LEMBAR PENGESAHAN

1.	Judul	: Difference Between Epithelial And Collagen In Second Degree Burns Between Human Cord Mesenchymal Stem Cell Extract And Silver Sulfadiazine In The Male White Rat (*Rattus Norvegicus*)
2.	Penulis	: **Dr. dr. Evi Kurniawaty, M.Sc.**, Efrida Warganegara, Ni Made Ariyuliami Savitri
3.	NIP	: 19760120 200312 2001
4.	Jabatan / Golongan	: Lektor/ III C
5.	Instansi	: Fakultas Kedokteran Universitas Lampung
6.	Publikasi	: Syntematic Riviews in Pharmacy Vol.11 issu 12 423-426 2020
7.	ISSN	: 0975-8453
8.	Website/Email	: http://www.sysrevpharm.org/
9.	Email	: evikurniawatyherlambang@yahoo.co.id

Bandar Lampung, 12 November 2020

Mengetahui,
Dekan FK Unila

Dr. Dyah Wulan SRW., SKM., M.Kes
NIP.197012082001121001

Penulis,

Dr. dr. Evi Kurniawaty, M.Sc.
NIP. 19760120 200312 2001

Menyetujui
Ketua Lembaga Penelitian dan Pengabdian
Universitas Lampung

Dr. Lusmeilia Afriani, D.E.A.
NIP.196505101993032008

Sys Rev Pharm 2020;11(12):423-426
A multifaceted review journal in the field of pharmacy

DIFFERENCE BETWEEN EPITHELIAL AND COLLAGEN IN SECOND DEGREE BURNS BETWEEN HUMAN CORD MESENCHYMAL STEM CELL EXTRACT AND SILVER SULFADIAZINE IN THE MALE WHITE RAT (*RATTUS NORVEGICUS*)

Evi Kurniawaty[a*], Efrida Warganegara[b], Ni Made Ariyuliami Savitri[c]

[a,b,c]Universitas Lampung
Corresponding author: evikurniawatyherlambang@yahoo.com[1*], Evikurniawaty@fk.unila.ac.id

Abstract

Purpose: Silver sulvadiazine is the gold standard in topical therapy for burn healing. Currently, other therapies have been developed to help the wound healing process, including using human umbilical cord mesenchymal stem cell extract because stem cells can accelerate the formation of epithelium and collagen, thereby accelerating the wound healing process.

Research Methodology: This study used 27 male white rats Sprague Dawley divided into 9 treatment groups, groups K4, K14, and K28 were control groups, groups SC4, SC14, and SC28 were groups that were given stem cell extract therapy, and groups SSD4, SSD14, and SSD28 were groups that were given silver sulvadiazine therapy. On days 4, 14, and 28 the rats were euthanized to take their skins and made preparations by staining hematoxylin-eosin and observed the epithelial and collagen formation at 40x magnification.

Results: Mean epithelialization score on day 28 K28: 5.33, SC: 7.67, SSD28: 8. Mean collagen score on day 14 K14: 6.67, SC14: 8.67, SSD14: 8. Mean collagen score on day 28 K28: 5, SC28: 4, SSD28: 3.67. There was a significant difference in epithelial thickness on day 28 and the amount of collagen on days 14 and 28

Keywords: burns, silver sulvadiazine, mesenchymal stem cells, epithelium, collagen

1. INTRODUCTION

Burns are tissue damage caused by contact with very high temperatures such as hot flames, chemicals, electricity, and radiation or contact with very low temperatures.Burns are 90% in countries with low income and minimal infrastructure to prevent burns. On year 2014, *World Health Organization* (WHO) estimates that more than 265,000 deaths occur annually due to burns and most of them occur in Africa, Southeast Asia and the Middle East. In Indonesia alone, burns are ranked 6th in accidental injury with a total of 0.7% of all injuries (Ministry of Health, 2013). Burns are a serious health problem, because not only cause local damage, but burns can cause systemic effects such as shock and can result *multi-system organ failure* (MOF) which requires intensive care.

2. LITERATURE REVIEW AND HYPOTHESIS DEVELOPMENT

The burn healing process is divided into 3 main phases that overlap one another, starting with an inflammatory phase which begins with an increase in blood vessel permeability and the migration of inflammatory cells, the proliferation phase is characterized by keratinocytes migrating to the wound area to assist tissue closure, phase maturation is the last phase starting from day 21 to about 1 year 3.

The skin is the main organ exposed to the outside world. The skin has functions in the form of protection, thermoregulation, metabolic, excretion, absorption and perception functions. Healing of the skin is important because when the skin loses its continuity, the skin's function cannot run as it should (Mescher, 2012). Hence, Healing wound burn need management and proper treatment so that the wound does not cause further damage.

One of the effective ways to treat burns is topical medication. *Silver sulfadiazine* (SSD) is the drug of first choice for the treatment of burns, SSD is a sulfonamide class of topical antibiotics that have properties *broad sprectrum* to prevent infection in the wound area. SSD is capable of producing a healing time of 8-15 days for *superficial burn* and 14-21 days for *deep dermal burn* 2 increases the thickness of the epidermis through accelerated epithelialization and increases migration of fibroblasts and keratinocytes thereby accelerating closure of the wound. (Lee *ea al.,* 2016).

Mesenchymal stem cells can be obtained from *wharton jelly* contained in the umbilical cord and blood in the placenta immediately after birth. The excess of these stem cells is a non-invasive removal procedure, does not use additional surgery and is taken from wasted tissue (Arno *et al.,* 2014). Experimental research on male white rats regarding the

administration of mesenchymal stem cell extracts of human umbilical cord to accelerate wound healing by Nur et al. showed significant results, in mice smeared topically with stem cell extracts occur acceleration time wound healing than the control group.

3. RESEARCH METHODOLOGY

This research is a laboratory experimental research with a research design *post test only control group design,* to determine the difference between epithelial and collagen in second degree burns between human umbilical cord mesenchymal stem cell extract and SSD in strained puith rats *Spragur-Dawley.*

This research was conducted from August 2017 to January 2018 at the Biomolecular Laboratory, Anatomical Pathology Laboratory, and *Animal House* University School of Medicine Lampung. The study population was male white rats *Spragur-Dawle* 23 months old and weighing about 250-300 grams. Samples were taken using the Federer formula with 3 Momenttis has beendeve loped samples per treatment group and 9 treatment groups, reatment using stem cells (*stem cells).* Stem cells are the latest medical technology in medicine, many scientists have researched the benefits of stem cell therapy, one of which is in skin healing therapy.

One type of stem cell could use in therapy healing of the skin are mesenchymal stem cells. Mesenchymal stem cells have a good ability to modulate the inflammatory response, accelerate remodeling of the extracellular matrix by stimulating increased production from collagen, so the total sample size was 27 rats, the sampling technique used was *simple random sampling.* The treatment group consisted of K4, K14 and K28 which became the control group without being given any therapy terminated on days 4, 14, and 28. Groups SC4, SC14, SC28 groups that given therapy topical using human umbilical cord mesenchymal stem cell extract terminated on the 4th da14, and 28. The SSD4, SSD14, and SSD28 groups are terminated on days 4, 14, and 28. Burns were made using coins with a weight of 5.34 grams, 1.83 mm thick, and 24 mm in diameter. The collagen thickness and amount of assessment are as follows:

Epithelial thickness assessment:

0: There is no epithelium with The metal was wrapped using gauze and soaked in boiling water at 98 ° C for 3 minutes and then attached to the skin of the rats, before it was shaved and anesthetized using ketamine 50mg / kg and xylazin 5mg / kg. After the burn is formed it is carried out *debridement* and rinse the wound

1: Very thin epithelium <30% normal skin epithelium

2: Thin epithelium ≥30% normal skin

3: Medium epithelium ≥60% normal skin epithelium

4: Good epithelium> 80% normal skin epithelium

Assessment of the amount of collagen: 0: No collagen formation 1: Very little collagen using distilled water.

On days 4, 14, and 28, rats were euthanized, and samples were sent to the Anatomical Pathology Laboratory and preparations were made. Use coloring *hematoxylin-eosin* and carried out an assessment 2: Little collagen, Medium collagen and Plenty of collagen.

4. RESULTS AND DISCUSSIONS

Following an overview epithelial and collagen differences: microscopic

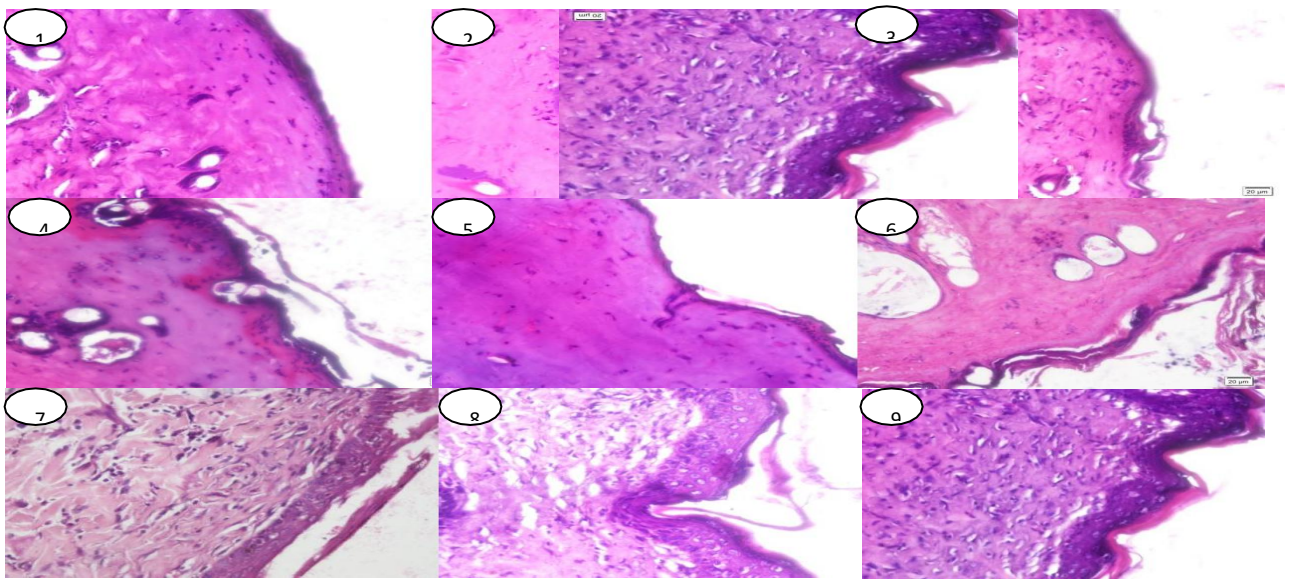

Figure 1. Epithelial and collagen microscopy
Description: 1. K4 2. SSD4 3. SC4 4. K14 5. SSD14 6. SC14 7. K28 8. SSD28 9. SC28

From the picture above, it is found that the day the epithelial thickness increases, on the 4th day the epithelial formation is still very thin in all treatment groups only getting a score of 1, as well as On the 14th day the average score obtained was 1 in the field of view, which was the presence of epithelium but the thickness was ≤30% normal epithelium, on the 28th day the thickest epithelium was in the SSD and extract group.

Stem cells with epithelial thickness category were ≥60% thickness of normal skin pithelium. The amount of collagen on day 4 and 14 has increased and on day 28 has decrease.

Table 1. Different is mean of collagen and biravariate analysis

Group	Days to 4	Days to 14	Days to 26
Control	4	.6.67	6
Stem Cell	5	8.67	4
SSD	4.67	8	3.67

P value	0.236*	0.005#	0.030*

In this research on formation analysis epithelium day to 4 not It was found that there was a significant difference in the mean epithelial formation between the 3 treatment groups. On the 4th day after the burn, it is an intermediate phase between the inflammatory phase and the proliferation phase. In this phase there is still release of inflammatory mediators in the burned skin so that epithelialization has not occurred properly. 3.

On the 14th day, the results of statistical analysis were not significant, but there were differences in the mean of the assessment parameters. On group SSD14 getting a mean of 2.00, the SC14 group got a mean of 2.00 and K14 got a mean of 1.67. On day 28, on day 28 there was a significant difference between groups K28 with SC28 and K28 with SSD28, in this group the mean score of the assessment parameters was 8 for the SSD28 group, 7.67 for the SC28 group, and in the K28 group got score of 5.33.

On statistical analysis *Mann Whitney* It was found that there was no significant difference between the SSD28 group and the SC28 group, but the mean value of the parameters was different. The group with the highest mean value after SSD on days 14 and 28 was the group given human umbilical cord mesenchymal stem cell extract, mesenchymal stem cells played a role in wound repair and healing by triggering differentiation, movement and paracrine secretion so that the proliferation of keratinocyte cells in the wound became faster and can accelerate the epithelialization process (Arno *et al.,* 2014; Lee *et al.,* 2016). When viewed macroscopic healing will appear wound burn that treated using cell extracts had a faster closure than the other groups. On the 28th day after euthanizing the rats, their hair was cut using a razor, it was a concern moment cutting fur causes epithelial damage so that the epithelialization score in the stem cell group is not optimal. In the control group, day 14 and 28 had the lowest mean values, the possibility of infection in the control group was because there was no treatment to prevent infection, thus prolonging the inflammatory process in the wound and causing epithelial formation and wound closure to be longer.b28 was the SSD group. SSD is the gold standard of topical therapy in burns. Eshafani *et al (* 2012) stated that SSD ointment has a positive effect on the proliferation phase so that SSD can accelerate the occurrence of epithelialization, besides because SSD is indeed a topical antibiotic (Setiabudy & Mariana, 2007) which prevents the occurrence of complications of burns, namely infections that can cause wounds to worsen.

Table 2. Differences in mean amount of collagen and bivariate analysis

Group	Day to 4	Day to 14	Day to 28
Control	4	6.67	5
Stem cell	5	8.67	4
SSD	4.67	8	3.67
P value	0.236*	0.005	0.000

On analysis difference average Collagen formation on the 4th day showed that there was no difference in the mean of collagen formation between groups, but when viewed from the parameter values there was a mean difference, namely the highest in the SC4 group, followed by SSD4 and the lowest in the K4 group. In contrast to analysis. on day 14, on day 14 there was a significant difference between groups K14 and SC14 and groups K14 with SSD14 but there was no significant difference between groups SC14 and groups SSD14.

The highest average collagen formation on day 4 and 14 was the group given human umbilical cord mesenchymal stem cell extract, Lee. *et al* stated that mesenchymal stem cells are able to stimulate an increase in fibroblast and collagen production which increases the repair of the extracellular matrix so that speed up the occurrence of wound healing. Fibroblasts Having an important role in the proliferation phase, fibroblasts produce an extracellular matrix which fills the wound cavity and prepares the basis for keratinocyte migration. Matrix extracellular will replaced by collagen type III which is also produced by fibroblasts in the proliferation phase.

The second highest average collagen formation is in the SSD4 and SSD14 groups, as has been explained in the discussion of SSD, SSD is the gold standard in topical treatment of burns, in addition because SSD is an antibiotic SSD can also stimulate the proliferation of fibroblasts which are the main source of formation. collagen (Esfahani *et al.,* 2012). The control group became the group with the lowest average collagen formation compared to stem cell extracts and SSDs, this could happen because the control group was not given any treatment so that the wound healing journey went on without stimulation from any drugs, unlike the stem cell and SSD groups.

On the 28th day of analysis, there was a significant difference in collagen between groups K28 and SC28 and groups K28 with SSD28, and there was no significant difference between groups SS28 and SSD28. There was a significant decrease in the amount of collagen on the 28th day.According to the normal course of wound healing, the 28th day is the journey of the wound to phase maturation, Where *matrix metalloproteinase (MMP*) produced by fibroblasts helps replace type III collagen with type I collagen. In the maturation phase there is a balance of synthesis and degradation. Collagen in excess amounts will be degraded by the collagenase enzyme and the end result of this maturation phase is scar tissue 13.

5. CONCLUSION

There is a difference in the mean epithelial thickness on day 28 and the difference in the amount of collagen on day 14 and 28 in second degree burns between administration of human umbilical cord mesenchymal stem cell extract with SSD.

LIMITATION AND STUDY FORWARD

It is hoped that this research will become the basis for further researchers regarding stem cell extract as another wound therapy

ACKNOWLEDGEMENT

The author thanks to Institute for Research and Community Service (LPPM) University of Lampung which had fully funded this research through DIPA BLU 2020.

REFERENCES

1. Arno AI, Amini-Nik S, Blit PH, et al. Human Wharton's jelly mesenchymal stem cells promote skin wound healing through paracrine signaling. *Stem Cell Res Ther*. 2014; 5 (1): 28-41.
2. Ashkani-Esfahani S, Faith MH, Lampung. 2017.

3. Djauhari T. Stem Cells. Biotechnology. 2010: 91-6.
4. Khoshneviszadeh M, et al. The healing effect of arnebia euchroma in second degree burn wounds in rat as an animal model. *Iran Red Crescent Med J.* 2012; 14 (2): 70-4.
5. Kim DW, Staples M, Shinozuka K, Pantcheva P, Kang SD, Borlongan C. Wharton's Jelly-Derived Mesenchymal Stem Cells: Phenotypic Characterization and Optimizing Their Therapeutic Potential for Clinical Applications. *Int J Mol Sci*. 2013; 14 (6): 11692-712. doi: 10.3390 / ijms140611692.
6. Lee DE, Ayoub N, Agrawal DK. Mesenchymal stem cells and cutaneous wound healing: novel methods to increase cell delivery and therapeutic efficacy. *Stem Cell Res Ther*. 2016; 7 (1): 37.
7. Moenadjat Y. Burns Problems and Management. Jakarta: FKUI. 2009.
8. Nur NN. The difference in incision wound healing in a manner macroscopic Among Topical administration of human umbilical cord mesenchymal stem cell extract with bioplacenton gel to male white rats *(Rattus norvegicus)* strains *Sprague Dawley [thesis].* Bandar lampung: University 2007.
9. Rowan MP, Cancio LC, Elster EA, et al. Burn wound healing and treatment: review and advancements. Crit Care. 2015; 19 (1): 1-12.
10. Setiabudy R, Mariana Y. Sulfonamides, cotrimoxazole, and urinary tract antiseptics. In: Gunawan SG, Setiabudy R, Nafrialdi, Elysabeth. *Pharmacology And Therapy*. Jakarta: FKUI Publishing Agency.
11. Tiwari V. Burn wound: howit differs from other wounds. *Indian J Plast Surg*. 2012; 45 (2): 364-373.
12. Velnar T, Bailey T, Smrkolj V. The Wound Healing Process: An Overview of the Cellular and Molecular Mechanisms. *J Int Med Res*. 2009; 37 (5): 1528-42
13. Vincy LAIW, Bachelor CM, Science B, Supervisor P, Zhang H. Comparison of MEBO with Silver Sulfadiazine (Ag-S) for the treatment deep injury. Chin *J Plast Burn Surg*. 2004.
14. VTIP Tanggo. Effect of topical administration of pomegranate peel extract on split thickness wound healing of rat skin [thesis]. Surabaya: Airlangga University. 2013.