Scientific Works. Series C. Veterinary Medicine. Vol. LXVIII (1), 2022
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

THE RAT AS AN ANIMAL MODEL FOR THE EVALUATION OF THE CUTANEOUS WOUND HEALING

Fabiola IONIȚĂ[1, 2], Cristin COMAN[1, 3, 4], Mario CODREANU[2]

[1]Cantacuzino National Medico-Military Institute for Research and Development, Bucharest Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independenței, Bucharest, Romania
[3]Fundeni Clinical Institute, Translational Medicine Centre of Excellence, 258 Fundeni Road, Bucharest, Romania
[4]Spiru Haret University, Faculty of Veterinary Medicine, 256 Basarabia Blvd, Bucharest, Romania

Corresponding author email: comancristin@yahoo.com

Abstract

The healing of cutaneous wounds is a dynamic process including overlapping phases of inflammation, proliferation, re-epithelialization and remodeling. Proper wound healing is essential for the reestablishment of structural and functional integrity of the damaged tissue. Rodents are valuable biological tools for understanding tissue repair process and for developing effective treatment strategies, despite anatomical and physiological differences between human and animal skin. The purpose of the study is to evaluate the cutaneous wound healing assessment for an excisional wound model in rats, for further testing with innovative medical devices loaded with biological active compounds. CD-SD female rats were surgically operated to excise one full-thickness circular skin patch, 20 mm diameter, in the dorsal region. Patches applied were changed every other day and samples of wounds/scars were collected on the 7th and 14th postoperative days. Macroscopic monitoring and histopathological examination assessed the wound healing process over time. Results showed that rats provide an optimal animal model for cutaneous wound healing, as data obtained can provide valuable translational information and can contribute in optimizing treatment protocols.

Key words: *wound healing, excision model, animal model, rat.*

INTRODUCTION

Skin, the largest organ of the human body, plays a crucial role in the protection against microorganisms, vitamin D synthesis initialization, excretion, thermal regulation and detection of sensory information about the external environment (Abdallah et al., 2017; Tottoli et al., 2020; Reinke & Sorg, 2012).

A wound is a breakdown in the protective function of the skin, a disruption of cellular, anatomical, and functional continuity of the cutaneous tissue. A wound may be described by its aetiology, anatomical location, whether it is acute or chronic, by the method of closure and by the appearance of the predominant tissue types in the wound bed. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue (Negut et al., 2018; Thakur et al., 2011; Wilhelm et al., 2016).

Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues (Gurtner et al., 2008). Wound healing involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines (Gonzales et al., 2016; Velnar et al., 2009). The healing process consists of a sequence of overlapping events including inflammatory responses, regeneration of the epidermis, shrinkage of the wound and finally connective tissue formation and remodeling (Alizadeh et al., 2007; Hasamnis et al., 2010).

Wound healing models have been developed over many decades in attempt to understand the tissue repair process and test new treatment protocols (Masson-Meyers et al., 2020). Although *in vitro* models have been important in underlying the mechanisms of this wound

repair process, *in vivo* models remain the most predictive models, allowing to obtain information on the multifactorial nature of the wound healing process, which may be influenced by external factors (Dorset-Martin 2004; Gottrup et al., 2000; Wong et al., 2011).
The advantage of using animal models is that the wound healing process is accelerated in animals and it is possible to study the process over days rather than longer periods of time needed in humans (Chang et al., 2019; Mogford, 2001). Currently used animal models for wound healing research are: rodents (mouse, rat), rabbit and pig.
Rats have been widely used in the study of skin wound healing by allowing the standardization of the type, size, shape, and depth of the wound injury (Dorset-Martin 2004). This particular animal species is often selected for its wide availability, tractable nature and cheapest cost in terms of housing, maintenance, and reproduction. Also, a wide variety of specific reagents are available for research purposes. Despite of their small size, rats are large enough to provide a suitable skin area for wound healing studies. (Grada et al., 2018; Masson-Meyers et al., 2020).
The purpose of the study was to evaluate the wound healing assessment for an excisional wound model in CD-SD female rats. This experiment is part of a study that will evaluate the *in vivo* healing potential of an innovative medical device loaded with biological active compounds. The following aspects were assessed during the experiment: clinical examination and general appearance, macroscopic wound monitoring and histopathological examination of samples collected at the end of the study. Blood samples were collected for hematological analysis.
Results showed that rats provide an optimal animal model for wound healing, as data obtained can provide information for a better understanding of the benefits and limitations of this model in translational applications.

MATERIALS AND METHODS

This study was carried out in compliance with the principles of ethics and in accordance with the provisions of EU Directive 63/2010 on compliance with the rules for the care, use and protection of animals used for scientific purposes.
This study was approved by the Ethics Committee of Cantacuzino National Medical-Military Development Research Institute and approved by the competent authority. The animals were provided by Băneasa SFP (Specific Pathogen Free) Animal Facility area for rats and mice of Cantacuzino National Medical-Military Development Research Institute, Bucharest.
All aspects related to animal housing and care were undertaken in accordance with the national and international regulations concerning animal testing. The food and the water were administered *ad libitum* during the entire experiment period The animals were kept under standard conditions, temperature 18-24°C, humidity 35-75% and in light controlled conditions (12 h/12 h light and dark cycles). During the study, the animals were housed into individual cages.
For this study, 20 CD-SD female rats, weighing 200-300 g, 12 weeks age were surgically operated to excise one full‑thickness skin patch, in the dorsal region. The animals were anesthetized by intraperitoneal injection of a cocktail of medetomidine (0.5 mg/kg; Biotur) and ketamine (75 mg/kg; Farmavet). The back of the animals was shaved and the selected area was disinfected using 70% ethanol and 3% betadine solution. The animal was placed on the lateral side and one circular full-thickness wound (20 mm in diameter) was made on the dorsum cervical region, using a sterile straight surgical scissors, a tissue forceps and a scalpel blade (no. 24). Bleeding was controlled with gauze compresses until hemostasis. Each wound was covered with an untreated textile patch (25 x 25 mm) and then covered with sterile gauze and flexible, self-adhesive bandage (Petflex). Patches were changed every other day after hydration with 0.9% saline solution (Figures 1 and 2).
Animals were randomly divided in 2 groups (n = 10 in each group), according to the moment of tissue sample collection. Half of the animals were euthanized using an anesthetic overdose on the 7th post-operative day and the other half of the animals on the 14th post-operative day.
Clinical examination of the animals was performed daily and wounds were measured using a vernier caliper (length and width). Wounds measurements, macroscopic descrip-

tion of the lesions and evaluation of the healing process were performed every 48 h. For reproducibility, the measurement of the wound area was performed by a single observer throughout the experimental time.

Figure 1- Surgical instruments used for skin patch excision and wound measurement

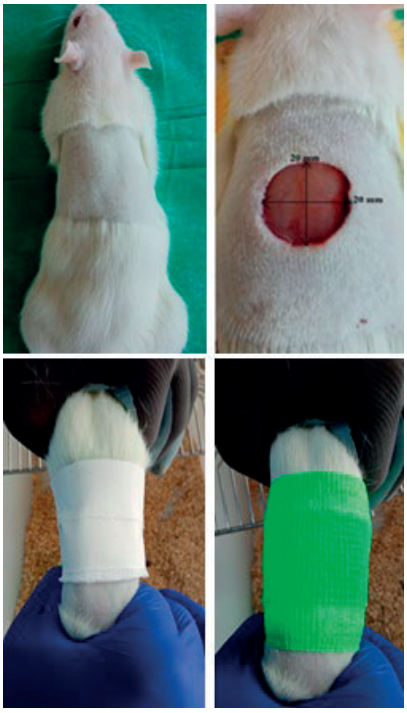

Figure 2- Surgical excision and wound coverage

At the end of the study, blood samples for hematological tests were collected from the retro-orbital sinus. For hematological tests, blood was sampled in EDTA pre-conditioned tubes and IDEXX ProCyte Dx 5 Diff analyzer was used.

Tissue specimens were obtained from the wound site by sharp dissection using the same instruments (surgical scissors and scalpel blade), on the 7th and 14 th post-operative days and histopathological examination was performed.

The full thickness wound tissues, including the adjacent skin, were fixed immediately in formalin and paraffin embedded for routine histological processing. A 4 μm section obtained from each paraffin block was stained with hematoxylin and eosin (H&E) and evaluated using a light microscope with specific image analysis software (Olympus SC 50).

RESULTS AND DISCUSSIONS

All the animals showed good general health condition throughout the study, as assessed by their weight gain, food consumption and mobility. Temporarily, some of the animals presented pruritus on the dorsal region, but without interfering with the wound healing process. No signs of intercurrences in wound healing, such as edema, erythema or suppuration in the wound area was observed during the experiment.

The average wounds area (mm^2) was calculated every 48/72 h by measuring the two dimensions (length x width). By day 7, the average wound area for group 1 had been reduced from 400.58 mm^2 to 170 mm^2, representing 42.43% from the initial excisional wound. By day 14, the average wound area for group 2 had been reduced from 400.82 mm^2 to 69.36 mm^2, representing 17.30 % from the initial wound. The measurement results are presented in Tables 1 and 2 and graphically represented in Figure 3. Macroscopic images of the wound-healing process over time are presented in Figure 4 (group1) and Figure 5 (group 2).

Table 1- Wounds area during the study for group 1 (mm^2)

Animal ID	Day 0	Day 2	Day 4	Day 7
1	400	360,99	239,94	164,64
2	403,6	384	285,65	171,2
3	400	364,7	255,76	166,44
4	400	362	269,01	195,3
5	400,8	354,9	277,24	149,03
6	400,2	355,12	251,12	219,45
7	400,6	361	264	163,8
8	400	299,28	159,6	129,36
9	400	329,42	225	142,74
10	400,6	370,54	250,56	198,12
Average	400,58	354,195	247,788	170,008
STDEV	1,04861	22,4723	33,844	26,0612

Table 2- Wounds area during the study for group 2 (mm^2)

Animal ID	Day 0	Day 2	Day 4
1	403,8	399,96	361,6
2	401,2	380,79	305,04
3	400,8	362,34	267
4	400	387,93	327,69
5	400,6	356,57	294,84
6	400,4	382	306,55
7	400,4	341,9	241,08
8	400	362,18	363,3
9	400,4	372,37	304
10	400,6	384,07	342,09
Average	400,82	373,01	311,31
STDEV	1,0486	16,377	37,054
Animal ID	**Day 7**	**Day 11**	**Day 14**
1	283,24	248,89	106,02
2	236,88	147,6	69
3	191,54	98,4	27,36
4	149,16	100	50,3
5	202,94	105,3	78,1
6	306,44	121,18	57,53
7	237,8	167,99	95,16
8	354,65	175,44	69,93
9	348,52	163,52	78,96
10	303,4	136,8	61,32
Average	231,1	146,512	69,368
STDEV	65,914	43,568	21,237

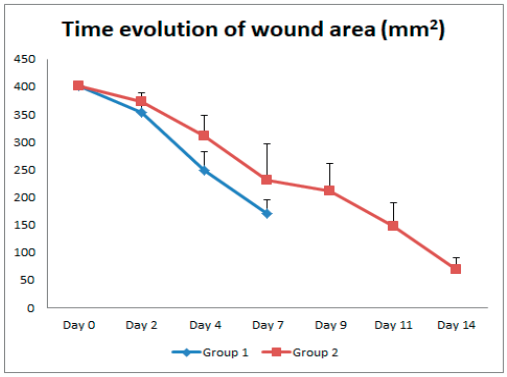

Figure 3 - Time evolution of wounds area for each group

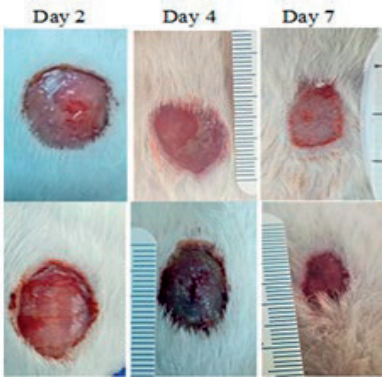

Figure 4 - Macroscopic images of wound-healing process on 2nd, 4th and 7th post-operative days (group 1)

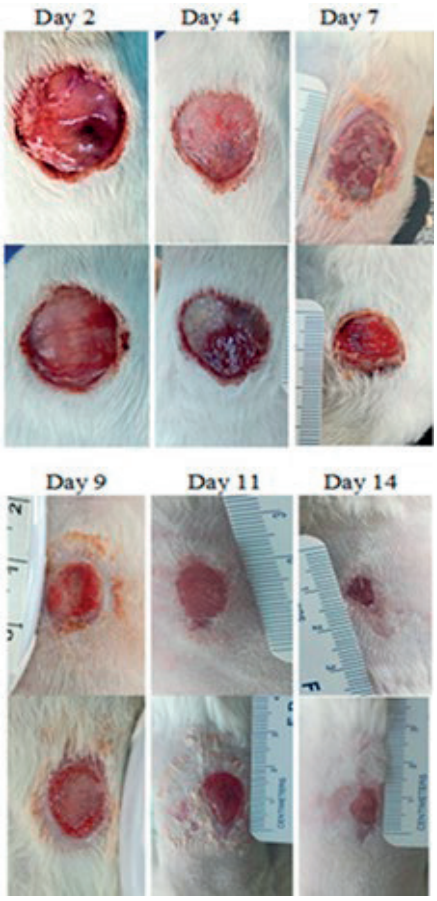

Figure 5- Macroscopic images of the wound-healing process on 2nd, 4th, 7th, 9th, 11th and 14th post-operatives days (group 2)

Hematological analyzes performed at the final day did not reveal significant differences between animals euthanized on 7th post-operative day and the ones on 14th day, for none of the determined cell lines (erythrocyte/leukocyte/platelet). Hematological inflammation markers (total WBC count, lymphocyte/granulocyte count) registered low degree variations, with values within the normal reference range and were not influenced by the wounds area.

Histopathological evaluation of tissues samples of wounds/scars collected at the end of the experiment included: bridging of cells and keratinization, inflammatory cells, neoangiogenesis, proliferation of fibroblasts and neocollagenesis. Histopathology aspects are presented in Figures 6 and 7.

Day 7

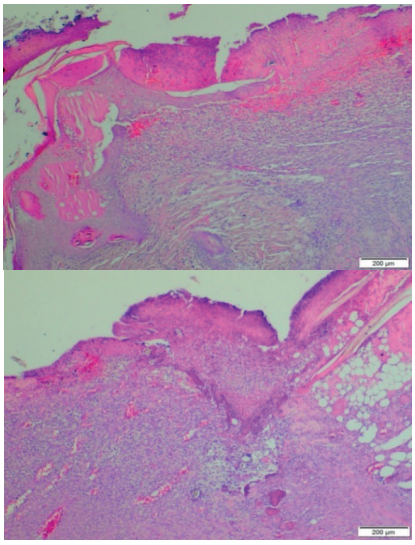

Figure 6 - Histopathology of tissue samples at 7th post-operative day, stained with H&E, 4X (Olympus SC 50) Early epithelialization with granulation tissue extending from the surface of the defect to the hypodermis and skeletal muscle layer; Abundance of polymorphonuclear and mononuclear inflammatory cells; Perivascular inflammatory cells and mast cells; Frequent fibroblasts and thin collagen fibers; Activation of local microvascular endothelial cells lining the inner surface of blood vessels (neoangiogenesis)

Day 14

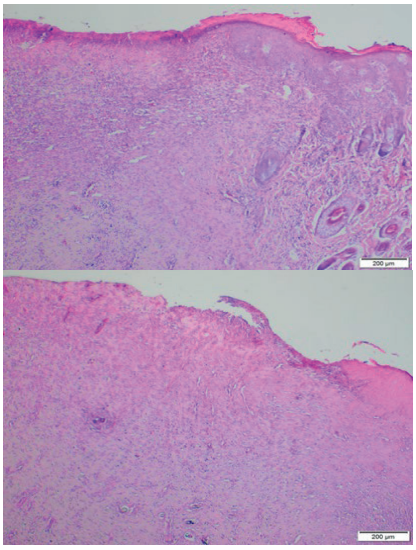

Figure 7- Histopathology of tissue samples at 14th post-operative day, stained with H&E, 4X (Olympus SC 50) Epidermal hyperplasia with parakeratosis, low granulation tissue, the defect being largely covered by epithelialization; moderate to low number of mononuclear inflammatory cells; dense collagen fibers, firmly orientated; Vertical orientation of the blood vessels in the granulation tissue with discreet angiogenesis

The purpose of this study was to establish a reproducible, predictable and quantifiable rat model of excisional wound healing, for further wound healing medical device testing.

Wound healing process was assessed by the measurements made during the experiment and histopathologic analysis in both time points (days 7th and 14th).

The gradual maturation of the granulation tissue and subsequent transformation into the primary fibrous scar is considered one of the important morphological features of wound healing progression. Neovascularization is critical for efficient wound healing, since it is required for the delivery of nutrients and maintenance of oxygen homeostasis, to allow cellular proliferation and tissue regeneration to occur (Pastar et al., 2021).

Murthy et al., (2013) created an excisional wound model by surgically removing a full-thickness skin area of 500 mm2 and assessed the wound healing process. The rate of wound healing in control rats was 21.6% to 68.3% from day 4 to day 12 and 80.6% to 98.1% from day 14 to day 20, while complete epithelialization and healing were observed on day 24.

Caetano et al. (2014) tested the efficacy of wound healing materials by performing two circular full-thickness wounds on rats dorsal region by using 15mm diameter punch and collected tissue samples on days 2,7,14 and 21. Macroscopic and histological analysis showed similar results to this study, most wounds being completely healed from the 14th day on.

Santos et al. (2021) performed 8 mm wounds on the back of Wistar rats for the wound healing assay. On day 7, granulation involved the full-thickness of dermal tissue and re-epithelialization was limited to the marginal area of the wound. On day 14, a remarkable increase in collagenesis, as well as reduction of the interfibrillary spaces was observed, with most of the defect being fully re-epithelialized.

Rat cutaneous wound healing does not perfectly mimic human skin wound healing because the skin morphology is different (Abdullahi et al., 2014, Petersen et al., 2016).) Rat skin is unique in having a subcutaneous panniculus carnosus layer (a thin muscle layer between the subcutaneous fat and dermal layer), that facilitates skin healing by both wound contraction and collagen formation

(Davidson & Opalenik, 2013). Consequently, wound contraction, which is usually more rapid than epithelialization, causes a decrease in the overall healing time of rat wounds (Chang et al., 2019; Masson-Meyers et al., 2020; Wong et al., 2011). In contrast, human wounds heal by re-epithelialization and granulation tissue formation, important differences to consider when assessing the translational relevance of rodent studies (Rouselle et al. 2018). The inherent differences between human and rat skin should be considered in determining whether rats are appropriate in wound-healing models. Rats have been classified as "loose-skinned animals", primarily because of their skin's elasticity and its lack of a strong adherence to the underlying structures compared to humans (Abdullahi et al., 2014). Efforts have been made to create modified models, where contraction is retarded to more closely mimic the physiology of human wound healing (Sharpe & Martin, 2013). Grada et al. (2018) discusses the limitations in using rats as a model due to contraction wound healing mechanism and mentions the use of splinting technique in order to avoid healing primarily via contraction. Son DO & Hinz, (2021) also describes a procedure to splint the edges of full-thickness rodent skin with a sutured plastic frame to prevent wound closure by granulation tissue contraction. Therefore, the wound will heal through granulation tissue formation and re-epithelialization, similar to the process in humans.

The diferences between human and rat skin are also present internally, as rats possess the enzyme l-gluconolactone that converts l-gluconogammalactone to vitamin C, therefore rats do not require diets with added vitamin C. This is particularly relevant in wound healing as vitamin C plays a vital role in collagen synthesis (DePhillipo et al., 2018). Main characteristics of human and rat skin are presented in Table 3.

Developing an animal model that can mimic the complexity of human healing process may seem an unattainable goal, because non-healing and delayed healing wounds in humans are often the result of a combination of external factors (Davidson & Opalenik, 2013; Dorset-Martin, 2004). Despite their limitations, rats are often selected for their availability, easy manipulation, low cost, and small size, as well as defined genetic backgrounds. Rats are large enough to provide a suitable area of skin for studies which require larger or more numerous wounds per animal (Grada et al., 2018).)

Table 3- Characteristics of human and rat skin

Trait	Human	Rat
Epidermis	Thick	Thin
Dermis Thick	Thick	Thin
Skin adherence	Tight	Loose
Panniculus carnosus	Absent	Present
Hait coat	Sparse	Dense
Hair growth	Mosaic	Patches
Vitamin C source	Exogenous	Endogenous
Keloid/ hypertrophic scar	Possible	No
Wound Healing Mechanism	Re-epithelialization	Contraction

Excisional wounds are the most commonly used wound healing models, generated by the surgical removal of all skin layers. Excisional models commonly use the rat's dorsum as the wound location as dorsal sites tend to be more effective in keeping the animal from reaching and manipulating the wound. This model allows the investigation of inflammation, granulation tissue formation, re-epithelialization, angiogenesis and remodeling tissue. (Masson-Meyers et al., 2020; Dorset-Martin, 2004).

CONCLUSIONS

Rats provide a valuable animal model for cutaneous wound healing and further research will improve wound assessment methods to provide a better understanding of the benefits and limitations of this model in translational applications.

ACNOWLEDGEMENTS

We would like to thank veterinarians Diana Ancuța and Vladimir Suhăianu for all the help provided with animal procedures and biochemist Petronica Gheorghiu for performing hematological tests.

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work. Authors contributed equally to this work.

All authors have read and approved the final manuscript.

Funding: Project PN-III-P1-1.2-PCCDI2017-0728 (TERAMED)

REFERENCES

Abdallah, F., Mijouin, L., & Pichon, C. (2017). Skin Immune Landscape: Inside and Outside the Organism. *Mediators of Inflammation*, 2017, 1–17. doi:10.1155/2017/5095293

Abdullahi, A., Amini-Nik, S., & Jeschke, M. G. (2014). Animal models in burn research. *Cellular and molecular life sciences, CMLS*, 71(17): 3241–3255. https://doi.org/10.1007/s00018-014-1612-5

Alizadeh, Alimohammad, Mohagheghi, Mohammadali, Khaneki, Mahmod, Saeedpour, Khadijeh (2007). Study of the effect of magnesium hydroxide on the wound healing process in rats. *Medical Journal of Islamic World Academy of Sciences,* 16 (4): 165-170.

Caetano, G. F., Frade, M. A. C., Andrade, T. A. M., Leite, M. N., Bueno, C. Z., Moraes, Â. M., & Ribeiro-Paes, J. T. (2014). Chitosan-alginate membranes accelerate wound healing. J*ournal of Biomedical Materials Research Part B: Applied Biomaterials,* 103(5):1013–1022. doi:10.1002/jbm.b.33277

Chang, S., Sartika, D., Fan, G., Cherng, J., & Wang, Y. (2019). *Animal Models of Burn Wound Management*. In E. Tvrdá, & S. C. Yenisetti (Eds.), Animal Models in Medicine and Biology. Intech Open. https://doi.org/10.5772/intechopen.89188

Davidson, J. M., Yu, F., & Opalenik, S. R. (2013). Splinting Strategies to Overcome Confounding Wound Contraction in Experimental Animal Models. *Advances in wound care*, 2(4), 142–148. https://doi.org/10.1089/wound.2012.0424

De Moura Estevão, L. R., Cassini-Vieira, P., Leite, A., de Carvalho Bulhões, A., da Silva Barcelos, L., & Evêncio-Neto, J. (2019). Morphological Evaluation of Wound Healing Events in the Excisional Wound Healing Model in Rats. *Bio-protocol,* 9(13), e3285. https://doi.org/10.21769/BioProtoc.3285

DePhillipo, N. N., Aman, Z. S., Kennedy, M. I., Begley, J. P., Moatshe, G., & LaPrade, R. F. (2018). Efficacy of Vitamin C Supplementation on Collagen Synthesis and Oxidative Stress After Musculoskeletal Injuries: A Systematic Review. *Orthopaedic journal of sports medicine*, 6(10), 2325967118804544. https://doi.org/10.1177/2325967118804544

Dorsett-Martin, W. A. (2004). Rat models of skin wound healing: A review. *Wound Repair and Regeneration*, 12(6), 591–599. doi:10.1111/j.1067-1927.2004.12601.x

Gonzalez, A. C. de O., Costa, T. F., Andrade, Z. de A., & Medrado, A. R. A. P. (2016). Wound healing - A literature review. A*nals Brasileiros de Dermatologia,* 91(5), 614–620. doi:10.1590/abd1806-4841.20164741

Gottrup F, Agren MS, Karlsmark T. (2000). Models for use in wound healing research: a survey focusing on in vitro and in vivo adult soft tissue. *Wound Repair Regen.* 8(2):83-96. doi: 10.1046/j.1524-475x.2000.00083.x. PMID: 10810034

Grada, A., Mervis, J., & Falanga, V. (2018). Research Techniques Made Simple: Animal Models of Wound Healing. *Journal of Investigative Dermatology,* 138(10), 2095–2105.e1. doi:10.1016/j.jid.2018.08.005

Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). Wound repair and regeneration. *Nature,* 453(7193), 314–321. doi:10.1038/nature07039

Hasamnis, A. A., Mohanty, B. K., Muralikrishna, & Patil, S. (2010). Evaluation of Wound Healing Effect of Topical Phenytoin on Excisional Wound in Albino Rats. *Journal of Young Pharmacists,* 2(1), 59–62. doi:10.4103/0975-1483.62215

Masson-Meyers, D. S., Andrade, T. A. M., Caetano, G. F., Guimaraes, F. R., Leite, M. N., Leite, S. N., & Frade, M. A. C. (2020). Experimental models and methods for cutaneous wound healing assessment. *International Journal of Experimental Pathology.* doi:10.1111/iep.12346

Mogford JE, Mustoe TA. (2001). Experimental models of wound healing. In: Falanga V, editor. *Cutaneous wound healing*. London: Martin Dunitz, 2001:109–22.

Murthy, S., Gautam, M. K., Goel, S., Purohit, V., Sharma, H., & Goel, R. K. (2013). Evaluation of In Vivo Wound Healing Activity of Bacopa monniera on Different Wound Model in Rats. *BioMed Research International,* 2013, 1–9. doi:10.1155/2013/972028

Negut, I., Grumezescu, V., & Grumezescu, A. (2018). Treatment Strategies for Infected Wounds. *Molecules*, 23(9), 2392. doi:10.3390/molecules23092392

Pastar, I., Stojadinovic, O., Yin, N. C., Ramirez, H., Nusbaum, A. G., Sawaya, A., Patel, S. B., Khalid, L., Isseroff, R. R., & Tomic-Canic, M. (2014). Epithelialization in Wound Healing: A Comprehensive Review. *Advances in wound care,* 3(7), 445–464. https://doi.org/10.1089/wound.2013.0473

Petersen, H., Tavakoli, F., Kruber, S., Münscher, A., Gliese, A., Hansen, N.-O., … Miller, R. J. D. (2016). Comparative study of wound healing in rat skin following incision with a novel picosecond infrared laser (PIRL) and different surgical modalities. *Lasers in Surgery and Medicine,* 48(4), 385–391. doi:10.1002/lsm.22498

Reinke, J. M., & Sorg, H. (2012). Wound Repair and Regeneration. *European Surgical Research*, 49(1), 35–43. doi:10.1159/000339613

Rousselle, P., Braye, F., & Dayan, G. (2018). Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. *Advanced Drug Delivery Reviews*. doi:10.1016/j.addr.2018.06.019

Santos, T. S., Santos, I. D. D. dos, Pereira-Filho, R. N., Gomes, S. V. F., Lima-Verde, I. B., Marques, M.N, Albuquerque-Júnior, R.L.C. de. (2021). Histological Evidence of Wound Healing Improvement in Rats Treated with Oral Administration of Hydroalcoholic

Extract of Vitis labrusca. *Current Issues in Molecular Biology*, 43(1), 335–352. doi:10.3390/cimb43010028

Santos, T. S., Santos, I. D. D. dos, Pereira-Filho, R. N., Gomes, S. V. F., Lima-Verde, I. B., Marques, M. N., … Albuquerque-Júnior, R. L. C. de. (2021). Histological Evidence of Wound Healing Improvement in Rats Treated with Oral Administration of Hydroalcoholic Extract of Vitis labrusca. *Current Issues in Molecular Biology*, 43(1), 335–352. doi:10.3390/cimb43010028

Sharpe, J. R., & Martin, Y. (2013). Strategies Demonstrating Efficacy in Reducing Wound Contraction In Vivo. *Advances in Wound Care*, 2(4), 167–175. doi:10.1089/wound.2012.0378

Son DO, Hinz B. (2021). A Rodent Model of Hypertrophic Scarring: Splinting of Rat Wounds. *Methods Mol Biol.,* 2299:405-417. doi: 10.1007/978-1-0716-1382-5_27. PMID: 34028757

Thakur, R., Jain, N., Pathak, R., & Sandhu, S. S. (2011). Practices in Wound Healing Studies of Plants. *Evidence-Based Complementary and Alternative Medicine*, 2011, 1–17.

Tottoli, E. M., Dorati, R., Genta, I., Chiesa, E., Pisani, S., & Conti, B. (2020). Skin Wound Healing Process and New Emerging Technologies for Skin Wound Care and Regeneration. *Pharmaceutics*, 12(8), 735. doi:10.3390/pharmaceutics12080735

Velnar, T., Bailey, T., & Smrkolj, V. (2009). The Wound Healing Process: An Overview of the Cellular and Molecular Mechanisms. *Journal of International Medical Research,* 37(5), 1528–1542. doi:10.1177/147323000903700531

Wilhelm, K.-P., Wilhelm, D., & Bielfeldt, S. (2016). Models of wound healing: an emphasis on clinical studies. *Skin Research and Technology*, 23(1), 3–12. doi:10.1111/srt.12317

Wong, V. W., Sorkin, M., Glotzbach, J. P., Longaker, M. T., & Gurtner, G. C. (2011). Surgical approaches to create murine models of human wound healing. *Journal of biomedicine & biotechnology,* 2011, 969618. https://doi.org/10.1155/2011/969618

Wong, V. W., Sorkin, M., Glotzbach, J. P., Longaker, M. T., & Gurtner, G. C. (2011). Surgical approaches to create murine models of human wound healing. 2011, 969618. https://doi.org/10.1155/2011/969618