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COMPARISON OF *SECRETOME DENTAL PULP STEM CELL* AND MEBO OINTMENT ON THE AMOUNT OF FIBROBLAST AND COLLAGEN IN SUPERFICIAL DERMAL BURNS

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ABSTRACT
Secretome Dental Pulp Stem Cells have been proposed as a new alternative for wound burn. The aim of this study is to compare *Secretome Dental Pulp Stem Cells* and MEBO ointment against several fibroblasts and collagen in the healing process of superficial dermal burns. This study used *a post-test only controlled group design* by using 15 rats that were given superficial dermal burned wounds. Rats were divided into a negative control group of NaCl 0.9% (K1), a treatment group of MEBO (K2), and a treatment group of DPSCs-secretome (K3). Histopathology was done on the 5th, 14th, and 21st days post-induction wound burn. Network-prepared histopathology by colouring *Messon Trichomes*. Then, it was observed under a microscope with 200x magnification. Scoring in a way semi-qualitative for assessing fibroblasts and collagen. The total score of fibroblasts in the MEBO group was higher than the DPSCs-*secretome* group on day 5th. In comparison, the score for increasing the amount of collagen in the DPSCs-*secretome* group on days 5th and 14th was higher than the MEBO group. DPSCs-*secretome* has better effectiveness in increasing the amount of collagen but not better in increasing fibroblasts compared to MEBO.

Keywords: Burn, Collagen**,** *Dental Pulp Stem Cell*, Fibroblast, *Secretome*

Introduction

Burned wounds are a major public health problem throughout the world. The high prevalence of cases and the risk of morbidity and mortality have an impact on permanent impairment of appearance and function which causes patients to lose their jobs and uncertainty about their future (Kemenkes, 2019). Burn healing treatment includes prevention infections, stimulate collagen formation, and ensuring that remaining epithelial cells can be removed grows so that it can cover the surface of the wound (Syamsuhidayat R dan W.D. Jong, 2004).

The wound-healing process will occur more quickly if new cells grow. Fibroblasts and collagen play a very important role in the wound-healing process because they repair damaged

or lost tissue. Recently, molecular biology has provided advancement in the medical field by utilizing stem cells (Thapak et al., 2022). The secreted bioactive chemicals produced by stem cells, particularly those originating from stem cells or known as the secretome (Kim et al., 2022). The secretome refers to the diverse collection of chemicals produced by stem cells, which consists of protein molecules, lipids, extracellular vesicles (exosomes, microvesicles, and apoptotic bodies), and nucleic acids (miRNA, mRNA, and lncRNA) which is involved in the physiological processes of cells and tissues (Madrigal et al., 2014) & (Vizoso et al., 2017) .(5,6)

Recently, DPSCs have been a promising cell source for many and varied regenerative medicine applications, and are being investigated for tissue repair.

Research Methods

This research employed a post-test-only control group design. The protocol of the animal study has been approved by the Ethics Committee of the Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia (No. 032/KEPK/UNPRI/VIII/2023).

The research subjects were 15 male *Wistar* rats, aged 2-3 months, 150-250 g b/w. Acclimatization of rats was conducted in a 12/12 hour light/dark cycle for one week, at a consistent temperature of 23±2°C. After the acclimation phase, the rats were divided into three groups, each consisting of five rats.

Preparation of DPSCs

Dental pulp stem cells were obtained from the Laboratorium Riset Terpadu Fakultas Kedokteran Gigi Universitas Gadjah Mada which had been isolated and stored in a refrigerator, being thawed, and cultured again in Petri dishes containing alpha-MEM medium, 20% fetal bone bovine serum, streptomycin, and penicillin fungizone, for 4-6 weeks until the third cycles before being used for this research. Dental pulp stem cell cultures were stored in an incubator at a temperature of 37°C and a CO_2 level of 5%.

Isolation of DPSCs secretome

After the dental pulp stem cell culture reaches 80-85% of confluence, the medium was replaced with 10 ml of serum-free Dulbecco's Modified Eagle's Medium (DMEM), and antibiotics were added (100 units/ml Penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B). The stem cells were incubated in the media for 48 hours. This cultured cell within conditioned media is defined as the secretome. The secretome that will be immediately used for research after 48 hours of incubation was centrifuged at 440xG, 5 minutes, 4°C. The supernatant was then separated and centrifuged again at a speed of 17,400xG, 3 minutes, 4°C and then filtered with a 0.22 µm filter. If it is to be stored, the medium is centrifuged for 5 minutes at 1500 rpm followed by 15,000 rpm for 1 minute to remove debris and dead cells. A total of 5 ml of culture medium was mixed with 45 ml of 100% ethanol and incubated at -20°C for 1 hour then centrifuged for 15 minutes at 15,000 rpm at 4°C to separate the supranatant. The precipitate was resuspended in cold 90% ethanol and centrifuged for 15 minutes at 15,000 rpm at 4°C. The resulting precipitate was then frozen at -80°C and stored in a refrigerator (Katagiri et al., 2016).

Superficial Burned Wounds and Treatments

A mixture of 1 ml ketamine and 10 ml NaCl 0.9% was used to anesthetize the rats. An iron measuring 2 x 2 cm was heated on the stove for 2 minutes and after epilation, it was placed on

the rat's back for 5 seconds. Then the wound was cleaned using 0.9% NaCl before being smeared with 0.9% NaCl (K1), MEBO (K2), and DPSCs secretome (K3).

Histopathology Examination

Skin samples were taken on the 5th, 14^{th,} and 21st days. The skin tissue samples were fixed in a 10% formaldehyde buffer solution for 3 hours and then washed with tap water. After that, tissue samples were dehydrated for 30 minutes using alcohol 70%, then for three times 30 minutes using alcohol 96%, three times one hour using pure alcohol, and one hour clearing with Xylol 1:1. Impregnation with paraffin was 60 min in an oven at 65°C, then staining with Masson's Trichrome. Tissues that had been paraffinized were cut into 5 µm-thick cross sections.

Microscopic Analysis

Table 1. Microscopic scoring parameters; (a) adopted from [8]; (b) adopted from [9,10].

	Parameters (per-view of field)	Score
a	**Collagen**	
	• Collagen density is more than normal tissues	3
	• Collagen density is equal to normal tissues	2
	• Collagen density is less than normal tissues	1
b	**Fibroblasts**	
	• More than 50 cells	3
	• 10-50 cells	2
	• 5-10 cells	1
	• No cells	0

Result and Discussion

A comparison of the number of fibroblasts and collagen in the healing process of superficial dermal burns on day 5th, day 14th and day 21st between the 0.9% NaCl control group, the *Secretome Dental Pulp Stem Cell* and MEBO treatment groups is shown in Table 2.

The number of fibroblasts in the MEBO treatment group was higher than that of *Secretome* on day 5th, while on day 14th and day 21s, the number of fibroblasts in all groups produced the same score. Meanwhile, on days 5th and 14th, the amount of collagen calculated in the *Secretome* was much higher than in the MEBO group. This shows that the Secretome has better effectiveness in collagen formation, which can be seen starting from day 5th.

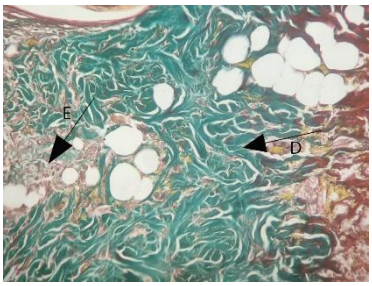
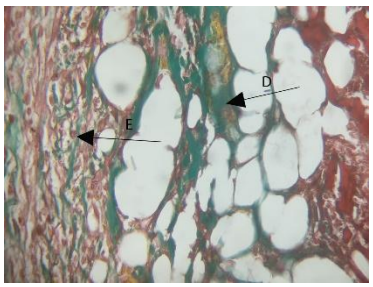
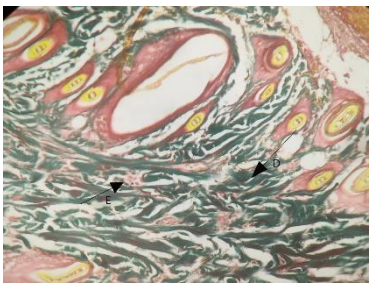
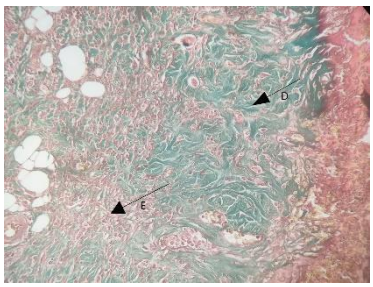
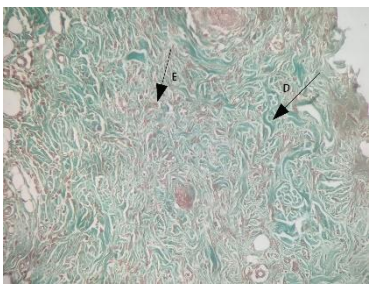
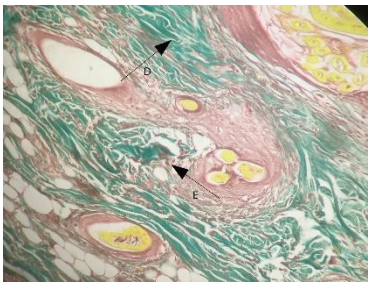
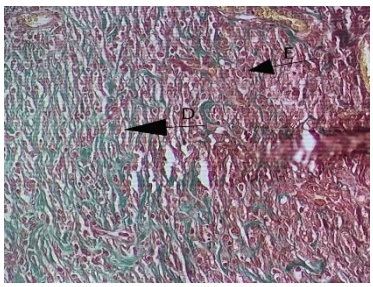
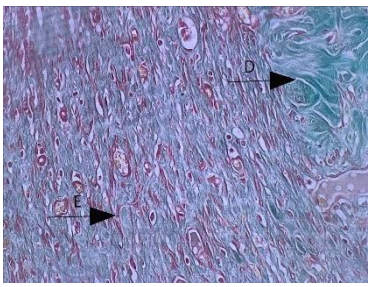
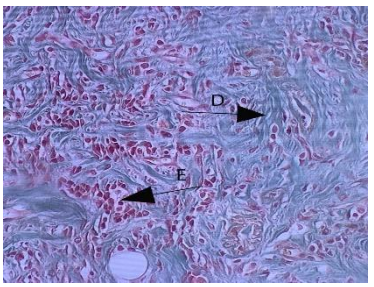
Table 2. Comparison of the Mean Number of Fibroblasts and Collagen in Each Group.

Variable	0,9 % NaCl (K1)	MEBO (K2)	DPSC Secretome (K3)	P Value
Days 5th				
Fibroblasts	2 ± 0	2 ± 0	1 ± 0	0,018
Collagen	1 ± 0	1 ± 0	2 ± 0	0,018
Days 14th				

Fibroblasts	3 ± 0	3,33 ± 0,577	3 ± 0	0,368
Collagen	1 ± 0	2 ± 0	2,67 ± 0,577	0,029
Days 21st				
Fibroblasts	3 ± 0	3 ± 0	3 ± 0	1.000
Collagen	2 ± 0	3 ± 0	3 ± 0	0,018

Kruskall–Wallis test sig. <0,05

Table 2. Histopathology images of all groups on the 5th, 14th, and 21st days

0,9 % NaCl (K1)	MEBO (K2)	DPSC Secretome (K3)
Days 5th		
		
Days 14th		
		
Days 21st		
		

Histopathological image of superficial dermal burns using *Messon Trichome* staining at 200x magnification. Image labels represent the presence of (D) collagen, (E) and fibroblasts.

On microscopic examination, it was seen that the DPSC secretome experienced collagen formation, which was the same as normal tissue on the 5th day, and the collagen formation was the same as normal tissue (Ahmed et al., 2016). This remained until the 14th day, the same as the MEBO group, and the collagen density was more than normal tissue on the 14th day. 21st in the DPSC and MEBO secretome groups**.** Our microscopic findings were corroborated by a prior study that found that Collagen expression increased in response to hGF and hGF-CM on day 7 compared with control groups. However, on day 14th, all treatments resulted in similar levels of collagen III expression (Ahangar et al., 2020). Regarding fibroblast formation, DPSC had more than 50 cells on day 14th, slower than the MEBO group, which had started to increase on day 5th. In vitro studies with Adipose Tissue Secretome (ASCs) showed higher concentration levels of fibroblast growth factor (FGF), which is known to be involved in wound healing and regeneration (Lombardi et al., 2019). Keratinocytes migrate during the proliferation phase to create a new epithelial layer to cover the wound. Epidermal growth factor (EGF), heparin-binding epidermal growth factor (HB-EGF), and transforming growth factor-α all directly activate this. In addition, fibroblasts play an important role in the synthesis of collagen, which supports vascular tissue (Soedjana, 2022).

Conclusion

This research demonstrated the therapeutic potential of DPSCs-secretome on superficial burns. DPSCs-secretome showed better performance on reducing wound area or increasing healing percentage during observation. Physiological observations and histopathological analysis with microscopic scoring methods revealed that DPSCs-secretome could increase the collagen density and fibroblasts, However, MEBO was faster in fibroblast formation than DPSC secretome on day 5^{th}.

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