[HP#74]

A Potential Halal Collagen-like Protein (RecCLPM-46) Exhibits Wound Healing Properties for Halal Pharmaceutical Industry

Faqihah Salleh, Azura Amid*, Noor Faizul Hadry Nordin

International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, Jln Gombak, 53100 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur. Malaysia

*Corresponding author's e-mail: azuraamid@iium.edu.my

ABSTRACT

Collagen is a powerful biomaterial that can be used in various industries, mainly, the medical and pharmaceutical industries. It is often used and incorporated to speed up the wound-healing process. A *halalan toyyiban* collagen uses for wound healing is in high demand for Muslims that abiding the *Syariah* law. In this study, the collagen-like protein, cloned from *Methylobacteria sp*. 4-46 (RecCLPM4-46) and purified using FPLC was tested for its wound healing property. The HaCaT keratinocyte skin cells were used to simulate the proliferation activity of the skin. Allantoin, the commercial wound healing aid in the market was used as the positive control, six different concentrations of RecCLPM4-46 (1.56 %, 3.13 %, 6.25 %, 12.50% and 25.0% v/v) were applied in the cell viability assay. Due to non-cytocompatibility, 12.50% and 25.0% v/v of RecCLPM4-46 were eliminated for wound closure assay. In the scratch assay, 6.25% v/v of RecCLPM4-46 has the highest wound closure percentage (65.4%) compared to the rest of the RecCLPM4-46 concentrations. In terms of wound closure percentage, 6.25% v/v of RecCLPM4-46 was not far off with 26.1% less wound closer from Allantoin. This result suggests that RecCLPM4-46 has a promising wound-healing feature for the biomedical and pharmaceutical industries.

Keywords: *Halalan and Toyyiban*; RecCLPM4-46; Collagen-like protein; Wound healing

1 Introduction

Collagen is a protein found abundantly in various parts of human and animal bodies such as ligaments, tendons, cartilage, and skins [1]. The production of collagen in human and animal bodies is decreasing over time therefore, it can be applied externally by taking it topically and orally to replace the internally produced collagen where it is depleted. There are 29 types of collagens that are found these days, and most collagen used externally in products is the Type I collagen which it can be found in 90% of animal bodies [2]. Based on regulations in Islam (*Shariah*) in Al-Quran (Al-Maidah 5:3), when the material involved in any daily product contains animal-based ingredients, it will eventually become the main concern for practicing Muslims. According to MS 2424:2019 [3], the animal-based material in halal pharmaceuticals must be from permissible livestock, poultry and fish and it must be halal-slaughtered according to *Syara'*. Aside from halal issue, dealing with animals to produce collagen exposed the risks of transmitting disease and sustainability issues [4].

DOI: 10.21467/abstracts.155 ISBN: 978-81-961472-9-7 (eBook)

The application of collagen is vast in different industries, mainly in the medical and pharmaceutical industries. In this particular industry, collagen is majorly used to heal wounds by being a drug-delivery medium, skin replacement and bone substitute [5]. There are four main phases of the wound healing process which are coagulation (homeostasis), inflammation, migration/proliferation, and remodelling [6]. After tissue injury, the keratinocyte cells at the boundary of the wound will display a high proliferative activity where the cells will migrate onto the wound bed and help to restore the epidermal barrier structure, therefore, continuing the main function of the organ/structure. To help speed up the process, collagen or any biomaterials with the wound-healing property were incorporated during the process. To simulate the effect of RecCLPM4-46 on proliferation and migration activity in the wound healing process *in vitro*, Keratinocyte HaCaT skin cells were used and 1 mg/mL (0.1% v/v) of Allantoin. The wound healing test carry out here is the standard test accepted by pharmaceutical industry, where it used Allantoin as positive control for the *in vitro* study due to the simplicity of the compound compared to the non-halal collagens. Although Allantoin can be synthetically produced but, naturally, allantoin is sourced from uric acid of urine, where consumer should aware off. Moreover, the test aims to observe the effectiveness of RecCLPM4-46 on wound healing process and not to compare it with non-halal collagen, therefore, Allantoin will be the best positive control for effectiveness since it is already known for its effectiveness for wound healing treated.

2 Materials and Methods

Previously, the cloning process was carried out by synthesizing the genetic sequence of *Methylobacteria sp*. 4-46 containing the Collagen-like Protein domain obtained from National Center of Biotechnology Information (NCBI) into pCold II plasmid and transformed into *Escherichia coli* BL21 (DE3). The RecCLPM4-46 protein was then expressed in *E. coli* using Minimal media (M9 media), induced with 10 mM of isopropyl β-D-1-thiogalacropyranoside (IPTG) and optimized to obtain the highest yield of RecCLPM4-46 with economical perspective considered. The RecCLPM4-46 was purified using Fast Protein Liquid Chromatography (FPLC) and to measure the collagen concentration of purified RecCLPM4-46, the EnzyFluo™ Collagen Assay Kit (ECOL-100) is used and read by spectrofluorometer at λex/em = 375/465 nm.

As no wound healing studies for RecCLPM4-46 have been conducted before, this study was undertaken by using scratch assay on HaCaT keratinocyte skin cell line. Allantoin, a commercial and natural wound healing aid accepted by pharmaceutical industries was used as the positive control and the negative control was the Dulbecco's Modified Eagle Medium (DMEM). The assay started with the HaCaT cells were seeded at the density of 2 X 10^5 cells into 24-well plates and incubated with DMEM supplemented with 10% v/v of Fetal Bovine Serum (FBS) at 37°C, 5% CO_2 until the cells reached 90% confluence. After 24 hr, the monolayer

ISBN: 978-81-961472-9-7
Series: AIJR Abstracts
DOI: 10.21467/abstracts.155

confluent cells were scrapped horizontally with a sterile P200 pipette tip. The debris was removed by washing it with Phosphate Buffer Saline (PBS). The cells were treated with 0.1% v/v Allantoin and 1.56 %, 3.13 %, 6.25 %, 12.50% and 25.0% v/v RecCLPM4-46 for 48 hr. The cell proliferation and migration were photographed at 0 hr and 48 hr using phase-contrast microscopy at 4X magnification. The migration rate was measured by analysing the photographed images with ImageJ software and the percentage of the closed area (μm^2) unit was measured and compared with the value of control at 0 hr. An increase in the percentage of the closed area indicated the cell migration. The assay was performed in triplicate manner.

3 Results

The scratch assay was done to observe the effect of RecCLPM4-46 on cell proliferation and migration of Keratinocyte HaCaT skin cells. Based on Table 1, in cell viability experiment, 12.5% and 25.0% v/v of RecCLPM4-46 showed non-cytocompatibility on HaCaT cells. The cytotoxicity effect was determined by comparison of 0.1% v/v of Allantoin (positive control) with the rest of the samples. The test system is acceptable only if the cell viability is higher than 80%. Therefore, to proceed with scratch assay, only 1.56%, 3.13% and 6.25% v/v of RecCLPM4-46 were chosen. In Figure 1, 6.25 % v/v of RecCLPM4-46 has the highest percentage of wound closure compared to 1.56 % and 3.13 % v/v RecCLPM4-46. Side to side, 6.25 % v/v RecCLPM4-46 with Allantoin, a natural wound healing aid that has been used in many pharmaceutical products, the percentage of wound closure of 6.25% v/v RecCLPM4-46 is not far off which is around 26.1 % less from 0.1% v/v Allantoin (Figure 1). The cell migration of these concentrations can be observed in Figure 2 where the 0 hr and 48 hr of each sample were shown side by side.

Table 1: *Cell Viability of 0.1% v/v (1 mg/mL) Allantoin and 1.56 %, 3.13 %, 6.25 %, 12.50% and 25.0% v/v RecCLPM4-46*

Sample	Concentration (%)	Cell Viability (%)	Wound closure (%)
Allantoin	0.1	95.8 ± 2.1	91.5 ± 3.7
RecCLPM4-46	1.56	98.3 ± 0.7	13.5 ± 3.9
	3.13	97.3 ± 2.6	38.1 ± 3.4
	6.25	98.4 ± 1.5	65.4 ± 3.2
	12.5	70.8 ± 3.9	n.a
	25.0	42.3 ± 5.1	n.a

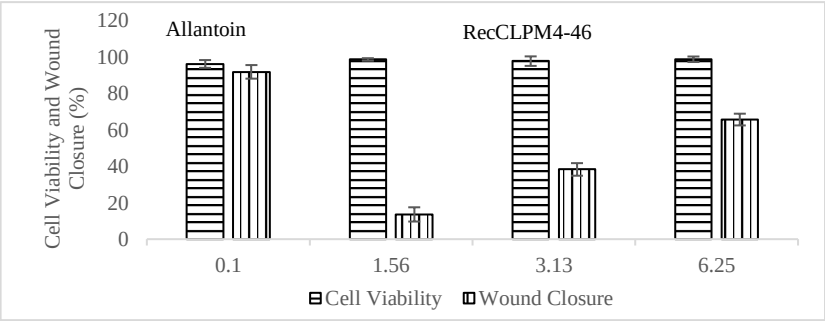

Figure 1: *Effects of positive control and RecCLPM4-46 on the rate of wound closure (%) and cell viability.*

ISBN: 978-81-961472-9-7
Series: AIJR Abstracts
DOI: 10.21467/abstracts.155

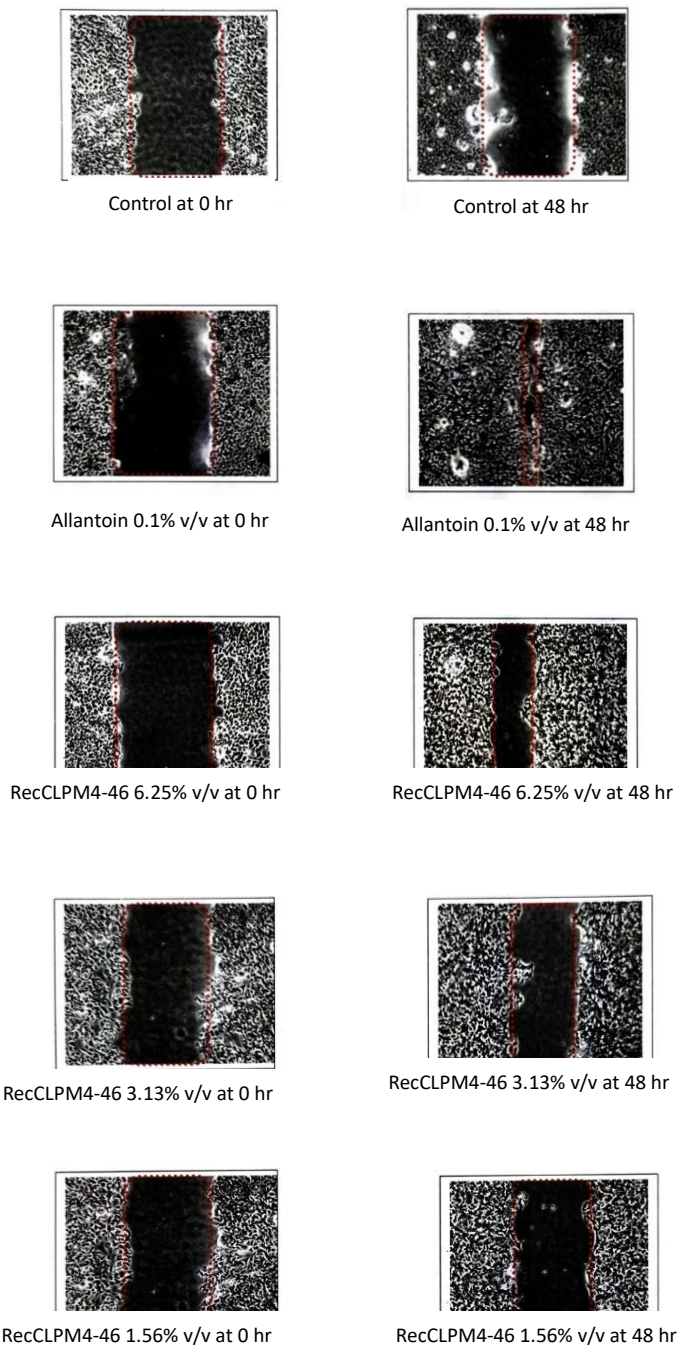

Figure 2: *The figure indicates HaCaT cells were scratched at 0 hr and treated with Allantoin (positive control) (Concentration 0.1% v/v or 1mg/mL) and RecCLPM4-46 (concentration 1.56%, 3.13% and 6.25% v/v). At 48 hr, the cell proliferation and cell migration were recorded and measured in µm2 using ImageJ software.*

4 Conclusions

Based on the results, RecCLPM4-46 exhibits the feature of accelerating the wound healing activity on human skin as simulated by HaCaT cells, thus making it a potential Halal collagen to cater for Halal pharmaceutical industry. In the future, this research warrants further investigation on different characterization studies such as thermostability, solubility, combination with additional biomaterials and fabrication to match the quality of collagen in the market today.

ISBN: 978-81-961472-9-7
Series: AIJR Abstracts
DOI: 10.21467/abstracts.155

5 Declarations

5.1 Acknowledgment

The authors would like to express their appreciation for the support of the sponsors (Ministry of Higher Education Malaysia) with Project No TRGS/1/2018/UIAM/01/1/1 for providing financial aid and laboratory facility for this research.

References

1. Mohs, A.; Silva, T.; Yoshida, T.; Amin, R.; Lukomski, S.; Inouye, M.; Brodsky, B. Mechanism of stabilization of a Bacterial Collagen Triple Helix in the absence of hydroxyproline. J. Biol. Chem. **2007**, 282, 29757–29765, doi:10.1074/jbc.M703991200.
2. Dewle, A.; Pathak, N.; Rakshasmare, P.; Srivastava, A. Multifarious fabrication approaches of producing aligned collagen scaffolds for tissue engineering applications. ACS Biomater. Sci. Eng. **2020**, 6, 779–797, doi:10.1021/acsbiomaterials.9b01225.
3. Department of Standards Malaysia MS 2424: Halal pharmaceuticals - General requirements (first revision). Malaysian Stand. 2019.
4. Gomez-Guillen, M.C.; Gimenez, B.; Lopez-Caballero, M.E.; Montero, M.P. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocoll. **2011**, *25*, 1813–1827, doi:10.1016/j.foodhyd.2011.02.007.
5. Lee, C.H.; Singla, A.; Lee, Y. Biomedical applications of collagen. Int. J. Pharm. **2001**, 221, 1–22, doi:https://doi.org/10.1016/S0378-5173(01)00691-3.
6. Enoch, S.; Leaper, D.J. Basic Science of Wound Healing. Surg. **2008**, *26*, 31–37, doi:https://doi.org/10.1016/j.mpsur.2007.11.005.

ISBN: 978-81-961472-9-7

Series: AIJR Abstracts

DOI: 10.21467/abstracts.155