Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 10, No. 4, October 2025

Research Paper

Potential of Secretome Hydrogel for Wound Healing in LPS- and Scratch-Induced BJ Cells as an Inflammation Model

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Abstract
Wound healing often requires specialized interventions to accelerate recovery due to prolonged inflammation and limited regenerative factors. Umbilical Cord Mesenchymal Stem Cells secretome (UCMSCs) comprises various cytokines and growth factors that can promote wound healing. This study aims to analyze the potential of a secretome-based hydrogel as a wound-healing agent using BJ fibroblast cells induced by lipopolysaccharide (LPS) and scratch injury as an inflammation model. The secretome hydrogel was formulated using Carbopol, Hydroxypropyl Methylcellulose (HPMC), Hydroxyethyl Cellulose (HEC), and secretome. Cytotoxicity was conducted using the WST-8 assay, while cell migration was evaluated through a scratch assay. Tumor Necrosis Factor-α (TNF-α), Nuclear Factor kappa-B (NF-κB), and Interleukin-8 (IL-8) genes expression were analyzed via qRT-PCR. Additionally, malondialdehyde (MDA) levels were measured for oxidative stress assessment, whereas Connective Tissue Growth Factor (CTGF) and Transforming Growth Factor-β1 (TGF-β1) levels were quantified using ELISA and colorimetric assays. The secretome hydrogel exhibited no cytotoxic effects on BJ fibroblast cells and significantly enhanced cell migration. Moreover, it reduced the TNF-α, IL-8, and NF-κB expression, indicating anti-inflammatory activity. The hydrogel also decreased MDA levels while increasing TGF-β1 and CTGF expression, suggesting antioxidant properties and enhanced tissue regeneration in the inflammatory model. The secretome-based hydrogel presents a promising therapeutic approach for promoting chronic wound healing by modulating inflammation, reducing oxidative stress, enhancing tissue regeneration, and stimulating fibroblast migration.

Keywords
Anti-Inflammatory, Fibroblast; Hydrogel, Regeneration, Secretome, Cells Migration

Received: 23 May 2025, Accepted: 26 August 2025
https://doi.org/10.26554/sti.2025.10.4.1242-1254

1. INTRODUCTION

Wounds represent disruptions to body tissues caused by physical injury, infections, or underlying medical conditions. Wound healing known as a complex process, dynamic biological phenomenon that involves an overlapping series and sequential phases, including hemostasis, proliferation, inflammation, and tissue remodeling. Under normal conditions, this process progresses efficiently; however, in certain cases, such as chronic or infected wounds, healing may be delayed or impaired, necessitating specialized interventions to accelerate tissue regeneration (Simader et al., 2017; Koob et al., 2013). Chronic wounds typically exhibit lower levels of growth factors compared to acute wounds, which can lead to impaired cellular responses and delayed healing (Koob et al., 2013; Hong and Park, 2012). Additionally, several intrinsic and extrinsic factors, including diabetes, advanced age, malnutrition, and biofilms, can exacerbate

wound chronicity by prolonging inflammation and promoting tissue degradation. These factors hinder the natural healing process and increase the risk of complications, highlighting the need for advanced therapeutic approaches to improve wound management and facilitate tissue repair (Monika et al., 2022; Frykberg and Banks, 2015).

Current wound healing strategies encompass modern wound dressings, topical medications, cell therapy, and biomaterials. While these approaches have demonstrated varying degrees of success, they are often associated with several limitations, including suboptimal efficacy, the risk of secondary infections, and high treatment costs (Simader et al., 2017; Kim et al., 2021; Yamakawa and Hayashida, 2019). Consequently, there is a critical need for alternative therapeutic solutions that are more effective, safe, and cost-efficient, particularly for managing complex or chronic wounds. One promising approach involves the utilization of secretome derived from mesenchymal stem cells, which has shown potential in accelerating wound healing. The secretome contains various bioactive factors that can enhance cellular migration, modulate inflammatory responses, and promote tissue regeneration, making it a viable candidate for improving wound healing outcomes (Ma et al., 2021; Hassan et al., 2014).

The wound healing biological mechanisms involve various molecular and cellular parameters, such as reduced inflammatory factors levels namely Interleukin-8 (IL-8) and TNF-α, increased antioxidant activity, stimulation of cell migration, and regulation of growth factors like Transforming Growth Factor-β1 (TGF-β1) and Connective Tissue Growth Factor (CTGF) (Noh et al., 2018; Yu et al., 2017). Signaling pathways namely NF-κB contributed in modulating inflammatory responses and facilitating tissue regeneration (Keshava and Gope, 2015; Nuraini et al., 2019). These molecular and cellular markers serve as important indicators for evaluating the novel agents therapeutic efficacy in promoting the wound healing process (Wei et al., 2022; Fadini et al., 2014).

Human Umbilical Cord Mesenchymal Stem Cells Secretome (HUCMSCS), bioactive molecules secreted by cells collection, has emerged as a potential candidate for wound therapy. It contains various cytokines, growth factors, and enzymes that support the wound-healing process by reducing inflammation, promoting cell migration, and stimulating tissue regeneration (Simader et al., 2017; Ma et al., 2021; Natallya et al., 2019). Its combination of bioactive properties makes the secretome represents a promising alternative to traditional therapeutic approaches. Studies have demonstrated that secretome derived from adipose-derived mesenchymal stem cells can enhance wound healing by stimulating fibroblast viability and accelerate their migratory capacity (Ma et al., 2021; Hassan et al., 2014).

Furthermore, the intregation of secretome with hydrogels offers novel approach to wound healing. Hydrogels possess high absorption capacity, biological compatibility, and the capability to release functional bioactive substances in a controlled manner (Simader et al., 2017; Kim et al., 2021; Natallya et al., 2019). The use of hydrogels ensures uniform distribution of the secretome at the wound site while simultaneously protecting its bioactive components from degradation, thereby enhancing its therapeutic efficacy (Simader et al., 2017; Kim et al., 2021). This ability can reduce the need for frequent use of hydrogels and offer a more efficient healing process (Doshi et al., 2024). Secretome hydrogels can expedite wound healing by boosting collagen deposition, enhancing angiogenesis, and modulating the inflammatory response (Kuncorojakti et al., 2024).

This study is highly relevant due to the limited available data on the application of secretome-based hydrogel as a wound-healing agent. By combining secretome and hydrogel innovatively, this research aims to evaluate the secretome hydrogel potential in supporting wound healing in BJ cells induced by Lipopolysaccharide (LPS) and scratch, by assessing its cytotoxicity, cell migration assay, inflammatory gene expression tests (IL-8, TNF-α, NF-κB) using qRT-PCR, and levels of malondialdehyde (MDA), TGF-β, and CTGF through ELISA and colorimetry. Additionally, the application of secretome-based hydrogels has the potential to address several existing challenges in wound care, including the demand for more effective, cost-efficient, and clinically applicable treatment options (Simader et al., 2017; Kim et al., 2021). The secretome hydrogel development may offer potential benefits to not only enhance patient outcomes but also reduce healthcare costs associated with prolonged wound healing (Yamakawa and Hayashida, 2019; Koob et al., 2013). This study contributes to the ongoing exploration of innovative wound healing strategies by investigating the secretome-based hydrogel therapeutic potential in a controlled inflammation model (Ma et al., 2021; Hassan et al., 2014).

2. EXPERIMENTAL SECTION

2.1 Materials

The materials applied throughout this study included Carbopol, Hydroxypropyl Methylcellulose (HPMC), Hydroxyethyl Cellulose (HEC), and double-distilled water (ddH_2O), which were utilized for the formulation of the secretome hydrogel. The human Umbilical Cord Mesenchymal Stem Cells secretome (hUCMSCs) was produced and packaged in the GMP-certified facility of Regenic PT. Bifarma Adiluhung (CN: PWS.01.04.1.3.333.09.21-0082), which also holds operational authorization from the Indonesian Ministry of Health (No: 11/1/10/KES/PMDN/2018). Human fibroblast BJ cells (ATCC®CRL-2522) were used for in vitro experiments and cultured in Minimum Essential Medium (MEM) from Biowest (L0416-500). Lipopolysaccharide (LPS) was employed to induce inflammation. Cytotoxicity assays were conducted using the Enhanced Cell Counting Kit-8 (WST-8/CCK8; Elabscience, E-CK-A362). RNA extraction was performed using TRI Reagent from Zymo Research, R2050-1-200 and Direct-zol^{TM} RNA Miniprep Plus from Zymo Research, R2073, while cDNA synthesis utilized the SensiFAST cDNA Synthesis Kit (Meridian Bioscience, BIO-65054). Quantitative real-time PCR (qRT-PCR) was performed with the AriaMx Real-Time PCR System from Agilent, G8830A. Enzyme-linked immunosorbent assays (ELISA)

were used to quantify MDA, TGF-β1, and cTGF using kits from Elabscience: MDA Kit (E-BC-K025-M), TGF-β1 Kit (E-EL-0162), and CTGF Kit (E-EL-H0828).

2.2 Intrumentation

This research utilized the following instruments: biological safety cabinet class IIA, CO_2, incubator (ThermoL-80XP, USA), inverted microscope (Nikon, Japan), microplate reader/spectrophotometer (Multiskan^{TM} GO, Thermo Scientific; 51119300), nanodrop spectrophotometer (Thermo, USA), and real-time thermal cycler (AriaMx Real-Time PCR System, Agilent, USA).

2.3 Methods

2.3.1 Secretome Hydrogel Formulation

The secretome hydrogel was formulated using Carbopol, Hydroxypropyl Methylcellulose (HPMC), ddH_2O, Hydroxyethyl Cellulose (HEC), and human Umbilical Cord Mesenchymal Stem Cells secretome (hUCMSCs). The secretome used in this study was produced and packaged in the Regenic PT. Bifarma Adiluhung laboratory, which is certified for Good Manufacturing Practices (GMP) by the Indonesian Food and Drug Authority (CN: PWS.01.04.1.3.333.09.21-0082). Additionally, the facility holds operational authorization for stem cell processing from the Indonesian Ministry of Health (No: 11/1/10/KES/PMDN/2018). The method for preparing the secretome hydrogel is based on several previous research studies with some modifications (Sarfraz et al., 2022; Betancourt et al., 2010). First, 50 g of Carbopol was gradually dissolved in 40 g of ddH_2O while stirring with a mechanical stirrer until homogeneous. Subsequently, 10 g of HPMC was administered to the solution, and the stirring process continued until it was fully mixed. Separately, 60 g of HEC was dissolved in 40 g of ddH_2O until a clear solution formed, which was then slowly incorporated into the main mixture while stirring. The secretome was gradually combined with the mixture and stirred until homogeneous. The secretome hydrogel was prepared in three concentrations: 5%, 25%, and 50%. If necessary, the pH of the solution was adjusted using a base solution, such as NaOH, to ensure the hydrogel's stability. The final hydrogel formulation was stored in a sealed container to prevent contamination and preserve its integrity.

2.3.2 Inflammation Cells Model with LPS and Scratch

This study employed BJ fibroblast cells derived from the ATCC ®catalog number CRL-2522. The cells were seeded in complete MEM medium (Biowest, L0416-500. Cells grown in T25 flasks were observed under an inverted microscope until they reached 70-80% confluence. The inflammation cell model was created by inducing LPS at 4 μg/mL. Additionally, a scratch assay was performed by creating using blue tips, a linear wound was created down the middle of the well to mimic physical damage and assess cell migration (Widowati et al., 2024)).

2.3.3 Cytotoxicity Assay

Cytotoxicity in BJ cells was analyzed following the instruction from the manufacturer using the Enhanced Cell Counting Kit 8. BJ cells were seeded at 1 × 104 cells per well in a 96-well plate then incubated at 37°C for 24 hours with 5% CO_2. The culture medium was then replaced with medium containing 1 μg/mL lipopolysaccharide (LPS) and incubated for 18 hours. Subsequently, the medium was changed to culture medium supplemented with various concentrations and percentages of hydrogel secretome derived from hUCMSCs, specifically 10, 50, and 100 μg/mL at 5%, 25%, and 50% concentrations. The cells were incubated for an additional 24 hours with 5% CO_2 at 37°C, followed by the 10 μL WST-8 reagent addition to each well. After 3 hours of incubation under the same conditions, absorbance was measured at 450 nm using a spectrophotometer (Multiskan GO Thermo Scientific, 51119300). Results were expressed as percentages of cell viability and proliferation inhibition (Sutjiatmo et al., 2021).

2.3.4 Scratch Assay

Assessment of cell migration was carried out via the wound healing assay (Krisnayanti et al., 2024). Cells were counted using a hemocytometer and seeded at 2 × 10^5 cells per well in 6-well plates, followed by incubation for 24 hours at 37°C with 5% CO_2. After this period, cell morphology was examined using an inverted microscope. The cells were then stimulated with a medium containing 4 μg/mL LPS and incubated for an additional 18 hours. A linear scratch wound was created down the center of each well using a 1 mL blue pipette tip. The culture medium was replaced with fresh medium that supplemented with 50 μg/mL hydrogel secretome at concentrations of 5%, 25%, and 50%, with a total volume of 2 mL per well. The plates were incubated for 24 hours at 37°C with 5% CO_2. Scar tissue formation was monitored at 0, 24, and 48 hours following serum addition. Cell migration percentages were quantified using the ImageJ software (Zulharini et al., 2018).

2.3.5 qRT-PCR Assay

RNA extraction was carried out using TRI Reagent in combination with the Direct-zol^{TM} RNA Miniprep Plus kit, according to the guidelines from the manufacturer. The quantity and purity of the extracted RNA were assessed using a micro-drop plate and measured by a spectrophotometer (Multiskan^{TM} GO Thermo Scientific; 51119300), with results presented in Table 1. This RNA was then used for complementary DNA (cDNA) synthesis via the SensiFAST cDNA Synthesis Kit, following a three-step process consisting of priming for 5 minutes at 25°C, reverse transcription for 20 minutes at 46°C, and enzyme inactivation for 1 minute at 95C° (Widowati et al., 2024).

Gene expression levels of TNF-α, NF-κB, and IL-8, along with the housekeeping gene GAPDH, were quantified using real-time quantitative PCR (qPCR) (see Table 2). GAPDH served as an internal reference to normalize the expression data (Widowati et al., 2024). PCR amplification was performed on the AriaMx Real-Time PCR System, with the detailed tem-

Table 1. RNA Purity of BJ Cells Treated with Hidrogel Secretom

Sample	Concentration (ng/µL)	Purity (λ260/λ280 nm)
Negative Control (NC)	50.72	1.8638
Positive Control (PC)	49.44	2.2915
Vehicle Control (VC)	33.68	2.3678
Secretome Control (SC)	51.52	2.3159
Secretome Hydrogel (SH50)	30.08	2.3000
Secretome Hydrogel (SH25)	36.24	2.3593
Secretome Hydrogel (SH5)	60.08	2.2176

*NC: Negative Control (untreated cells); PC: Positive control (LPS induction and Scratch); VC: Vehicle Control (PC + Basic Hydrogel); SC: Secretome Control (PC+ Secretome 50 µg/mL); SH50: PC + SH 50% 50 µg/mL; SH25: PC + SH 25% 50 µg/mL; SH5: PC + SH 5% 50 µg/mL.

perature profiles, reaction durations, and cycling parameters provided in Table 3.

2.3.6 ELISA Assay

MDA, TGF-1β, and cTGF levels were analyzed following the method outlined by Widowati et al. (2024) with modifications. The MDA, TGF-β1, and CTGF ELISA Kit were utilized in the research following the instructions from manufacturer.

2.3.7 Statistical Analysis

Statistical analyses were performed using SPSS software (version 20.0; SPSS Inc., USA). Data were evaluated by one-way analysis of variance (ANOVA), then followed by Tukey's HSD post-hoc test for datasets that met normality and homogeneity assumptions (Rozirwan et al., 2024). For data that were normally distributed but exhibited unequal variances, Dunnett's T3 post-hoc test was employed. Statistical significance was defined as $p \leq 0.05$. The results were presented as mean ± standard deviation and visualized in histograms generated with GraphPad Prism (version 8.0.244).

3. RESULTS AND DISCUSSION

3.1 The Non-cytotoxic Effect of Secretome Hydrogel on BJ Cells

The choice of polymers for the hydrogel in this research was based on their biocompatibility and ability to form a stable hydrogel matrix that can effectively release bioactive molecules over time. Carbopol, a common hydrogel component, provides a strong network structure, while HPMC and HEC enhance the hydrogel's moisture retention and mechanical properties (Sarfraz et al., 2022; Kim et al., 2021; Natallya et al., 2019). The cytotoxicity assay revealed that the secretome hydrogel was non-toxic to LPS-induced BJ cells, maintaining cell viability close to normal conditions (Figure 1A) without causing cell death or triggering excessive proliferation (Figure 1B).

Based on the cytotoxicity test results, all the hydrogel treatments were classified as non-cytotoxic or minimally cytotoxic. Furthermore, some treatments showed a negative inhibition result regarding their ability to induce proliferation. This possibility arises because the secretome also contains some cytokines that are important for inducing cell growth. The safest concentrations of secretome hydrogel for further testing were identified as 5%, 25%, and 50% at a concentration of 50 μg/mL. These concentrations were selected based on their ability to support cell viability while minimizing potential adverse effects, which are crucial for wound healing applications. The absence of cytotoxic effects suggests that secretome hydrogel could be safely applied in clinical settings, potentially reducing the adverse reactions risk that associated with other treatments (Yamakawa and Hayashida, 2019).

In addition to the cytotoxicity test, we also conducted observations of cell morphology to evaluate the cell shapes related to their condition. These observations revealed that treatment with secretome hydrogel resulted in healthier cell structures compared to the positive control group (LPS induction), indicating a protective effect against inflammation-induced damage (Figure 2). High cell density was associated with increased cell viability and a higher proportion of healthy cells, whereas low cell density corresponded to reduced viability and increased cellular damage. This enhancement in cell morphology is consistent with the role of secretome in supporting cellular health and facilitating tissue regeneration. (Ma et al., 2021; Hassan et al., 2014).

3.2 The Effect of Secretome Hydrogel on BJ Cells Migration

Cell migration is a critical msechanism that contributes in accelerating the wound healing process. The scratch assay shown that secretome hydrogel significantly enhanced cell migration in BJ cells. Observations of cell migration showed that all treatments reduced the area of the scratch, indicating that the cells were migrating to close the gaps (Figure 3A). After 48 h, the gaps were closed in most of the treatments with secretome hydrogel. The quantification of cell migration indicated that after 24 h the group treated with 50% secretome hydrogel (SH50) exhibited the most substantial increase in cell migration compared to other groups, while the vehicle control (VC) and 5% secretome hydrogel (SH5) showed lower migration rates (Figure 3B). At 48 h, SH50 maintained the highest level of cell migration, suggesting that higher concentrations of secretome hydrogel are more effective in enhancing cell migration, a criti-

Table 2. Primer Sequences Used in qRT-PCR.

Gen	Reference	Sequence Primer (5'–3')	Primer Length (bp)
TNF-α *(Homo sapiens)*	NM_000594.4	F: CACACTCAGATCATCTTCTC	20
		R: GAGTAGACACAAGGTACAACCC	20
NFKB *(Homo sapiens)*	NM_199267.2	F: GGACTTGAAGTGATGCGG	20
		R: ACAACCCTAAGTCTTCTTGTG	20
IL-8 *(Homo sapiens)*	NM_000584.4	F: GTGCAGTTTTGCCAAGGAGT	20
		R: TTTCTACGCCCTCTTCAAAAACTT	23
GAPDH *(Homo sapiens)*	NM_001357943.2	F: GGGGCTGTTTAAACTTGGT	20
		R: TGGGCAGGGACTTCTTACAGGG	20

* F: Forward primer; R: Reverse primer

Table 3. Temperature, Time, and qRT-PCR Cycle Settings

Genes	Pre-denaturation	Denaturation	Annealing	Pre-elongation	Elongation ~
NF-κB	95°C; 5'	95°C; 30"; 40 cycles	57°C; 30"; 40 cycles	72°C; 50"	72°C; 5' 4°C
TNF-α	95°C; 5'	95°C; 30"; 40 cycles	57°C; 30"; 40 cycles	72°C; 50"	72°C; 5' 4°C
IL-8	95°C; 5'	95°C; 30"; 40 cycles	58°C; 30"; 40 cycles	72°C; 50"	72°C; 5' 4°C
GAPDH	95°C; 5'	95°C; 30"; 40 cycles	58°C; 30"; 40 cycles	72°C; 50"	72°C; 5' 4°C

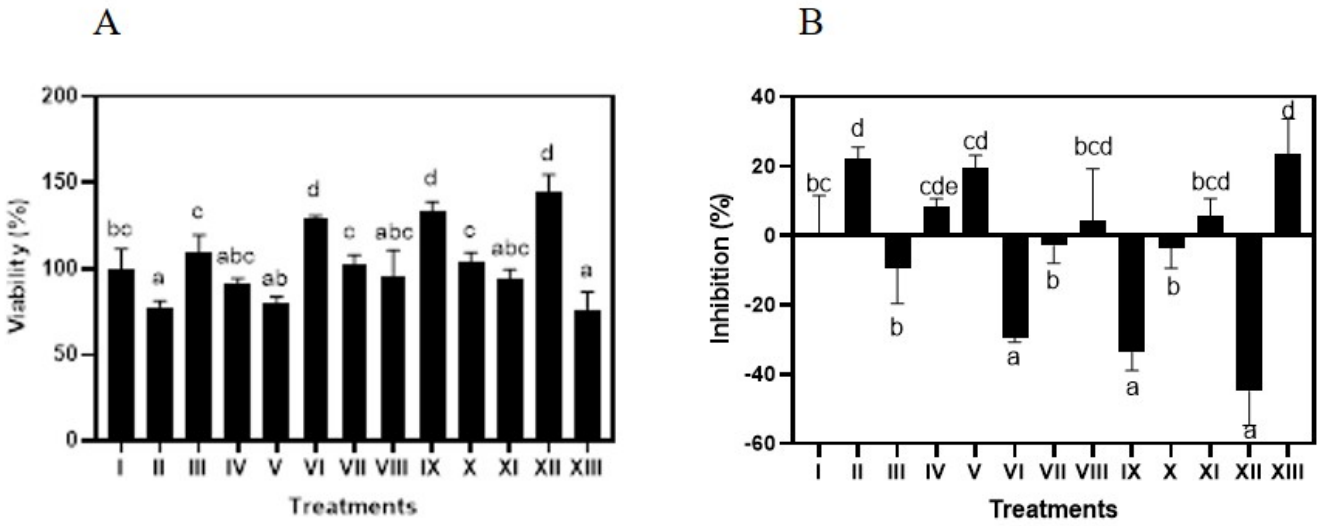

Figure 1. Effect of Different Secretome Hydrogel Concentrations Toward Cells Viability and Cells Inhibition in LPS-Induced BJ Cells

The quantification comparison of cells viability (A) and proliferation inhibition (B) from several group treatment to determine the cytotoxicity of the secretome hydrogel (SH) treatment. Data are presented as averages with standard deviation from 3 replications. I: Negative Control; II: Positive Control (LPS Induction); III: PC + SH 5% 100 µg/mL; IV: PC + SH 5% 50 µg/mL; V: PC + SH 5% 10 µg/mL; VI: PC + SH 25% 100 µg/mL; VII: PC + SH 25% 50 µg/mL; VIII: PC + SH 25% 10 µg/mL; IX: PC + SH 50% 100 µg/mL; X: PC + SH 50% 50 µg/mL; XI: PC + SH 50% 10 µg/mL; XII: Vehicle Control (Basic Gel); XIII: Secretome Control. The difference in notation on the graph shows significance based on Tukey's ANOVA test ($p < 0.05$).

cal aspect of wound healing (Zulharini et al., 2018). This enhanced migration supports the potential of secretome hydrogel to accelerate wound closure by facilitating the cells movement into the wound area (Simader et al., 2017).

3.3 Effect of Secretome Hydrogel Toward Inflammatory Related Genes on LPS- and Scratch-Induced BJ Cells

Inflammation is a crucial phase in the wound healing process, and its effective regulation is essential for optimal tissue recov-

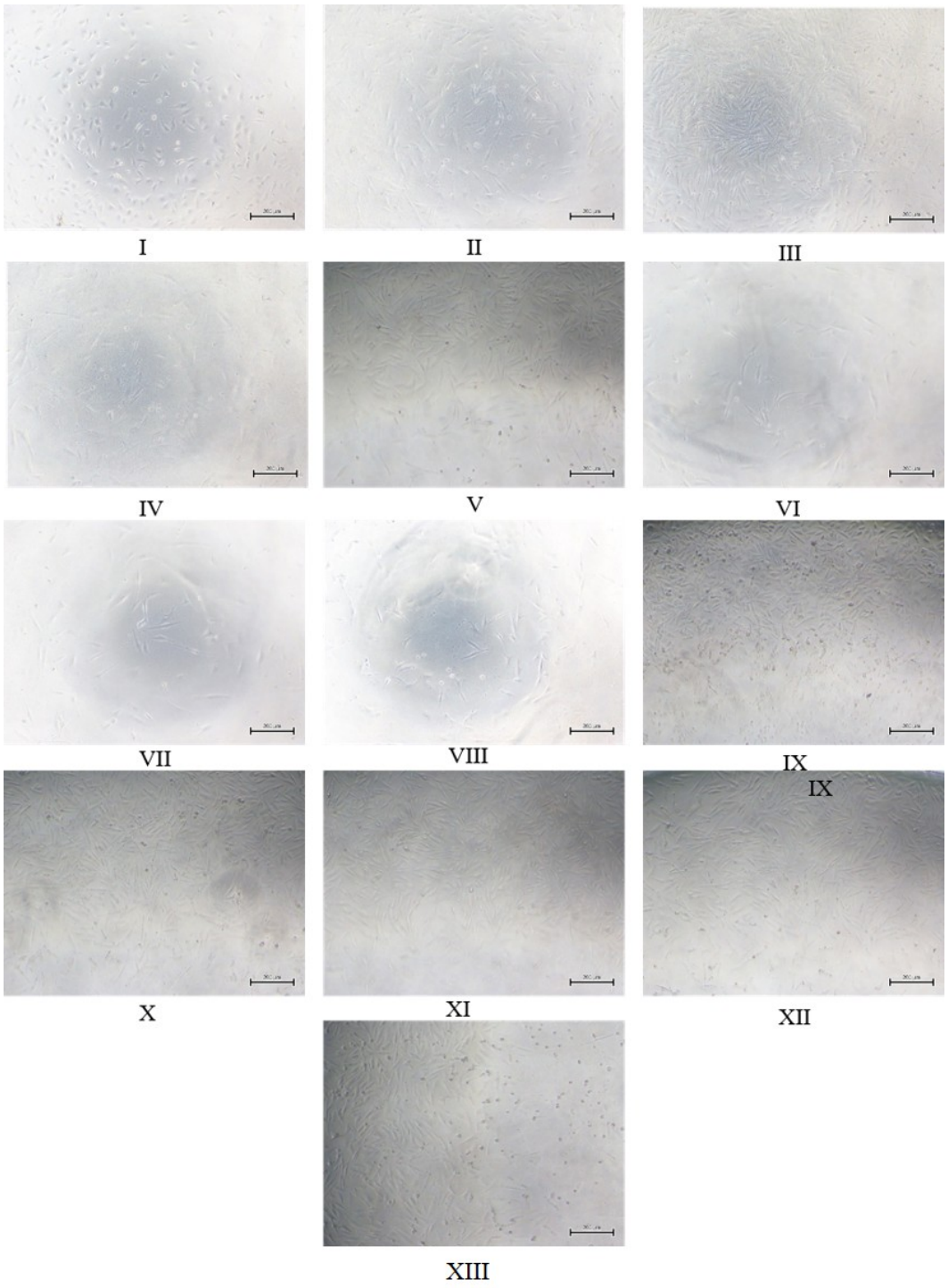

Figure 2. Effect of Different Secretome Hydrogel Concentrations toward Cell Morphology and Cell Density in LPS-Induced BJ Cells

Morphology observation was conducted to evaluate the effect of secretome hydrogel (SH) to the cell condition. I: Negative Control; II: Positive Control (LPS Induction); III: PC + SH 5% 100 µg/mL; IV: PC + SH 5% 50 µg/mL; V: PC + SH 5% 10 µg/mL; VI: PC + SH 25% 100 µg/mL; VII: PC + SH 25% 50 µg/mL; VIII: PC + SH 25% 10 µg/mL; IX: PC + SH 50% 100 µg/mL; X: PC + SH 50% 50 µg/mL; XI: PC + SH 50% 10 µg/mL; XII: Vehicle Control (Basic Gel); XIII: Secretome Control.

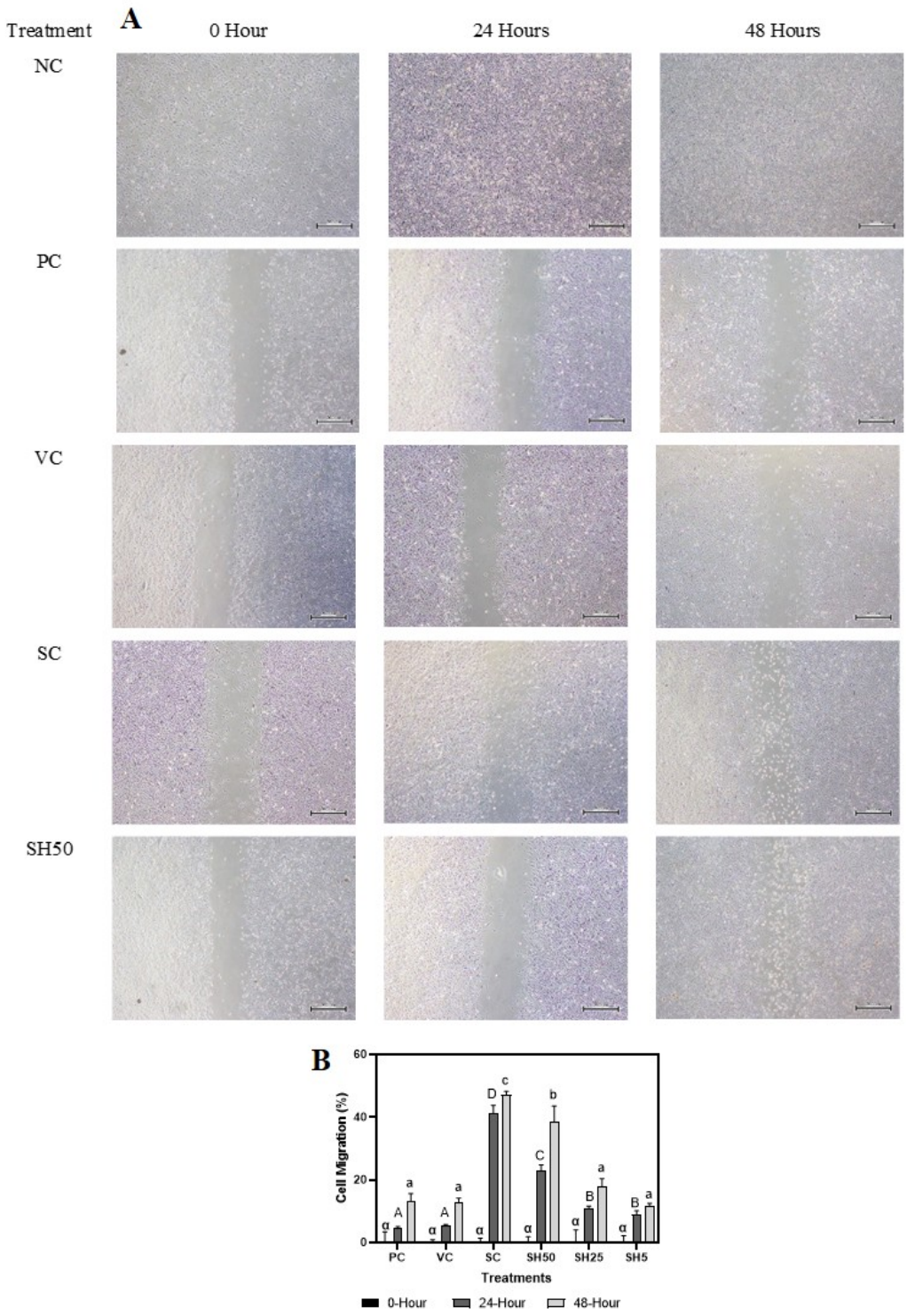

Figure 3. Effect of the Secretome Hydrogel on Cell Migration in Scratch-Induced BJ Cells

(A) The cell cultures were observed at 24 hours and 48 hours after scratching to compare the gaps between each treatment. (B) The quantification of cell migration for each treatment at 24 and 48 h is presented. Data are shown as averages with a standard deviation from three replicates. PC: Positive Control (LPS and Scratch Induction); VC: Vehicle Control (PC + Basic Hydrogel); SC: Secretome Control (PC + Secretome 50 µg/mL); SH50: PC + 50% SH 50 µg/mL; SH25: PC + 25% SH 50 µg/mL; SH5: PC + 5% SH 50 µg/mL. Differences in notation on the graph indicate significance based on ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

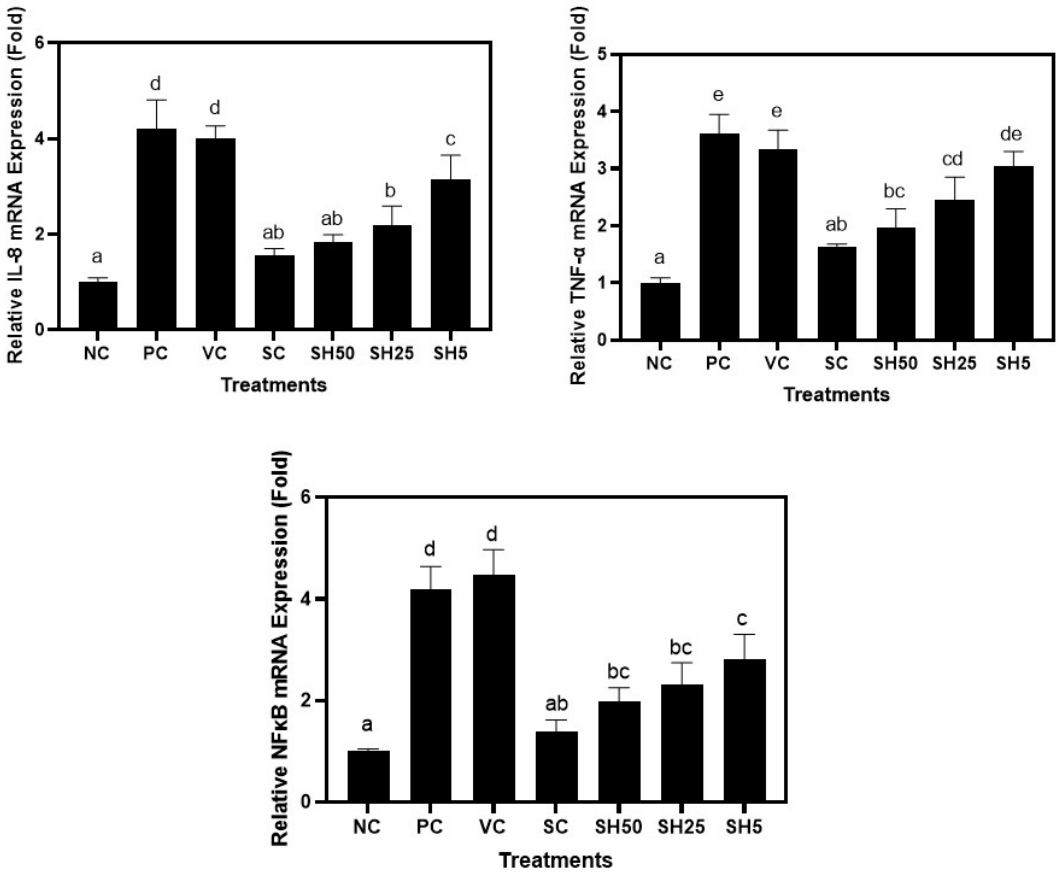

Figure 4. Effect of Different Secretome Hydrogel Concentrations Toward Inflammation Markers in LPS- and Scratch-Induced BJ Cells

The mRNA levels were measured using qRT-PCR to evaluate the impact of secretome hydrogel on the inflammation markers IL-8, TNF-α, and NF-κB. Data are presented as averages with standard deviations from three replicates. PC: Positive Control (LPS and Scratch Induction); VC: Vehicle Control (PC + Basic Hydrogel); SC: Secretome Control (PC + Secretome 50 µg/mL); SH50: PC + 50% SH 50 µg/mL; SH25: PC + 25% SH 50 µg/mL; SH5: PC + 5% SH 50 µg/mL. Differences in notation on the graph indicate significance based on ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

ery. Secretome hydrogels have emerged as a promising therapeutic strategy by modulating inflammation and enhancing tissue repair. Their ability to provide a controlled the anti-inflammatory factors release and growth factors fosters a conducive environment for wound healing, highlighting their potential in the development of advanced treatment approaches.

We analyzed the mRNA levels of several inflammation markers. The qRT-PCR analysis showed that SH significantly reduced the expression of inflammatory genes, namely IL-8, TNF-α, and NF-κB, in LPS-induced BJ cells (Figure 4). The PC group exhibited the highest IL-8 expression, indicating an inflammatory response induced by LPS, while the SH-treated groups showed a significant decrease in IL-8 expression, particularly in SH50. Similar trends were observed for TNF-α and NF-κB, with elevated levels in PC and marked reductions in SH50. This reduction in inflammatory markers highlights the anti-inflammatory properties of SH, which may help alleviate excessive inflammation that often impedes the wound healing process (Keshava and Gope, 2015; Nuraini et al., 2019).

3.4 Effect of Secretome Hydrogel Toward Fibrosis and Cellular Signalling Pathways on LPS- and Scratch-Induced BJ Cells

Fibrosis is a major concern in wound healing, as it can result in excessive scar formation and compromise tissue integrity. To investigate this, an ELISA assay was conducted to assess the proteins associated with fibrosis expression and key cellular signaling pathways involved in the wound healing process. The findings provide insights into the potential cellular mechanisms modulated by the secretome hydrogel. The results demonstrate the effects of various treatments on MDA levels, TGF-β concentration, and CTGF concentration (Figure 5).

LPS and scratch induction caused an increase in MDA in BJ cells, while treatment with the secretome hydrogel was able to reduce MDA levels. MDA is an indicator of oxidative stress, with the most optimal concentration being SH50.

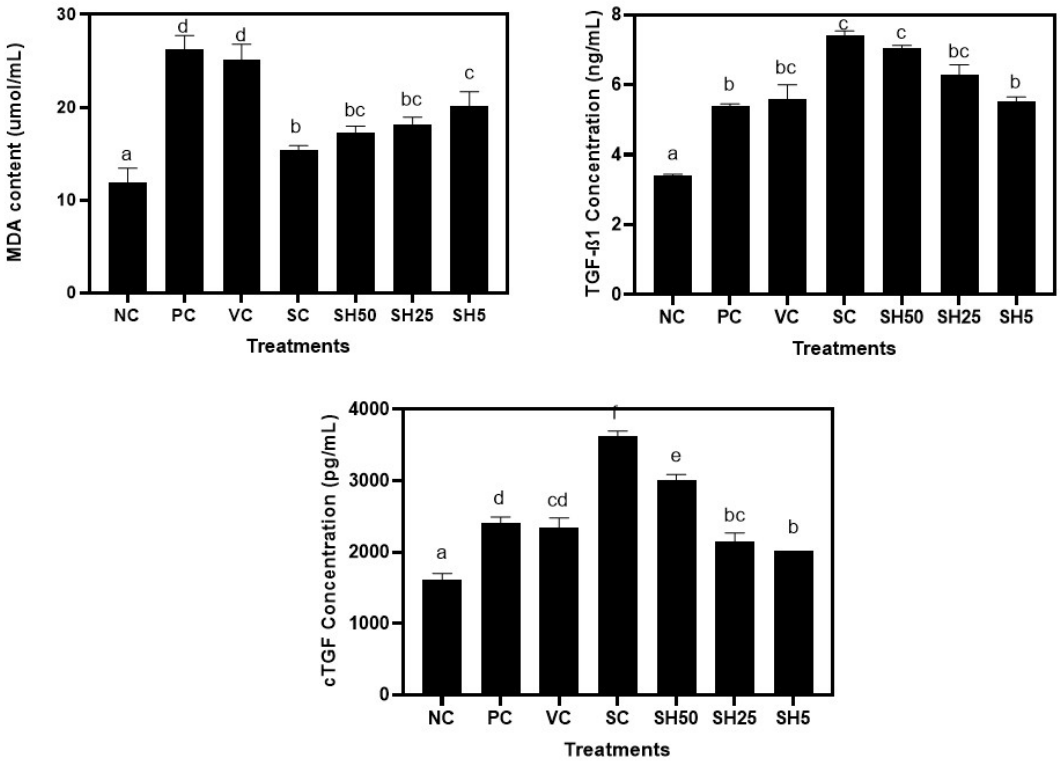

Figure 5. Effect of Different Secretome Hydrogel Concentrations toward CTGF, TGF-β1, MDA Levels on LPS- and Scratch-Induced BJ Cells

The concentrations of TGF-β1, CTGF, and MDA were measured following treatment with the secretome hydrogel. Data are presented as the average with a standard deviation from three replicates. PC: Positive Control (LPS and Scratch Induction); VC: Vehicle Control (PC + Basic Hydrogel); SC: Secretome Control (PC + Secretome 50 µg/mL); SH50: PC + 50% SH 50 µg/mL; SH25: PC + 25% SH 50 µg/mL; SH5: PC + 5% SH 50 µg/mL. Differences in notation on the graph indicate significance based on ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$)

Furthermore, we also measured TGF-β, which contributed in fibroblast proliferation for wound healing. The TGF-β concentration significantly increased in the positive control compared to the negative control, indicating an inflammatory response. Treatment with the SH further increased TGF-β concentration, thereby triggering the positive feedback mechanism to enhance the wound healing process. We also measured cTGF levels, which also influence tissue formation. The results demonstrate that treatment with the secretome hydrogel increases cTGF concentration in LPS- and scratch-induced BJ cells. In general, the SH decreased MDA levels while increasing TGF-β1 and CTGF levels, which are crucial for tissue regeneration and wound healing. These results suggest that the SH not only reduces inflammation but also promotes tissue repair by enhancing the growth factors production that essential for wound closure (Noh et al., 2018; Yu et al., 2017).

3.5 Discussion

Wounds are often difficult to heal due to prolonged inflammatory responses and the limited regenerative factors at the wound site. Secretome of MSCs has proven to be an effective therapeutic agent because it contains various bioactive factors that support cell migration, proliferation, and regeneration. Studies have demonstrated that secretome hydrogel exhibits significant potential in accelerating the wound healing process (Simader et al., 2017; Tan et al., 2023). Previous research also revealed that secretome combined with biomaterials can enhance wound healing through similar mechanisms (Deshpande et al., 2018; Koh et al., 2022).

To ensure the biocompatibility and safety of secretome hydrogel materials for biomedical applications, cytotoxicity tests have become an important part of the initial steps in developing the secretome hydrogel treatment. The significance of these tests lies in their ability to assess how hydrogels interact with various cell types, which is essential for understanding their potential impact on human tissues. For instance, studies have shown that certain hydrogels, such as those based on chitosan, do not affect the viability of mesenchymal stem cells (MSCs) and allow them to maintain their paracrine activity, including the release of micro vesicles (Boido et al., 2019). Similarly, wound-healing hydrogels are non-toxic to human skin-derived cells, including fibroblasts and endothelial cells,

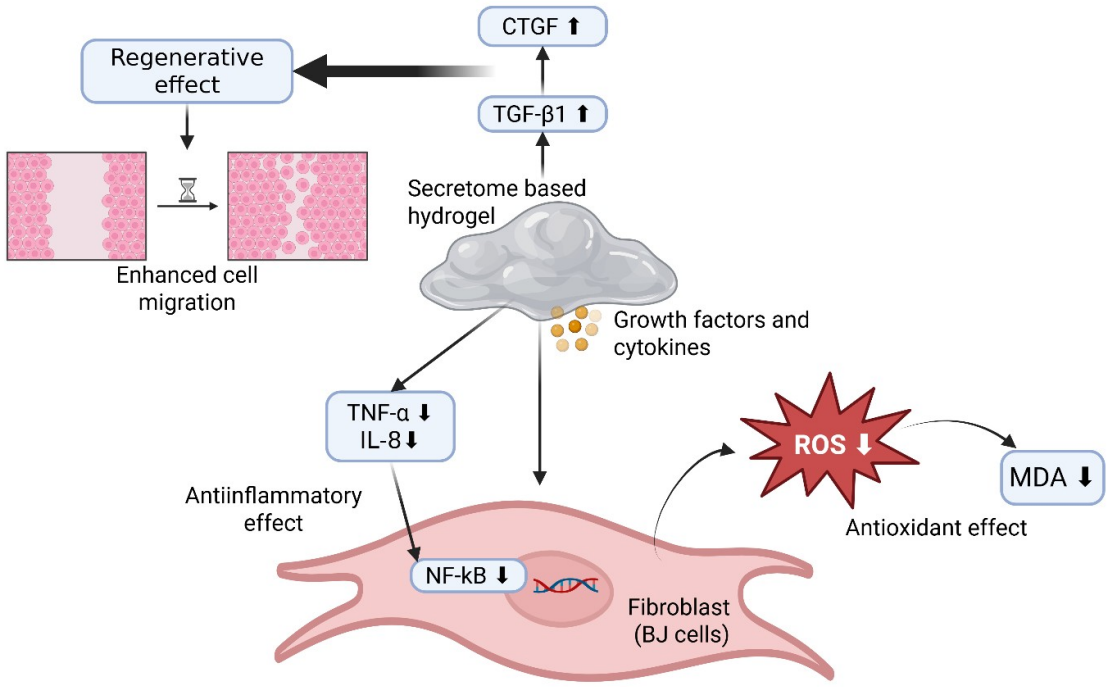

Figure 6. Proposed Mechanism of Secretome-Based Hydrogel in Promoting Wound Healing in LPS- and Scratch-Induced BJ Cells

The hydrogel promotes regeneration via TGF-β1 and CTGF upregulation, reduces inflammation by lowering TNF-α, IL-8, and NF-κB, and provides antioxidant effects through decreased ROS and MDA levels.

reinforcing their potential for clinical applications (Kraskiewicz et al., 2021). Conducting cytotoxicity assays, such as MTT or CCK-8 tests, helps confirm that hydrogels do not induce adverse cellular responses, thereby validating their suitability for therapeutic applications (Suhail et al., 2022). The safety of using secretome in wound therapy is a crucial aspect, and this study demonstrates that the secretome hydrogel is not cytotoxic to LPS-induced BJ cells, maintaining high cell viability at certain concentrations. These results are consistent with other reports which indicate that secretome-based gels do not cause toxic effects and support human fibroblast viability (Natallya et al., 2019).

During wound healing, cells such as epithelial cells and dermal fibroblasts migrate either collectively or individually into the wound site to facilitate tissue repair. Collective migration, particularly among epithelial cells, involves the coordinated movement of large cell sheets with tight intercellular junctions, which is essential for maintaining epithelial integrity and promoting effective wound closure (Li et al., 2022). Fibroblast migration is equally critical, as these cells contribute to wound closure by proliferating and filling the cell-free area (Monfared et al., 2020). This migratory process is regulated by various biochemical and biophysical factors, including extracellular matrix (ECM) composition and stiffness, as well as chemotactic signals such as growth factors (Qu et al., 2019). Results from this study show that the 50% secretome hydrogel (SH50) group significantly enhanced cell migration at 24 and 48 hours compared to the control, indicating its effectiveness in supporting wound healing. These findings align with research by Koh et al. (2022), which demonstrated the role of the secretome in enhancing fibroblast migration.

Inflammation is a significant barrier to wound healing, with TNF-α often elevated in chronic wounds and hindering the healing process. Secretome hydrogels have been demonstrated to lower TNF-α levels, indicating their efficacy in mitigating local inflammation (Dedier et al., 2023). Chen et al. (2015) also shown that bioactive factors within the secretome can suppress TNF-α expression, thereby facilitating tissue repair. Likewise, secretome hydrogels have been shown to reduce IL-8 levels, further highlighting their potent anti-inflammatory properties (Deshpande et al., 2018). This aligns with findings by Zhang et al. (2023), which showed that bioactive biomolecule-based therapies can reduce IL-8 to facilitate the resolution of inflammation. NF-κB, known as a crucial regulator of the inflammatory cascade, is also suppressed by secretome hydrogels, further corroborating their role in inflammation suppression (Dedier et al., 2023). Simader et al. (2017) reported similar outcomes, where secretome exhibited the capacity to inhibit NF-κB activation in an inflammatory wound model.

Oxidative stress, indicated by MDA levels, is often elevated

in chronic wounds. Secretome hydrogels possess antioxidant properties, contributing to wound healing by reducing MDA levels (El-Sawah et al., 2021). These findings support research by Kwon (2023), which demonstrated that reducing oxidative stress can expedite tissue regeneration. Conversely, growth factors such as TGF-β play a crucial role in collagen synthesis and fibroblast proliferation, accelerating tissue remodelling during wound healing (Kim et al., 2021). An increase in TGF-β levels in the secretome hydrogel group suggests stimulation of faster tissue regeneration (Saheli et al., 2020). Additionally, CTGF, which regulates extracellular matrix production, is elevated, promoting quicker tissue formation (Wei et al., 2022; Deshpande et al., 2018).

The proposed mechanism illustrated in Figure 6 demonstrates how secretome-based hydrogel facilitates wound healing through multiple coordinated pathways. The hydrogel contains a rich composition of cytokines and growth factors that contribute to fibroblast activation. Upon application, the hydrogel enhances cell migration, which accelerates wound closure. It also upregulates TGF-β1 and CTGF expression, key mediators of tissue regeneration, thereby promoting fibroblast proliferation and extracellular matrix formation. Additionally, the hydrogel suppresses pro-inflammatory markers namely TNF-α, IL-8, and NF-κB, reflecting its anti-inflammatory effect. Furthermore, the hydrogel reduces intracellular ROS and MDA levels, indicating its antioxidant activity. Together, these processes create an optimal microenvironment for wound repair by simultaneously enhancing regeneration, reducing inflammation, and mitigating oxidative stress. These results support the concept that integrating biomaterials with bioactive factors can create an optimal microenvironment for wound healing, as also reported by Li et al. (2022).

4. CONCLUSIONS

The formulated secretome hydrogel demonstrates significant potential as a wound-healing agent. It is non-cytotoxic to LPS-induced BJ fibroblast cells and enhances cell migration. The hydrogel promotes tissue regeneration by upregulating TGF-β and CTGF gene expression, while also exhibiting antioxidant properties through the reduction of MDA levels. Furthermore, it downregulates inflammatory markers including IL-8, TNF-α, and NF-κB. Further research is needed to investigate its clinical applicability across various wound conditions.

5. ACKNOWLEDGMENT

This research was financed by Ministry of Higher Education, Research, and Technology for Matching Fund 2024. We are grateful to Regenic Cell Therapy Manufacturing, PT. Bifarma Adiluhung, Jakarta, Indonesia who supported the hUCMSCs-Secretome, PT Nanotech Serpong, PT Aretha Medika Utama, Indonesia, for providing the laboratory resources for this research. The authors acknowledge the Vini Ayuni contributions from Aretha Medika Utama.

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