy agent. The freeze-dried gel formulation increases the stability of bioactive substances, thus ensuring their effectiveness over a longer period (Bari et al., 2020; Damayanti et al., 2021). In addition, FDSG is able to release bioactive factors like growth factors and cytokines are released gradually and in a controlled manner, ensuring optimal concentrations to support the healing process (Mirfendereski et al., 2025; Bari et al., 2020). FDSG demonstrates potential in regulating inflammation through maintaining equilibrium between pro- and anti-inflammatory mediators' activities, and creates a microenvironment conducive to tissue regeneration. In addition, FDSG also stimulates collagen production through stimulation of fibroblasts by bFGF and TIMP-2 content, strengthening the newly formed tissue (Chandra et al., 2022; Fadhilah, 2023). The angiogenesis process is also supported by molecules such as VEGF and PDGF contained in FDSG, which play a role in the development of new blood vessels for oxygen and nutrient supply (Deng et al., 2018). With high biocompatibility, FDSG minimizes the risk of side effects on body tissues (Fadhilah, 2023). Research by Widowati et al. (2024a, 2024b) also validated that FDSG contains growth factors, cytokines, and antioxidants in higher concentrations than NFDS formulations, thus increasing its potential as an effective burn therapy. This combination of characteristics makes FDSG an innovative and promising formulation in supporting the burn healing process. The proposed mechanism of FDSG as a burn wound therapy agent can be seen in Figure 4.

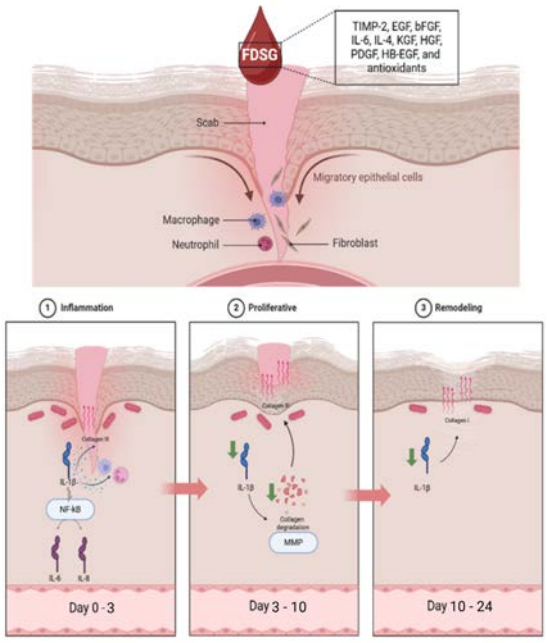


Figure 4. Proposed mechanism of FDSG treatment as a burn wound therapy

FDSG contains various growth factors, cytokines, and antioxidants that act as burn wound therapy agents. FDSG plays a role in wound reduction through IL-1β regulation and increased collagen density in the inflammatory, the cell growth and tissue reconstruction stages during the healing process.

This study has several limitations. First, the wound healing effects of the FDSG were only evaluated in a rat model, which may not fully replicate human wound healing processes. Second, the relatively small sample size and limited observation period may influence the generalizability of the results. Future studies with larger animal groups, longer-term monitoring, and molecular

profiling are needed to validate the findings and explore clinical applications in human subjects.

5. Conclusion

FDSG is known to have potential as a burn wound therapy agent, with twice daily application being an effective treatment. On day 24, FDSG treatment significantly reduced wound size. In addition, FDSG caused IL-1β regulation and increased collagen density in the wound healing phases including inflammation, proliferation, and remodelling. Overall, these findings confirm that FDSG reduces inflammation, increases collagen synthesis, and supports skin regeneration in burn wounds, which warrants further research on its mechanisms and broader clinical applications in wound healing.

6. Ethical Clearance:

This research received ethical approval from Research Ethic Committee, Faculty of Medicine, Maranatha Christian University (No: 046/KEP/V/2024, dated on May 3rd 2024).

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Conflicts of Interests:

None.

Supplementary Material:

None

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