ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Comparative Study of Effect of Silver Nitrate Gel and Differentiated Dermal Precursors (BMSCs) on Third Degree Burn in Wistar Rats

Balaji K*[1,2], Perumal Saraswathi[3], Prabhu K[1], Shila Samuel[4], Melani Rajendren[5], Siva T[6]

[1]Department of Anatomy, Sree Balaji Medical College, Chennai-600044, Tamil Nadu, India
[2]Department of Research, Bharath Institute of Higher Education and Research, Chennai-600073, Tamil Nadu, India
[3]Department of Anatomy, Tamil Nadu Dr. M.G.R Medical University, Guindy, Chennai-600032, Tamil Nadu, India
[4]Department of Biochemistry, VRR Institute of Biomedical science, Chennai-600056, Tamil Nadu, India
[5]Department of Anatomy, Madha Dental College and Hospital, Chennai-600069, Tamil Nadu, India
[6]Department of Anatomy, Sri Ramachandra Medical College & Research Institute, Porur, Chennai-600116, Tamil Nadu, India

Article History:

Received on: 12 Aug 2019
Revised on: 01 Nov 2019
Accepted on: 20 Nov 2019

Keywords:

Burn,
Bone marrow differentiated dermal precursors (DDP),
Silver nitrate gel

ABSTRACT

Skin is an ectodermal derivative that maintains internal homeostasis of the body. Any damage to the skin like burn injury internal homeostasis is lost, resulting in delayed healing. The aim is to study the histoarchitecture comparative effect of silver nitrate gel, and BMSCs (DDP) on third-degree burns in Wistar rats. A burn wound of size 2.5 cm (length) x 2.5 cm (breadth) x 6 mm (depth) was created using a preheated metal plate on flanks of Wistar rat. Every burn wound was treated with silver nitrate gel (commercially available as silverex), bone marrow differentiated dermal precursors, and monitored for 1, 7, 14, 21 days until wound healing. Wound surface area was measured and compared among groups with histological and gross observations. The healing time was faster in bone marrow differentiated dermal precursors (DDP) group compared to control. Prolonged silver nitrate gel usage heals burn wound with no infection, but silver toxicity was noted. Wound contraction is slower but steady using bone marrow differentiated dermal precursors (DDP) cell when compared to the group treated with silver nitrate gel. The data from this study help use to use bone marrow differentiated dermal precursors (DDP) cells as an alternate and effective way to treat burn wounds.

*Corresponding Author

Name: Balaji K
Phone:
Email: balajismch1@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i1.1906

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Burns are acquired tissue injuries when the skin is subjected to chemicals, radiation, hot liquids, hot solids, flames, etc. (Bosman, 2019). WHO states, burn injuries results in mortality and morbidity (Barillo *et al.*, 2019). Depending on severity of burns and thickness involved with epidermis, dermis, and hypodermis of skin, burns are divided into first degree – burn lesion restricted to epidermis of skin, second degree – burn involving epidermis and superficial dermis, third-degree – burns comprising dermis and subcutaneous layer of

skin (Tredget *et al.*, 2017). An autologous skin graft is used as a golden standard in the treatment of burn injuries (Varkey *et al.*, 2019). Wound healing initiates the process of inflammation, odema, hypertrophy of burn site, leaving behind scar tissues (Chiang *et al.*, 2016).

Silver cream on topical wound application is a beneficial antibacterial agent (Branski *et al.*, 2018) but maybe cytotoxic to target cells that it comes in contact. A stem cell has the remarkable potential to grow into many differentiated body cell types during early life and growth. They act as an internal repair system in many tissues, dividing essentially devoid of the limit to replenish other cells as long as person or animal live. Once the stem cell divides, every new cell remains as stem cell or become another cell type with a particular function such as cartilage, bone, muscle, nerve, and tooth.

This differentiation potential of stem cells has enumerable therapeutic applications so that it can be benefited by common people in trauma burns and old age patients. This study compares the effect of silver nitrate gel and DDP (BMSCs) cells on third-degree burn in Wistar rats.

MATERIALS AND METHODS

Animals

The test facility is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment, Forest and Climate Change, Animal Welfare Division, Govt. of India (No.1182/PO/Rc/S/08/CPCSEA). The IAEC approval number of this study is MTR/IAEC/PRU/013-15. Animals were housed individually with stainless steel grill cages and provided with standard rodent pellet feed and daily UV purified RO filtered water *ad libitum.* Sterilized clean paddy husk was taken as the bedding material. Room Temperature and relative humidity were maintained. The test room facility was provided with 12 hrs light and 12 hrs of dark conditions throughout the experiment.

Third-degree Burn Model

Rats were anesthethetized using a combination of 10 mg/kg xylazine and 80 mg/kg ketamine intraperitoneally with a total average volume of 1.5 ml/animal. Third-degree Burn wound model was made on a shaved area by keeping a heated metal plate of 300ºC for 10 min on the flank of each rat with dimension 2.5 cm (length) x 2.5 cm (breadth) x 6 mm (depth).

Procedures

The third-degree burn Model rats (18nos) were classified into three random groups containing 6 animals each. Group 1 – injured/ negative control, Group 2 treated with silver nitrate gel once daily for 21 days, Group 3 was treated once intralesionally, with 1 ml dermal precursors (DDP) from centre to periphery of the wound. No oral or parenteral antibiotics were directed to Wistar rats. Animals were weighed and observed for gross and histopathology observations.

Gross observation

Erythema, edema, and clinical signs was grossly observed under the burn model. The wound surface area was measured by radial planimetry (Foltynski *et al.*, 2015). Wound contraction was observed every day by tracing the wound margins on acetate film.

Wound contraction percentage is calculated using formula, initial wound area-specific day wound area / initial wound area x 100 (Demilew *et al.*, 2018).

Histopathology

At the end of the experiment, the third degree burn treated rats were given an overdose of Co2 anaesthesia. The treated area of wounded skin samples was taken from each group on day 7, 14, and 21. The Skin tissue measuring 5-6 μm thickness was collected and stored in neutral buffered formalin (10%) for the Histopathological process. Tissue, once embedded with wax, were subjected to microtomy and stained using hematoxylin and eosin dye for histological examination.

Statistics

The wound half-lives were compared using the Kruskal-Wallis one-way ANOVA and by Scheffe's technique for multiple comparisons. All analyses were accomplished with the help of a SPSS 15 on a personal computer. Using the simple linear regression analysis, absolute area measurements showed that the initial wound area created no remarkable difference to half-life calculations.

RESULTS AND DISCUSSION

There was no mortality/morbidity for all animals were observed throughout the experimental day. The bodyweight of the individual animal was recorded during randomization, anaesthesia, and necropsy. There was no substantial difference in the weight gain between all treatment and control groups. None of the animals showed erythema, edema, and were observed individually for any clinical signs/toxicity signs once daily throughout the experimental days. The burnt wound rats have been depicted in Figure 1.

Group 1- Control

Day 1 Day 7 Day 14 Day 21

Group2- Silver Nitrate

Day 1 Day 7 Day 14 Day 21

Group 3- DDP injected

Day 1 Day 7 Day 14 Day 21

Figure 1: Effect of silver nitrate and DDP in burnt wound healing rats

Wound healing measurement and closure of the wound

Healing measurement of an individual animal was measured and recorded before application on day 1 of treatment followed by day 7, 14 and 21 (Figure 2). A better wound healing pattern with wound closure was observed in treated rats with 21 days of treatment in all the group. The wound contraction and reduction in healing on different days. There was a remarkable reduction in the size of a wound from 7^{th} day in treated rats and also, the closure rate was much faster in group 3 (DDP cells), in contrast to group 2 (silver nitrate gel) and group 1(control group) in the later days.

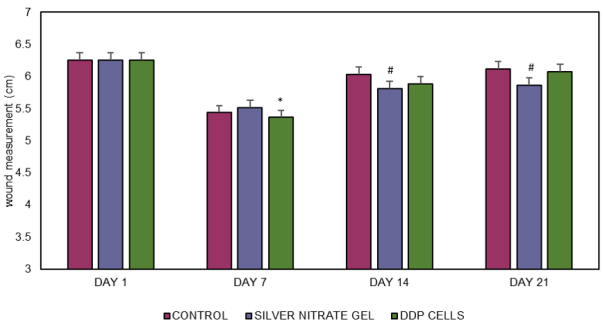

Figure 2: Wound measurement in DDP and silver nitrate gel treated rats. Results were expressed as Mean ± SEM. $^{*,\#}p<0.05$ statistically significant as compared with control rats

Histopathology

Figure 3 depicts an extensive loss of epidermis with large scab consisting of necrotic cellular debris in fibrin coagulum bordered with numerous dege erated and intact neutrophils, increased mast cells, and histiocytes with moderate fibroblasts in the hypodermis (Figure 3 A). Extensive necrosis of epidermis with fatty changes di-organization of collagen and loss of adnexa in the dermis ; large scab with necrotic debris a d fibrin coagulum, numerous neutrophils and bacterial clumps distorting the muscle and adipose tissue in the hypodermis; increased fibroblasts, neovessels, mast cells and histiocytes in

the hypodermis (Figure 3 B). Extensive loss of epidermis with a scab, increased fibroblasts, neovessels in the dermis with immature collagenisation, and a moderate number of neutrophils, mast cells, and histiocytes (Figure 3 C). Complete loss of epidermis and dermis and replacement with abundant scab tissue; numerous degenerated and intact neutrophils ; mild fibroblastic activity, increased mast cells, histiocytes, and neovascularization in the hypodermis (Figure 3 D).

Epidermis and dermis intact; reduction in the size of scab and inflammatory cells; intense fibroblastic activity with collagenisation and neovascularization; a few scattered mast cells and histiocytes in the hypodermis (Figure 3 E). Loss of epidermis and scab formation; intense fibroblastic activity and neovascularization with numerous mast cells, histiocytes, and a few scattered neutrophils in the dermis; mild collagenisation (Figure 3 F).

Reduction in scab in hypodermis; increased neutrophils and fibroblastic activity with neovascularization ; mild collagenisation (Figure 3 G). Reduction in scab in hypodermis increased neutrophils and fibroblastic activity with neovascularization ; mild collagenisation (Figure 3 H). Loss of epidermis with scab; partial mild re-epithelization; increased fibroblastic activity, neovascularization, and moderate collagenisation; a few scattered neutrophils and mast cells (Figure 3 I).

The epidermis of thin skin is exposed to moderate sunburn or ultraviolet light. It causes capillary, venules in the papillary and sub papillary region to dilate by making it turn red - (erythema). In severe burns, the capillaries and venules allow plasma to leak through them, resulting in accumulation of fluid between the epidermis and dermis, resulting in blisters. Under natural conditions, the epidermal cells surviving in the lower part of hair follicles regenerate over the blisters surface resulting in healing.

Acute wound fluids have a positive effect of the wound. This could be due to increased stem cells (Krzyszczyk *et al.*, 2018). If the burn injury involves the superficial part of the dermis, epithelial cells from a deep part of the hair follicle proliferate to grow along with the interface between living and the dead dermis. However, if the burn damage extends beyond the base of the hair follicle, regrowth of epidermis can occur only at the perimeter of the burns. Delayed healing in chronic wounds is due to stem cell deficiency (Ghieh *et al.*, 2015).

Healing of chronic burn wounds occurs so slowly that a skin graft is necessary. Autologous Skin graft transplanted from the different sites of the body is used as a golden standard in treating chronic burn injuries (Gardien *et al.*, 2016). Epidermis and superficial part of the dermis is transplanted. Skin grafts derive its vascularity and nutrients from tissue fluid exudate, where the skin graft is placed, resulting in a healing area covered with new skin. Slow regrowth of epidermis occurs at the periphery of the burn site. Systemic transplantation of dermis derived multipotent cells has no superior effect in the healing of irradiated wounds (Francis *et al.*, 2019). Mesenchymal stem cells were applied in addition to surgical flaps (Linard *et al.*, 2018). The bone marrow mesenchymal stem cells application reduces inflammation and healing time (Dehkordi *et al.*, 2019). BMSCs were used as an artificial dermal substitute to wound healing by increasing the rate of epithelisation and vascular density. After burns injury, healing of wound progress with haemostasis, inflammation, proliferation, and tissue remodelling.

In the present study, a third-degree burn model was created and was divided into group 1 – injured control, group 2 -treated with silver nitrate gel, and group 3- treated with bone marrow differentiated dermal precursors. Wound healing was noted on day 1, 7, 14, and 21 by gross and histological observations. Under gross observation, all animals were healthy, and none of them reported with erythema, edema, weight loss, morbidity, mortality, & clinical signs/toxicity signs.

Wound contracture was greater in group 2 treated with silver nitrate gel, followed by group 3 treated with bone marrow differentiated dermal precursors (DDP) when compared to the control group. This could be due to the daily application of silver nitrate gel. Whereas bone marrow differentiated dermal precursors (DDP) was injected once intralesionally from the periphery to central on third-degree burn wound. Under histological observations, day 7: group 1 showed extensive loss of epidermis with large scab consisting of necrotic cellular debris in fibrin coagulum bordered with numerous degenerated and intact neutrophils when compared to group 2 & 3. Stem cells injected to rectangular thermal burns with unburned interspaces did not necrose after 7 days, thereby preventing the progression of burn injuries. Day 14: histological observations of group 1 shows epidermal necrosis with collagen disorganization and loss of adnexa in the dermis with bacterial clumps distorting the muscle and adipose tissue in the dermis. Group 2 shows a reduction in the size of scab and inflammatory cells. This could be due to the potent antimicrobial and anti-inflammatory properties of silver (Burdușel *et al.*, 2018).

Argyria, a perpetual disorder caused by the deposi-

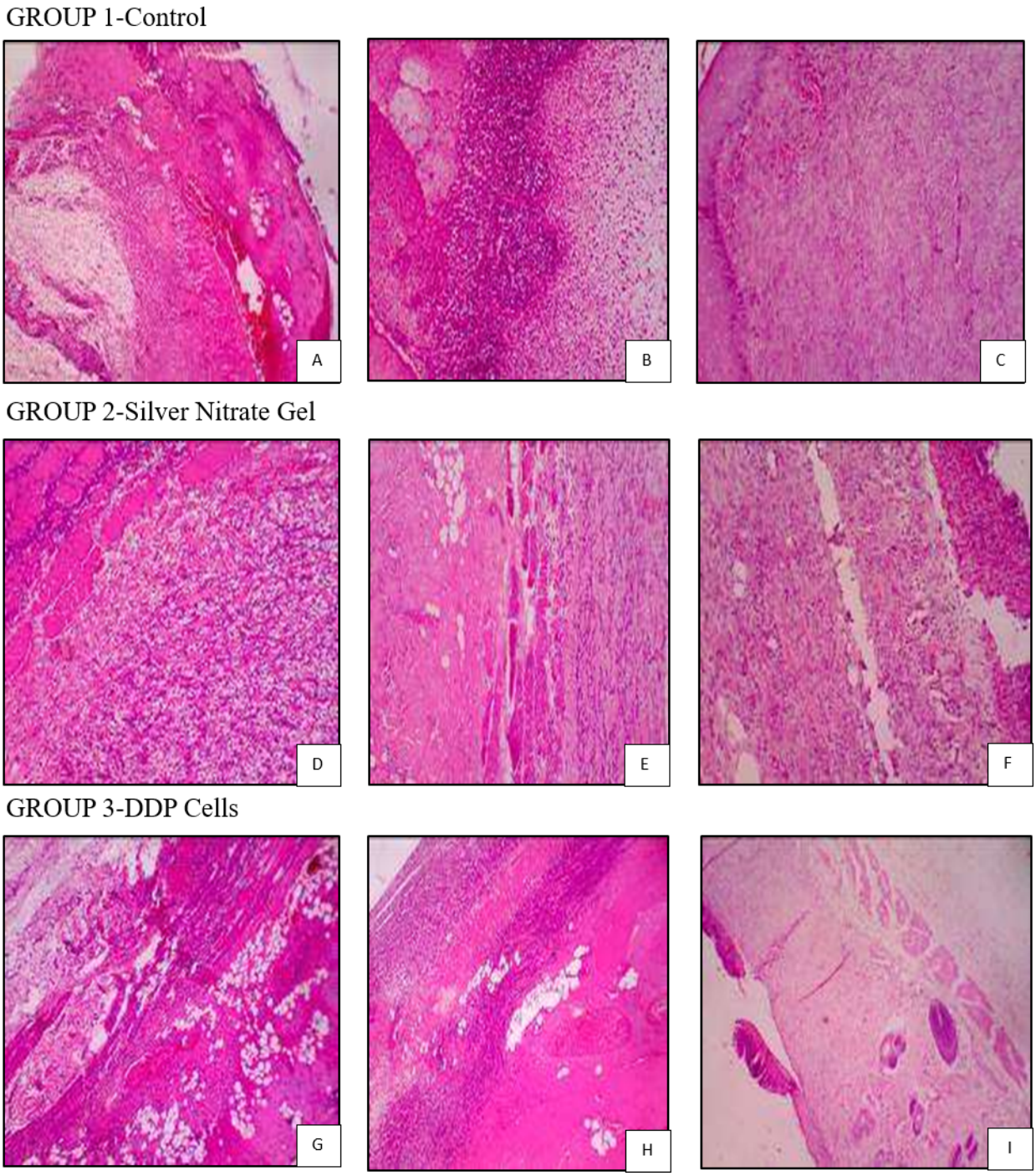

Figure 3: Histopathology results of wound healing control and treated rats (magnification at 20x)

tion of silver in the skin's micro-vessels in people who are exposed to chronic silver toxicity (Maghsoudi *et al.*, 2011). Injection of stem cells into rat accelerates wound healing by decreasing the count of inflammatory cells, down regulating interleukins 1 & 6, increased level of interleukin 10, and TSG-6 (Qi *et al.*, 2014). Day 7: Group 3 showed a reduction in scab in the hypodermis, increased neutrophils, and fibroblastic activity with neovascularisation and mild collagenisation. Day 14: group 3 showed partial mild re-epithelisation; this could be due to the proliferation of stem cells to keratinocytes, thereby promoting re-epithelisation and wound healing (Yang *et al.*, 2019). epithelial cells on wound margin move to seal the entire wound by process of "epiboly". Day 14 of group 3 showed increased fibroblastic activity. Bone marrow cells exhibit fibroblast-like morphology. Fibroblast moves to the injured target tissue to secrete collagen matrix. Collagen provides strength

and stability to underlying tissue in order to retain structural and functional anatomy. There exists a homeostasis between normal and abnormal collagen deposition.

Group 3 showed mild to moderate collagenisation from day 7, 14, and 21 days. Fibroblast adhere to collagen and elastin fibre which they laid down. Bone marrow differentiated dermal precursors are a principal source of dermal fibroblast. Dermal fibroblast secretes type 1 collagen (Tracy *et al.*, 2016). Fibroblast particularly active during wound repair, multiplying, and laying down a fibrous matrix which becomes invaded by numerous blood vessels (Caballé-Serrano *et al.*, 2019). Neovascularisation was observed in group 3 & group 2 on 7, 14, and 21^{st} day. This is to attain Cell nourishment. The nutrients to the building block are transferred by the process of "diffusion". Mesenchymal stem cells injected in the burn-injured mouse model showed faster healing and denser collagen (Revilla *et al.*, 2016). Mesenchymal stem cell medium is chemo attractant invitro, thereby recruiting specific cells like epidermal keratinocytes and dermal fibroblast to wound via Mesenchymal stem cell paracrine signalling. This mechanism of paracrine signalling favours angiogenesis and tissue remodelling such as fibroblast proliferation and epithelisation in all phases of wound repair (Lee *et al.*, 2016). BMSCs differentiated into dermal cells when injected into the targeted dermal injury progresses wound healing by maintaining tissue homeostasis, inflammation, proliferation, and tissue remodelling. BMSCs differentiated dermal fastens healing time, so as to reject and overcome graft and minor surgeries.

CONCLUSIONS

Culturing DDP cells ex-vivo is a long process which needs a GMP environment. DDP cells are expensive and cannot be afford by common people. Bone marrow differentiated dermal precursors fulfill the DDP functions by accelerating wound closure with increased epithelisation, granulation tissue formation, and angiogenesis. The BMSCs differentiated dermal precursors (DDP) cells showed faster tissue healing than the control group. Continuous silver nitrate gel usage causes silver toxicity. Intralesional administration of DDP cells once from inside out of lesion showed steady wound healing when compared with control groups. However, healing is incomplete and required some more cells for it to be complete and replace tissue damage. Therapeutic administration of BMSCs differentiated dermal precursors (DDP) on third-degree burns will replace damaged skin and can be used as an alternate therapeutic option in treating dermal injuries with reduced treatment duration.

REFERENCES

Barillo, D. J., Rizzo, J. A., Broger, K. P. 2019. Burn Casualties. *Aeromedical Evacuation*, pages 265–288.

Bosman, R. 2019. Burn wound challenges: wound care. *The Specialist Forum*, 19:30–34.

Branski, L. K., Herndon, D. N., Barrow, R. E. 2018. A Brief History of Acute Burn Care Management. *Total Burn Care*, pages 1–7.

Burdușel, A. C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A., Andronescu, E. 2018. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. *Nanomaterials*, 8(9):681–681.

Caballé-Serrano, J., Zhang, S., Ferrantino, L., Simion, M., Chappuis, V., Bosshardt, D. D. 2019. Tissue Response to a Porous Collagen Matrix Used for Soft Tissue Augmentation. *Materials*, 12.

Chiang, R. S., Borovikova, A. A., King, K., Banyard, D. A., Lalezari, S., Toranto, J. D., Widgerow, A. D. 2016. Current concepts related to hypertrophic scarring in burn injuries. *Wound Repair and Regeneration*, 24(3):466–477.

Dehkordi, A. N., Babaheydari, F. M., Chehelgerdi, M., Dehkordi, S. 2019. Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. *Stem Cell Research & Therapy*, 10(1):111–111.

Demilew, W., Adinew, G. M., Asrade, S. 2018. Evaluation of the Wound Healing Activity of the Crude Extract of Leaves of Acanthus polystachyus Delile (Acanthaceae). Evidence-Based Complementary and Alternative Medicine. pages 1–9.

Foltynski, P., Ladyzynski, P., Ciechanowska, A., Migalska-Musial, K., Judzewicz, G., Sabalinska, S. 2015. Wound Area Measurement with Digital Planimetry: Improved Accuracy and Precision with Calibration Based on 2 Rulers. *PLOS ONE*, 10(8).

Francis, E., Kearney, L., Clover, J. 2019. The effects of stem cells on burn wounds: a review. *International Journal of Burns and Trauma*, 9(1):1–12.

Gardien, K. L. M., Marck, R. E., Bloemen, M. C. T., Waaijman, T., Gibbs, S., Ulrich, M. M. W., Middelkoop, E. 2016. Outcome of Burns Treated with Autologous Cultured Proliferating Epidermal Cells: A Prospective Randomized Multicenter Intrapatient Comparative Trial. *Cell Transplantation*, 25(3):437–448.

Ghieh, F., Jurjus, R., Ibrahim, A., Geagea, A. G., Daouk, H., Baba, B. E., Jurjus, A. 2015. The Use of Stem Cells in Burn Wound Healing: A Review. *BioMed Research International*, pages 1–9.

Krzyszczyk, P., Schloss, R., Palmer, A., Berthiaume, F. 2018. The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes. *Frontiers in Physiology*, 9.

Lee, D. E., Ayoub, N., Agrawal, D. K. 2016. Mesenchymal stem cells and cutaneous wound healing: novel methods to increase cell delivery and therapeutic efficacy. *Stem Cell Research & Therapy*, 7(1).

Linard, C., Brachet, M., Strup-Perrot, C., L'homme, B., Busson, E., Squiban, C., Benderitter, M. 2018. Autologous Bone Marrow Mesenchymal Stem Cells Improve the Quality and Stability of Vascularized Flap Surgery of Irradiated Skin in Pigs. *STEM CELLS Translational Medicine*, 7(8):569–582.

Maghsoudi, H., Monshizadeh, S., Mesgari, M. 2011. A Comparative Study of the Burn Wound Healing Properties of Saline-Soaked Dressing and Silver Sulfadiazine in Rats. *Indian Journal of Surgery*, 73(1):24–27.

Qi, Y., Jiang, D., Sindrilaru, A., Stegemann, A., Schatz, S., Treiber, N., Scharffetter-Kochanek, K. 2014. TSG-6 Released from Intradermally Injected Mesenchymal Stem Cells Accelerates Wound Healing and Reduces Tissue Fibrosis in Murine Full-Thickness Skin Wounds. *Journal of Investigative Dermatology*, 134(2):526–537.

Revilla, G., Darwin, E., Yanwirasti, Rantam, F. A. 2016. Effect of Allogeneic Bone Marrow-mesenchymal Stem Cells (BM-MSCs) to Accelerate Burn Healing of Rat on the Expression of Collagen Type I and Integrin $\alpha 2\beta 1$. *Pakistan Journal of Biological Sciences*, 19(8):345–351.

Tracy, L. E., Minasian, R. A., Caterson, E. J. 2016. Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound. *Advances in Wound Care*, 5(3):119–136.

Tredget, E. E., Shupp, J. W., Schneider, J. C. 2017. Scar Management Following Burn Injury. *Journal of Burn Care & Research*, 38(3):146–147.

Varkey, M., Visscher, D. O., Van Zuijlen, P. P. M., Atala, A., Yoo, J. J. 2019. Skin bioprinting: the future of burn wound reconstruction? Burns & Trauma. 7.

Yang, R., Liu, F., Wang, J., Chen, X., Xie, J., Xiong, K. 2019. Epidermal stem cells in wound healing and their clinical applications. *Stem Cell Research & Therapy*, 10(1):229–229.

