An Easy, Rapid, and Reproducible Way to Create a Split-Thickness Wound for Experimental Purposes

Nazim Gümüş, MD, Neşe Kurt Özkaya, MD,* Hüseyin Eray Bulut, MD,† and Sarper Yilmaz, MD**

Abstract: Partial-thickness wound models of rat skin have some difficulties in creating the wounds in equal size and depth. Moreover, making a split-thickness wound on the rat skin seems not to be simple and rapid. A new alternative method was presented here to overcome these obstacles, by using a waterjet device to create a split-thickness wound on rat skin.

Twenty-four male Wistar rats were randomly divided into 3 groups. An area of 4 × 4 cm in diameter was marked on the center of the dorsal skin. Waterjet hydrosurgery system was used to create a wound on the dorsal rat skin, by removing the outer layers of the skin. In group 1, rat skin was wounded with setting 1 to create a superficial skin wound. In group 2, it was injured with setting 5 to make a deeper wound, and in group 3, skin wound was performed with setting 10 making the deepest wound in the experiment. After the wounds were created on the rat skin, a full-thickness skin biopsy was taken from the middle of the cranial margin of the wound, including both the wound surface and the healthy skin in a specimen. Healing time of the wounds of animals was recorded in the experiment groups. Then, the results were compared statistically between the groups. In the histologic assessment, both the thickness of the remnant of the epidermis in the wound surface and the thickness of the healthy epidermis were measured under light microscope. Thickness of the epidermis remaining after wounding was statistically compared among the groups and with the healthy epidermis.

The mean thickness of the remaining epidermis was determined for each group. It was higher in the superficial wounds than in the deep wounds, because of the removal of the skin from its outer surface through the deep layers of the skin with waterjet device. The most superficial wound in the experiment was observed in group 1, which was statistically different from the wounds of group 3, whereas there was no difference between the wounds of groups 1 and 2. Compared with the wounds of groups 1 and 2, the wounds in group 3 were significantly deeper than the wounds of other groups, which was statistically significant. In all groups, mean thickness of epidermis in the wound surface showed statistically significant difference from that in the healthy skin. When compared with the healing times of the wounds in the groups, a statistically significant difference was found between them.

Creation of a split-skin wound, by using the waterjet system, provides a wound in reproducible size and depth, also in a standardized and rapid manner. Moreover, it makes precise and controlled wound creation in the rat skin.

Key Words: Versajet, rat, experiment, split-thickness wound

(*J Craniofac Surg* 2014;25: 1898–1901)

Skin wounds created on the rat skin have been widely used for various experimental purposes including spesific researches on wound healing and epitelialization, dressing materials, and drug investigations. Experimentally, most frequently used wounds on rat skin may be divided into 3 groups. The first, standard full-thickness skin defects are excisional wounds usually created on the back of the rats by removing the skin completely. The second incisional wounds are made up of a cut into the skin layers. Finally, partial-thickness wounds consist of the remaining skin after tangential excision of the upper skin layers including the epidermis and various thicknesses of the dermis. Experimental design of the first and second wound forms is quite simple, making them preferable and reproducible in a standardized fashion; so in most of the experiments, full-thickness skin wounds, namely excisional or dermal wounds, have been widely used.[1,2] Partial-thickness wound models of rat skin have some difficulties in creating the wounds in equal size and depth, so standardization of them may be considered a significant problem to be overcome in the planning of the models. Moreover, making a split-thickness wound on the rat skin seems not to be simple and rapid and requires an extra person for assistance in most instances; additional surgical incisions and dissections; and some spesific instruments such as clips, plates, and tongue depressors.[3–8] As a result, partial-thickness wound models are less preferred than excisional wounds in the experimental studies.

Challenges for making a split-thickness wound on a rat skin mainly arise from the properties of rat skin, which has a panniculus carnosus tissue consisting of a striated muscle tissue. Skin and underlying panniculus carnosus layer cause high mobility and laxity in rodent skin, which complicates the cutting of the skin tangentially.[8]

Presented here is a new alternative method to overcome these obstacles, by using a waterjet device to create a split-thickness wound on rat skin. This method offers not only a rapid, standardized, and reproducible way but also 3 different options for wound depth.

MATERIALS AND METHODS

This study enrolled 24 male Wistar rats, 110 to 120 days old, weighing between 270 and 300 g. The rats were randomly divided into 3 groups, each of which was composed of eight. This experiment was approved by the ethical committee of the University for Animal Researches. The animals were housed in individual cages and fed standard rat chow and water ad libitum upon completion of the experiments.

From the Departments of *Plastic, Reconstructive and Aesthetic Surgery and †Histology and Embriyology, Cumhuriyet University Medical Faculty, Sivas, Turkey.
Received January 4, 2014.
Accepted for publication February 10, 2014.
Address correspondence and reprint requests to Nazim Gümüş, MD, Cumhuriyet Üniversitesi Plastik Rekonstrüktif ve Estetik Cerrahi AD, Sivas, Turkey; E-mail: gumus1970@hotmail.com
The authors report no conflicts of interest.

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000000921

After the rats were anesthetized with intramuscular injections of 10 mg/kg of ketamine and subcutaneous injections of 3 mg/kg of xylazine hydrochlorid, the dorsal skin was shaved using an electric clipper. Then, the stubbles were removed with a wet shave. An area of 4 × 4 cm in diameter was marked on the center of the dorsal skin and then disinfected with povidone-iodine. During the surgical procedure, a local sterile environment and asepsis were provided. Water-jet hydrosurgery system known as Versajet device was used to create a wound on the marked area of the dorsal rat skin, by removing the outer layers of the skin. A standard handpiece with a 45-degree angled tip and a 14-mm working window was used in the intervention. As versajet device has 10 settings in use, 3 settings—1, 5, and 10—were preferred to perform skin wounds in various depths. Tip of the waterjet system passed 10 times over each site of the wound being created to make a standardized wounding procedure for each of the rats in all groups. In group 1, the rat skin was wounded with setting 1 to create a superficial skin wound (Fig. 1). In group 2, it was injured with setting 5 to make a deeper wound, and in group 3, the skin wound was performed with setting 10 making the deepest wound in the experiment (Figs. 2, 3). After the wounds were created on the rat skin, a full-thickness skin biopsy, which was 5 × 10 mm, was harvested from the middle of the cranial margin of the wound, including both the wound surface and healthy skin in the same specimen. After the biopsy site was sutured, all wounds were dressed, by using the same materials and techniques, and dressings were changed within the same time intervals. Healing time of the wounds was recorded in the experiment groups. Then, it was compared statistically among the groups. After the wounds healed completely, a similar biopsy sample was obtained from the opposite site of the wound, namely middle of the caudal margin of the healed wound.

For the histologic assessments, biopsy samples were fixed in 10% of formaldehyde solution for 24 hours, embedded in paraffin, sectioned, and stained with routine hematoxylen eozin (HE) stain. Both the thickness of the remnant of the epidermis in the wound surface and the thickness of the healthy epidermis next to the wound surface were measured under light microscope, revealing the depth of the wounds. Measurement was performed between the surface of the epidermis beginning under the stratum lucidum layer and basal lamina deep to the epidermis. Thickness of the epidermis remaining after wounding was statistically compared among the groups and with the healthy epidermis. This measurement method for the evaluation of wound depth was used to show wound depth indirectly, using the epidermal thickness over basal lamina, because morphometrical measurements in a soft tissue for the determination of wound depth were very difficult and misleading without using a well-known and standard structure such as the basal lamina. In the study, wound depth was measured a few times along the wound surface, and mean values were calculated for each wound.

Morphometrical Method

The aim of the morphometrical measurements used in this study was to obtain quantitative structural information on the depth of experimentally developed wounds in rats. To do this, 5-μm-thick rat skin tissue sections were evaluated morphometrically under an Olympus BX51 (Tokyo, Japan) light microscope with camera and LCD monitor attachments. Other instruments used for morphometrical measurements were a millimetric ruler, a grating replica (Graticules Ltd, Tonbridge Kent, England) with 100 × 0.01 = 1-mm scale, and a test grid with 5-cm-spaced vertical parallel lines to sample the measurement points on the tissue.

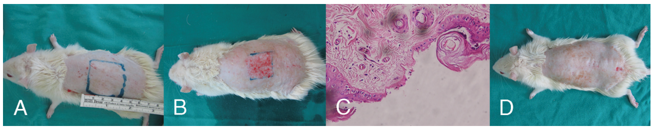

FIGURE 2. View of the planning of a wound and obtained results in the group 2 rats. A, Preoperative marked area on the dorsum of the rat skin. B, Created wound. All of the marked surface area was uniformly wounded. C, Histologic findings of the wound showing a deeper wound than that of group 1, but there was no significant difference (HE, ×40). D, Healed wound area.

Before the morphometrical measurements, the exact magnifications were evaluated using the grating replica (Graticules Ltd, Tonbridge Kent, England) and the millimetrical ruler. Epidermis thickness measurements in millimeters were done by a conventional millimetric ruler, using the intersection points on the edge of the outer epidermal layer determined by a test grid with 5-cm-spaced vertical parallel lines. The tissue images were captured on the LCD monitor attachment of the light microscope on which the test grid was overlapped. All values measured in millimeters were converted to micrometers, using the exact magnification value to derive the true length of wound depths. All values in micrometers were obtained in all groups, and statistical analyses were applied to those values to determine if the differences were significant or not between the groups.

For the statistical analysis, $P < 0.05$ was regarded as indicating statistical significance, and data were expressed as mean (SD). The SPSS for Windows 16.0 software package (IBM, Armonk, NY) was used for the statistical analyses of the data. Kruskal-Wallis Test was used to detect differences between the groups.

RESULTS

All animals survived throughout the study, and no sign of infection was observed during the wound healing in the animals. The mean time to perform a split-thickness skin wound on 1 animal was only a few minutes. The use of the waterjet device was very simple and easy, and there was no need for experience in using this device. Learning curve for this technique was quite short.

The mean thickness of the remaining epidermis was determined for each group, reflecting the wound depth created with this device (Table 1). Thickness of the epidermis was higher in the superficial wounds than in the deep wounds, because of the removal of the skin from its outer surface through deep layers of the skin with waterjet device. In group 1, mean (SD) thicknesses of the remaining epidermis were 9.95 (1.84) μm in the wound surface

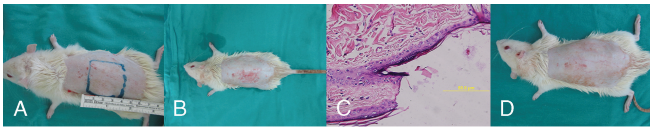

FIGURE 1. Apperance of the planning of a wound and obtained results in the group 1 rats. A, Preoperative marked area on the dorsum of the rat skin. B, Created wound. Note that it did not involve all of the marked surface area. C, Histologic findings of the wound showing partial loss in the epidermis (HE, ×40). D, Healed wound area.

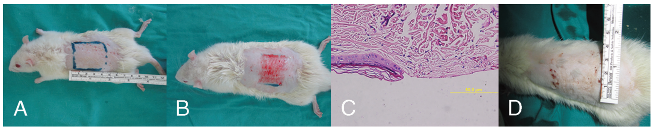

FIGURE 3. Apperance of the planning of a wound and obtained results in the group 3 rats. A, Preoperative marked area on the dorsum of the rat skin. B, Created wound. C, Histologic findings of the wound showing complete loss of the epidermis (HE, ×40). D, Healed wound area showing some of surface irregularities and contraction due to the healing of a deep wound.

TABLE 1. The Mean Thicknesses of the Remnant Epidermis Remaining After Creation of the Wounds in the Groups

Rat No.	Group 1, μm	Group 2, μm	Group 3, μm
1	8.91	7.63	2.18
2	12.9	6.0	1.64
3	7.82	18.18	3.27
4	9.27	5.09	1.27
5	10.0	10.73	1.09
6	8.18	8.91	2.54
7	12.36	14.18	1.82
8	10.18	15.82	2.0
Mean (SD)	9.95 (1.84)	10.81 (4.78)	1.98 (0.71)*

*Significant difference versus the groups 1 and 2 ($P < 0.05$).

and was 32.9 (5.39) μm in the healthy skin area. In group 2, the mean (SD) thicknesses of it were 10.81 (4.78) μm in the wound surface and 29.7 (5.19) μm in the healthy skin next to the wound area. In group 3, the mean (SD) thicknesses were 1.98 (0.71) μm in the wound surface and 29.61 (9.59) μm in the healthy skin area. A superficial wound was observed in group 1, which was statistically different from the wounds of group 3, whereas there was no difference between the wounds of groups 1 and 2. Compared with the wounds of groups 1 and 2, the wounds in group 3 were significantly deeper than the wounds of other groups, which was statistically significant ($P < 0.05$). In all groups, mean thickness of epidermis in the wound surface showed statistically significant difference from that in the healthy skin ($P < 0.05$). In addition, there was no significant difference between the groups for the mean thickness of epidermis in healthy skin ($P > 0.05$). In the skin biopsies taken after the wound healing completed, there was no difference in the measurements of epidermis thickness among the groups.

The progress of healing was examined in the groups, by using the same wound care approaches in all groups. The time of complete epithelialization in the animals was recorded, and mean days of epithelialization was calculated (Table 2). The mean (SD) times for epithelialization were 5.37 (0.91) days in group 1, 8.87 (0.99) days in group 2, and 12.0 (0.92) days in group 3. When compared with the healing times of the wounds in the groups, a statistically significant difference was found between the groups, suggesting that 3 different wounds in various depths were created in the experiment ($P < 0.05$) (Fig. 4).

DISCUSSION

Waterjet system works with a high-pressure jet stream of saline, which is oriented parallel to the working plane. It creates a vacuum that removes debris, contaminants, and tissues from its contact surface; therefore, it is capable of cleansing the wound deeply while working for debridement. As a result of these properties of the system, it has been widely used in daily clinical practice of burn surgery.[9–11] Waterjet system makes precise and controlled tangential excision, so it has been known to be a helpful device to debride some burned surfaces such as face, groin creases, axillae, hands, or feet where excision of the necrosis is very difficult with conventional tangential excision methods using a handheld dermatome such as Watson knife.[10,11]

TABLE 2. Wound Healing Times After Wounding the Rat Skin in the Groups

Rat No.	Group 1, days	Group 2, days	Group 3, days
1	5	9	11
2	7	9	13
3	5	9	11
4	5	8	12
5	4	8	13
6	5	8	12
7	6	11	13
8	6	9	11
Mean (SD)	5.37 (0.91)	8.87 (0.99)	12 (0.92)*

*Significant difference was observed among the groups ($P < 0.05$).

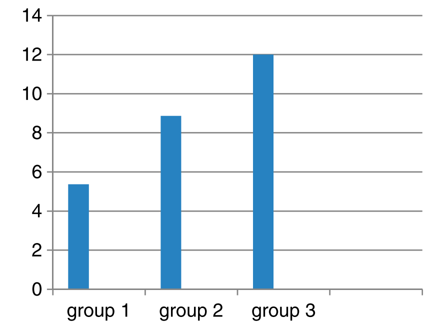

FIGURE 4. The mean healing days of the wounds. Note that there was statistically significant difference among the groups, and standard deviations were 0.91, 0.99, and 0.92 days in the groups, respectively.

Waterjet device has also been used for the excision of skin layers tangentially in reduction mammaplasty operations in which deepithelialization was made.[12] Similarly, in this study, waterjet device was used for removing the skin layers of the rat tangentially as a knife to create a partial-thickness skin wound. As well known in the clinical studies, on the lower settings, it is able to remove the epidermis and the superficial dermis, and on the higher settings, it can excise the skin layers down to the deep dermis.[13,14] As a result of these features of this device, we designed 3 different wound models in this study. The first wound was a superficial wound such as a skin abrasion, which was made with setting 1. The second model had a wound with intermediate depth, which was created with setting 5. The third wound was the deepest wound in the experimental models, which was made with setting 10. Although statistically significant difference was not found between the wound depth of groups 1 and 2, healing times of wounds showed significant difference between the groups, and appearence of the wounds revealed significant difference, suggesting that these 2 groups differed from each other. In the apperance of the group 1 and 2 wounds, the wounds did not involve all of the marked surface area in group 1, although morphometrical measurement showed similar wound depth with group 2. Group 2 wounds spreaded uniformly over all wound surfaces. This wound difference possibly arises from the working properties of the device. Therefore, group 1 wounds may be considered as too superficial wounds such as an abrasion, and group 2 wounds may be accepted as a uniform superficial wound.

The most using way of making a split-skin wound in rats is harvesting a split-skin graft from the rat skin. However, taking a split-skin graft, by using a dermatome, has some difficulties in the rodents. Presence of panniculus carnosus tissue, which is striated muscle in rat skin, makes the skin highly mobile and lax, complicating the harvesting of split-skin grafts significantly. For the fixation of the rat skin during graft harvesting, some techniques have been advocated, providing easy, rapid, and standardized graft taking.[3–8] Unfortunately, many of these methods described mainly for graft harvesting, not for the creation of a split wound, need additional surgical incisions and dissections and many instruments such as clamps, tongue depressors, plates, and devices; so ideally, they seem not to be suitable for the creation of a split wound. They injure the experiment area with the surgical incisions and dissections, being capable of interfering the results of the planned experiments. To overcome these challenges, in many experiments, a small skin wound, a few milimeter in size, is used or experiment is planned in a full-thickness wound model. A recently published article described a new method for harvesting split-skin graft in rodents.[8] This method avoids additional injuries outside the planned area and takes advantage of the elasticity of rat skin. A metal support plate is used and held laterally beside the skin area where the split skin is to be harvested. Then, the skin is mobilized and pulled over the plate, and using a dermatome, the split-skin graft is taken on the metal plate. This method seems to be the most effective approach to create a split-skin wound in a rat in the other tecniques described; however, it needs experience for taking a graft, permits taking a split skin only from the lateral sides of the dorsal rat skin, is not suitable for creating a wound on the central of the rat dorsum or on the other sites of the rat body, and seems to bear difficulty for making a superficial wound such as an abrasion. Our approach offers 3 different wound models, which can be created on everywhere of the rat skin based on the need of the planned experiment. In addition, it does not require experience because of the simplicity of using the device and is gentle to the animal without needing sacrification.

In this experiment, we had to make a superficial skin wound by means of passing the tip of the device 10 times over an area because of the need of basal lamina for the measurement of wound depth. This number of tip pass, namely 10 times, provided both the protection of basal lamina and the removal of epidermis as much as possible, so wound depth could be measured a few times by using a stable structure. Obtained results were used for the statistical analyses of groups with which effectivity of device in creating a partial skin wound was investigated. If more than 10 times of pass such as 15 or 20 times had been used for the wound creation, the depth of the wound would have increased significantly, involving deeper layers of the dermis. However, if basal lamina and epidermal remnants had been removed, correct measurement of wound depth would have been impossible. In this experiment, in view of histologic findings, epitelial remnants seemed as if the device was able to create superficial wounds such as erosions, and it was not capable of making deep wounds; however, being well known, the device is capable of removing tissues tangentially and can remove nearly all dermis if it works in sufficient time over the wound area; so deeper skin wounds can easily be performed by passing the tip of the device over the skin surface for a longer time. The number of the tip pass used in the experiment should be considered as another important factor for wound depth.

Financially, as Versajet is an expensive instrument, its cost may restrict its use in an animal laboratory.

In conclusion, the creation of a split-skin wound, by using a waterjet system, provides a wound in reproducible size and depth, also in a standardized and rapid manner. Moreover, it makes precise and controlled wound creation in the rat skin.

REFERENCES

1. Takzare N, Hosseini MJ, Hasanzadeh G, et al. Influence of aloe vera gel on dermal wound healing process in rat. *Toxicol Mech Methods* 2009;19:73–77
2. Konya D, Gercek A, Akakin A, et al. The effects of inflammatory response associated with traumatic spinal cord injury in cutaneous wound healing and on expression of transforming growth factor-beta1 (TGF-beta1) and platelet-derived growth factor (PDGF)-A at the wound site in rats. *Growth Factors* 2008;26:74–79
3. Rudolph R, Linnevold R. Rapid harvesting of precise split thickness skin grafts in small animals. *J Invest Dermatol* 1971;57:180–183
4. James MI. A simple and reliable method for rapidly harvesting split skin grafts in rats. *Eur J Plast Surg* 1989;12:258–260
5. Millington GM, Moore TC. A rapid method for mass pattern skin grafting in the rat. *J Surg Res* 1968;8:379–383
6. Pan YC, Ozcan G, Chahadeh CG, et al. A simple technique for harvesting standardized skin grafts in the rodent. *Ann Plast Surg* 1993;30:186–189
7. Gustavson EH. A simple aid to taking split-thickness skin grafts in small experimental animals. *Br J Plast Surg* 1974;27:165–166
8. Rahmanian-Schwarz A, Knoeller T, Held M, et al. A new, rapid, standardized method for harvesting split skin grafts in rodents. *Plast Reconstr Surg* 2011;127:1494–1497
9. Rennekampff HO, Schaller HE, Wisser D, et al. Debridement of burn wounds with a water jet surgical tool. *Burns* 2006;32:64–69
10. Tenenhaus M, Bhavsar D, Rennekampff HO. Treatment of deep partial thickness and indeterminate depth facial burn wounds with water-jet debridement and a biosynthetic dressing. *Injury* 2007;38S:S39–S45
11. Cubison TC, Pape SA, Jeffery SL. Dermal preservation using the Versajet hydrosurgery system for debridement of paediatric burns. *Burns* 2006;32:714–720
12. Lonergan I, Moquin K. Use of the Versa Jet for pedicle deepithelialization during breast reduction surgery. *Aesthetic Plast Surg* 2009;33:250–253
13. Klein MB, Hunter S, Heimbach DM, et al. The Versajet water dissector: a new tool for tangential excision. *J Burn Care Rehabil* 2005;26:483–487
14. Jeffery SL. Device related tangential excision in burns. *Injury* 2007;38S:S35–S38