Validation of a Model for the Study of Multiple Wounds in the Diabetic Mouse (db/db)

Stephen R. Sullivan, M.D., Robert A. Underwood, B.F.A., Nicole S. Gibran, M.D., Randall O. Sigle, Ph.D., Marcia L. Usui, B.S., William G. Carter, Ph.D., and John E. Olerud, M.D.

Seattle, Wash.

The genetically diabetic db/db mouse exhibits symptoms that resemble human type 2 diabetes mellitus, demonstrates delayed wound healing, and has been used extensively as a model to study the role of therapeutic topical reagents in wound healing. The purpose of the authors' study was to validate an excisional wound model using a 6-mm biopsy punch to create four full-thickness dorsal wounds on a single db/db mouse. Factors considered in developing the db/db wound model include reproducibility of size and shape of wounds, the effect of semiocclusive dressings, comparison with littermate controls (db/−), clinical versus histologic evidence of wound closure, and cross-contamination of wounds with topically applied reagents. The size of wounds was larger, with less variation in the db/db mice (31.11 ± 3.76 mm^2) versus db/− mice (23.64 ± 4.78 mm^2). Wounds on db/db mice that were covered with a semiocclusive dressing healed significantly more slowly (mean, 27.75 days) than wounds not covered with the dressing (mean, 13 days; $p < 0.001$), suggesting the dressings may splint the wounds open. As expected, wounds healed more slowly on db/db mice than db/− mice (covered wounds, 27.75 days versus 11.86 days, $p < 0.001$; wounds not covered, 13 days versus 11.75 days, $p = 0.39$). Covered wounds, thought to be closed by clinical examination, were confirmed closed by histology only 62 percent of the time in the db/db and 100 percent of the time in the db/− mice. Topical application of blue histologic dye or soluble biotinylated laminin 5 to one of the four wounds did not spread locally and contaminate adjacent wounds. Multiple, uniform, 6-mm wounds in db/db mice heal in a relatively short time, decrease the number of animals needed for each study, and allow each animal to serve as its own control. The db/db diabetic mouse appears to be an excellent model of delayed wound healing, particularly for studying factors related to epithelial migration. (*Plast. Reconstr. Surg.* 113: 953, 2004.)

Genetically diabetic (db/db) mice have an autosomal recessive mutation that results in defective leptin receptors in the hypothalamus. The defect leads to increased appetite, decreased energy expenditure, obesity, and development of marked hyperglycemia that resembles human type 2 diabetes mellitus.[1–3] This mouse also demonstrates delayed wound healing and has been used extensively as a model for studying the role of therapeutic topical reagents.[4–7] Despite wide use, methods and models vary among studies. Some factors to consider in developing a wound model include reproducibility of size and shape of wounds, number of wounds per animal, wound dressings, and cross-contamination of wounds with treatment reagents.

Whereas normal murine wounds heal approximately 90 percent by contraction, db/db mice exhibit less contraction and a greater degree of epithelization,[4] thus serving as a valuable model for studying keratinocyte migration following cutaneous injury. Migration of keratinocytes at the margin of wounds is essential in the epithelization of cutaneous human wounds.[8] We have demonstrated that in chronic wounds from patients with diabetes, keratinocytes proliferate at the wound edge but migrate poorly.[9] Laminin 5, an epithelial basement membrane adhesive ligand,[10] may have a crucial role in signaling keratinocyte migration in wounds.[11] We evaluated variables

From the Departments of Medicine (Division of Dermatology), Surgery, and Pathobiology, University of Washington, and the Fred Hutchinson Cancer Research Center. Received for publication January 22, 2003; revised May 13, 2003.

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in healing of db/db mouse excisional wounds to improve our analysis of the effects of exogenous topical reagents, including laminin 5.

The purpose of our study was to systematically characterize an excisional wound model using a 6-mm biopsy punch to create four full-thickness dorsal wounds on a single mouse. Other investigators have used both control and treatment wounds on the same db/db mouse.[5,7,12] They have not reported whether topically applied reagents spread locally to affect control wounds.

Semiocclusive dressings have been shown to shorten wound-healing time of full-thickness wounds in both pig and human models.[13,14] Human and pig wounds heal less by contraction than rodents such as mice.[15] The effect on wound healing kinetics of Tegaderm (3M, St. Paul, Minn.), a transparent, semiocclusive, polyurethane dressing that transmits oxygen and moisture vapor, was examined in db/db mice. Our study provides data to validate a db/db mouse model of wound healing with regard to reproducibility of size and shape of wounds, comparison with littermate controls (db/−), the effect of semiocclusive dressings, clinical versus histologic evidence of wound closure, and cross-contamination of wounds with topically applied reagents.

Materials and Methods

Animals and Wounding

Genetically diabetic 8- to 12-week-old male mice (db/db; C57BL/Ks J-*m*+/+ Lepr^{db}) and heterozygous nondiabetic littermates (db/−) were purchased from The Jackson Laboratory (Bar Harbor, Me.). Strain and age of mouse were chosen because the mutant mice exhibit severe diabetic conditions, with plasma insulin concentration and hyperglycemia peaking between 8 and 12 weeks.[1,16] Ninety-three mice were used in the preliminary studies to develop this model and test the multiple variables for our study. Mice were housed individually in the University of Washington Department of Comparative Medicine vivarium, maintained on a 12-hour light/dark cycle, and allowed ad libitum access to rodent chow and water. All procedures were approved by the University of Washington Animal Care Committee.

Mice were anesthetized with an intraperitoneal injection of ketamine (150 mg/ml) and xylazine (10 mg/ml) (Phoenix Pharmaceuticals, Inc., St. Joseph, Mo.). The dorsal skin was shaved, treated with depilatory cream, and cleansed with povidone-iodine solution. Mice were kept warm during anesthesia and surgery using a heat lamp and heating pad maintained at approximately 30°C. Four full-thickness 6-mm punch biopsy (Acuderm, Inc., Ft. Lauderdale, Fla.) wounds were created on the dorsal surface of the mice (Fig. 1). A template was used to mark the sites of biopsy 1 cm apart. The skin was then scored with the biopsy punch followed by excision through the skin and panniculus carnosus with surgical scissors. Wound

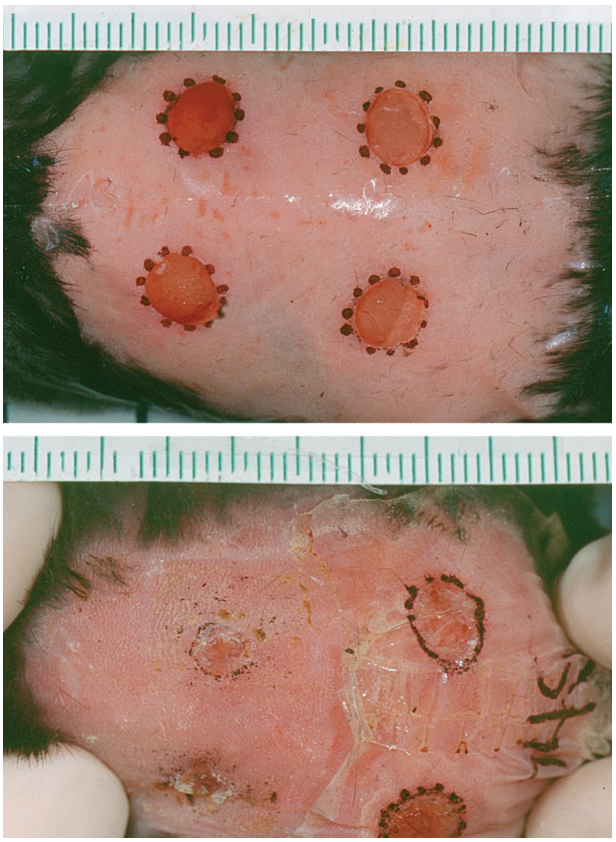

FIG. 1. (*Above*) On both db/db and db/− mice, four full-thickness wounds were created with a 6-mm biopsy punch, marked with ink, and covered with Tegaderm. Wound size created with a 6-mm punch biopsy on db/db mice was reproducible, with a mean area of 31.11 ± 3.76 mm^2, which corresponds to 10.0 percent wound enlargement. Wounds on db/− mice had a mean area of 23.64 ± 4.78 mm^2, which corresponds to 16.4 percent wound contraction. Wounds on the db/db mice were significantly larger than those on db/− mice at the time of wounding ($p < 0.001$). (*Below*) A db/db mouse 14 days after wounding. Preliminary studies suggested that Tegaderm frequently peeled off wounds and altered the rate of wound healing. Wounds that remained covered (posterior two wounds) closed more slowly than wounds that became uncovered (anterior two wounds). The indelible ink delineating the original margin of the wounds indicates that this difference is attributable in part to splinting of the wound by the adhesive dressing.

margins were then marked with indelible nontoxic ink. Depending on experimental goals, wounds were next covered with a semiocclusive dressing.

Testing the Effect of Wounds Covered and Not Covered with a Dressing

To test the effect of a semiocclusive dressing, all four wounds of eight db/db and eight db/− mice were covered with Tegaderm, whereas six db/db and five db/− mice were not covered. Covered wounds could be visualized through the transparent dressing. Scabs were gently removed from the wounds not covered because they decrease the rate of wound contraction[17] and impair assessment of wound closure.[18] Digital photographs of wounds, including a metric ruler, were taken on day zero, weekly, and on the wound closure date. Original wound size was measured using a scripted process of calibration, tracing, and area calculation in square millimeters, using Adobe Photoshop (Adobe Systems, Inc., San Jose, Calif.) with the Image Processing Tool Kit (Reindeer Games, Asheville, N.C.) plug-ins.

Partial Purification and Biotinylation of Laminin 5

Normal human foreskin keratinocytes were prepared as described by Boyce and Ham[19] and maintained in serum-free keratinocyte growth medium (KGM; Clonetics, San Diego, CA) containing insulin, epidermal growth factor, hydrocortisone, and bovine pituitary extract (50 μg of protein per ml). Conditioned culture medium from confluent cultures of human foreskin keratinocytes was passed over gelatin Sepharose to remove fibronectin. Laminin 5 was removed from the medium on the final column by adherence to wheat germ agglutinin.[20] The result of this process was a soluble form of partially purified laminin 5 with a protein content fluorometrically determined to be 65 μg/ml. Presence of laminin 5 was confirmed by Western blot. To permit detection by fluorescence after application to mouse wounds, some of the laminin 5 was conjugated with NHS-LC-Biotin (biotinylated laminin 5) using EZ Link (Pierce Chemical Company, Rockford, Ill.) according to the manufacturer's protocol.

Testing Local Spread of Topically Applied Soluble Reagents

To study the local spread to adjacent wounds of topically applied soluble reagents, wounds were covered with Tegaderm and the left cephalad wound of the group of four (Fig. 1, *above*) was treated daily with one of four different soluble reagents applied to six db/db mice. Reagent (0.1 ml) was infused subjacent to the Tegaderm using a syringe and 30-gauge needle. Blue histology dye was applied to two db/db mice and biotinylated laminin 5 to two db/db mice. For control, nonbiotinylated laminin 5 was applied to one db/db mouse and phosphate-buffered saline was applied to one db/db mouse.

Wound Harvesting

Mice were killed with an intraperitoneal injection of sodium pentobarbital (210 mg/kg) (Abbott Laboratories, North Chicago, Ill.). Mice used to test time to closure with or without Tegaderm were killed when all four wounds appeared grossly to be completely epithelized. Dorsal mouse skin, including all four wounds and a 0.5-cm margin of unwounded skin, was removed en bloc and fixed in 10% neutral buffered formalin. To confirm epithelization, wounds were bisected in a sagittal plane through the widest margin of the wound and embedded in paraffin for routine hematoxylin and eosin histologic evaluation.

Mice used to study spread of topically applied soluble reagent were killed either 24 hours or 7 days after wounding. Dorsal mouse skin, including all four wounds and a 0.5-cm margin of unwounded skin, was removed en bloc. Wounds from mice treated with blue histology dye were photographed, fixed in 10% neutral buffered formalin, bisected, and embedded in paraffin. To assess spread of biotinylated laminin 5 or nonbiotinylated laminin 5 to adjacent wound beds, tissue was fixed in 4% paraformaldehyde at 4°C for 1 hour. Tissue was rinsed and en face wound beds were incubated with streptavidin-Cy5 (1:500 dilution; Jackson Immuno Research Laboratories, West Grove, Pa.), at room temperature for 1 hour. Nuclei were stained with 4',6-diamidino-2-phenylindole (Sigma Chemical Co., St. Louis, Mo.) for 5 minutes at room temperature. The wound beds were dissected out using a 4-mm biopsy punch and mounted in an antiquench medium for fluorescence microscopy.

Photography

Gross specimen photographs of wounds before and after excising were captured using a Nikon D1 digital camera equipped with a Mi-

cro Nikkor macro lens and dual electronic flash (Nikon, Tokyo, Japan). Polarizing filters were fitted over both the flash and lens. Cross-polarization of these filters was found to be essential to remove spectral reflections from the Tegaderm or wound surface. A ruler was included in each image for spatial calibration.

Photomicrography was performed using a Nikon Microphot SA microscope equipped with brightfield, differential interference contrast and epifluorescence illumination. Images were captured using a Photometrics Sensys Monochrome digital camera controlled by IP Lab software (Scanalytics, Vienna, Va.).

Hematoxylin and eosin–stained wound sections were imaged using brightfield illumination. Individual grayscale images captured through red, green, and blue separation filters were merged and saved as 24-bit color image files.

Wound beds, incubated with streptavidin-Cy5 to assess spread of topically applied biotinylated laminin 5 and nonbiotinylated laminin 5, were first imaged with differential interference contrast to identify and focus on the dorsal surface followed by epifluorescence illumination to record the Cy5 fluorescence signal. A minimum of six nonoverlapping fields were captured to record the Cy5 signal at the surface of the wound bed.

Image Analysis

Photoshop 5.0 software loaded with the Image Processing Tool Kit 2.5 was used to analyze wound closure and wound bed Cy5 fluorescence signal. Wound closure was determined by opening the image in Photoshop, spatially calibrating the image using the scale bar, demarcating the wound edge, filling the interior with black, and measuring this area in square millimeters.

Images captured for the study of the spread of topically applied laminin 5 were opened in Photoshop. Using the Image > Histogram command, the mean pixel value and SD was recorded for each image. Each set of images representing a wound bed was charted as mean ± SD.

Data Analysis

The *t* test was used to test for potential differences in the size of day-zero wounds between db/db and db/− mice and wound was the unit of analysis. We used one-way analysis of variance to test for potential differences in time to wound closure between mice with wounds covered or not covered with Tegaderm, and mouse was the unit of analysis. We used repeated-measures analysis of variance to test both groups for potential differences in fluorescent signal intensity of wound beds treated with biotinylated laminin 5, nonbiotinylated laminated 5, control, and adjacent wounds. Multiple images of each wound were captured. Results were corrected for multiple comparisons between wound treatments using the Bonferroni method. Because data were not normally distributed, the natural logarithm of wound area, days to closure, and pixel value for fluorescent signal was used in the statistical analysis. Data in the Results section and Table I, however, are presented as mean ± SD in the original untransformed units. All analysis was performed with Stata Statistical Software 6.0 (Stata Corp., College Station, Texas).

RESULTS

Mice and Wounds

As with previous studies, the db/db mice were obese, weighing 40 to 44 g, whereas their nondiabetic littermates weighed 25 to 30 g. The mice tolerated the anesthesia, wounding procedure, and application of soluble reagents without problems. Mice did not experience large weight change after wounding or during the study. More than 90 percent of the mice survived the anesthesia and experiments. When mice died, death most frequently occurred if repeated anesthesia was used.

By gross examination, wounds created on the db/db mice appeared uniform (Fig. 1,

TABLE I
Days to Wound Closure

Wound Treatment	No.	Mean	SD	Maximum	Minimum
db/db mice covered with Tegaderm	8	27.75	1.49	31	27
db/db mice not covered with Tegaderm	6	13.00	1.73	14	11
db/– mice covered with Tegaderm	8	11.86	3.02	15	8
db/– mice not covered with Tegaderm	5	11.75	3.61	15	8

above). A 6-mm biopsy punch has an area of 28.27 mm^2. Wounds created on db/db mice with biopsy punch were quite reproducible, with a mean area of 31.11 ± 3.76 mm^2 immediately after wounding. These wounds enlarged after excision and became 10.0 percent larger than the punch biopsy instrument. No significant difference in initial wound area was found when the four wounds, cranial and caudal, were compared between db/db mice ($p = 0.39$).

Wounds created on db/− mice were less reproducible, with a mean area of 23.64 ± 4.78 mm^2 immediately after wounding. These wounds contracted after excision and became 16.4 percent smaller than the biopsy punch instrument. No significant difference in initial wound area was found when the four wounds, cranial and caudal, were compared between db/− mice ($p = 0.92$). When wounds on db/− mice were compared with db/db mice immediately after wounding, they were 31.6 percent smaller on average, a difference that was statistically significant ($p < 0.001$).

Wounds Covered and Not Covered with a Dressing

In preliminary studies, the rate of wound healing was altered when the semiocclusive dressing peeled off the mouse wounds. Wounds that remained covered closed more slowly than wounds that became uncovered (Fig. 1, *below*). Therefore, we tested the effect of a semiocclusive dressing on wound healing by either covering or not covering the wounds immediately after surgery. The wound dressings on db/db mice remained in place for the duration of the study, as mice were docile and rarely had hair regrowth after initial shaving and depilatory cream application. Wounds covered with a dressing did not develop an eschar, whereas wounds not covered did develop an eschar. Large differences in time to closure were found between mice with wounds covered and not covered. The db/db mice with wounds covered healed significantly more slowly (mean, 27.75 days) than wounds not covered (mean, 13 days; $p < 0.001$) (Table I).

The db/− mice were quite active and frequently demonstrated hair regrowth after removal by shaving and depilatory cream. Therefore, the dressings frequently peeled off within 3 to 4 days. Dressings were replaced each time they peeled off. The db/− mice with wounds covered healed slightly, although not significantly, more slowly (mean, 11.86 days) than db/− mice with wounds not covered (mean, 11.75 days; $p = 0.70$).

The db/db mice with wounds covered healed significantly more slowly than db/− mice with wounds covered (mean, 27.75 versus 11.86 days, respectively; $p < 0.001$). The db/db mice with wounds not covered healed more slowly (mean, 13 days) than db/− mice with wounds not covered (mean, 11.75 days), although this difference did not reach statistical significance ($p = 0.39$). Comparisons between wounds covered and not covered on db/− mice and between wounds covered on db/db and db/− mice were complicated by the fact that dressings frequently peeled off of db/− mice and remained in place on db/db mice.

Clinical versus Histologic Wound Closure

Wound closure as defined by gross examination was inaccurate, especially in wounds covered with a dressing. Wounds determined to be closed by gross examination showed that only 62.5 percent of wounds covered and 91.7 percent of wounds not covered on db/db mice were actually completely epithelized when histology was reviewed. Histology of wounds from db/− mice determined to be closed by gross examination showed 100 percent of wounds covered with a dressing and 87.5 percent of wounds not covered were completely epithelized. Therefore, we underestimated the time to closure as assessed by gross examination and harvested wounds before complete closure by epithelization.

Spread of Topically Applied Soluble Reagents

Blue histology dye applied daily to one wound did not spread to any of the three adjacent wounds on mice killed at 24 hours or 7 days after wounding (Fig. 2). The lack of reagent spread to adjacent wounds was also demonstrated in wounds harvested on day 7 after daily application of biotinylated laminin 5 or nonbiotinylated laminin 5. Fluorescent emission of streptavidin-Cy5 signal from the biotinylated laminin 5–treated wound bed was significantly greater than all other wounds ($p < 0.001$) (Fig. 3, *above*). Images of the wound beds with Cy5 immunofluorescence are shown in Figure 3, *below*. These results confirm that topically applied blue dye and biotinylated laminin 5 do not spread locally to adjacent wounds on the same mouse when applied daily for 7 days.

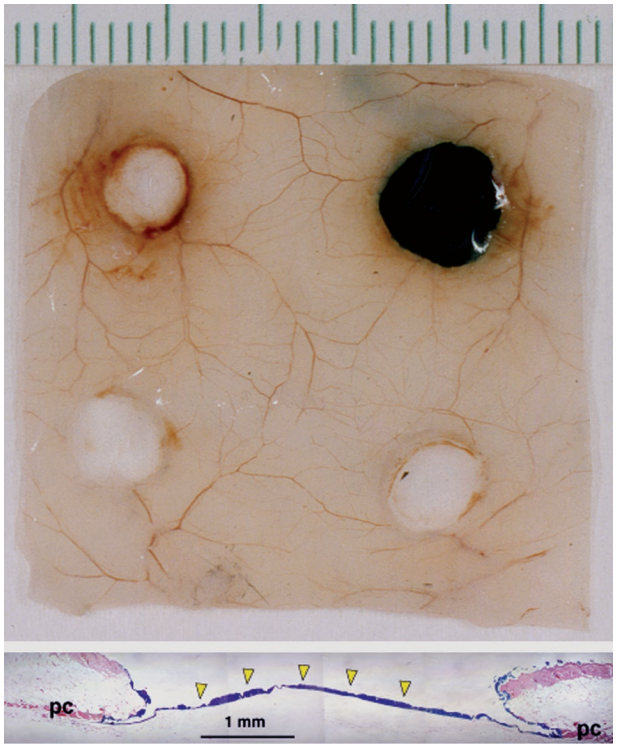

FIG. 2. (*Above*) View of subcutaneous surface of dorsal skin en bloc. Blue dye applied to wound 2 does not spread to any of the three adjacent wounds. (*Below*) Hematoxylin and eosin–stained section of wound confirms that the blue dye is retained in the wound bed. *Arrowheads* indicate wound bed and *pc* denotes the panniculus carnosus.

DISCUSSION

Many variables influence the outcome and interpretation of wound-healing studies in murine models. Unless the wound model is well characterized, the results of a wound-healing study may have less to do with the therapeutic intervention than some of the other variables described in this study. On the basis of our observations in this study, investigators should carefully consider methods of wounding, reproducibility of size and shape of wounds, use of control and treated wounds on the same animal, and effects of the wound dressing.

Many studies have examined wound healing in db/db mice using 1.5 × 1.5-cm excisional wounds.[4,21–23] The smaller 6-mm wounds heal more quickly than 1.5 × 1.5-cm wounds (28 days versus up to 50 days),[4,22] reducing experiment time and vivarium costs. Smaller wounds require less reagent, which can be expensive or in limited supply. Smaller wounds allow up to four wounds on the dorsal surface of a single mouse. Comparisons of multiple wounds should be limited to wounds that are in a similar cranial-caudal position because cranial-caudal differences in wound healing have been suggested.[24]

Reproducibility of wound size is also important in comparing treated and control wounds or wounds on db/db and db/− mice. We have found that wounds created with a 6-mm biopsy punch are uniform in size. Wounds on db/db mice were significantly larger after excision than wounds created on db/− mice. Wounds on db/db mice enlarge after excision, because of the obesity and distended skin of the db/db mouse, but were uniform, with a reproducible area. By contrast, wounds on db/− mice contract after excision. These data show that meaningful comparisons of endpoints such as time to closure between db/db and db/− mice are difficult to interpret. Comparisons among treatment groups in db/db mice are much more methodologically sound.

Measuring percent of wound healing at fixed time intervals after wounding[4–6] circumvents problems inherent in judging the clinical endpoint in time-to-closure studies. Image analysis software can be used for serial measurements of wound closure from photographs at fixed time points after wounding. With small

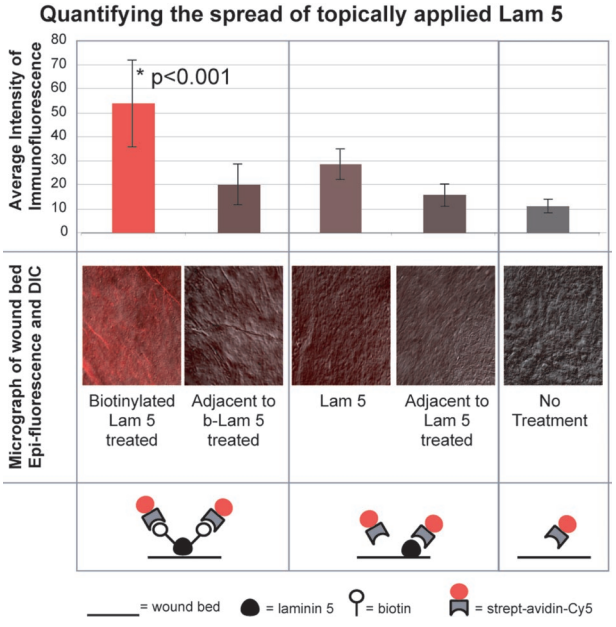

FIG. 3. (*Above*) Bar graph represents quantification of fluorescent emission of streptavidin-Cy5 remaining on the wound beds. Wounds treated with biotinylated laminin 5 had significantly greater signal than all other wounds ($p < 0.001$). Comparison of the wounds adjacent to the biotinylated laminin 5–treated wound to all other wounds revealed no significant differences. (*Below*) Differential interference contrast was used to reveal the surface of the wound bed and is shown as gray. The Cy5 fluorescence signal captured at this level of the wound bed is depicted in red.

wounds, however, slight errors in tracing the area of the open wound can result in large differences in the calculated percent closure and emphasize the need for evaluation by blinded observers.

The db/db mice with wounds covered by an adherent dressing heal more slowly than mice with wounds not covered. Given the magnitude of the difference in wound-healing rate, it is important that wounds in different treatment groups are dressed similarly. The healing time between wounds covered and not covered may be attributable to splinting by the adhesive dressing. Splinting by an adhesive has been previously described by Korula et al.[25] They found that skin grafts covered with an adhesive contracted at a significantly slower rate than wounds not covered. Splinted wounds rely more on epithelization than on contraction for healing. Splinting of the wound by an adhesive dressing, therefore, is a good choice for studies of keratinocyte migration with reagents such as laminin 5. Dressings retain soluble reagents on the wounds,[26] prevent introduction of salivary growth factors,[27] prevent contamination of control wounds from reagents applied to test wounds, and reduce infection.[28,29] Despite its being transparent, we found that Tegaderm obscures clinical evaluation of the wound bed and reduces accuracy in grossly determining complete epithelization.

Our observations about the lack of local spread of the blue histology dye and the biotinylated laminin 5 after daily application for 7 days demonstrates that treatment and control wounds can be placed on the same animal. This allows each animal to serve as it own control, reduces the number of animals needed for study, and reduces experimental time and cost. Other investigators have used both control and treatment wounds on the same db/db mouse[5,7,16] but have not reported whether topically applied reagents spread locally to affect control wounds.

CONCLUSIONS

The db/db mouse is an excellent model for studying delayed wound healing in an animal with features of type 2 diabetes mellitus. It is a particularly good model for the study of epithelial migration if an adhesive dressing is kept in place to splint the wounds open. This study validates the use of four 6-mm punch biopsy wounds per animal and points out the inherent difficulty of trying to compare wound-healing rates between db/db and db/− animals.

John E. Olerud, M.D.
Department of Medicine
Division of Dermatology
University of Washington
1959 N.E. Pacific Street
Box 356524
Seattle, Wash. 98195-6524
olerudje@u.washington.edu

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REFERENCES

1. Coleman, D. L. Obese and diabetes: Two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* 14: 141, 1978.
2. Mordes, J. P., and Rossini, A. A. Animal models of diabetes. *Am. J. Med.* 70: 353, 1981.
3. Debray-Sachs, M., Dardenne, M., Sai, P., et al. Anti-islet immunity and thymic dysfunction in the mutant diabetic C57BL/KsJ db/db mouse. *Diabetes* 32: 1048, 1983.
4. Greenhalgh, D. G., Sprugel, K. H., Murray, M. J., and Ross, R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *Am. J. Pathol.* 136: 1235, 1990.
5. Tsuboi, R., Shi, C. M., Rifkin, D. B., and Ogawa, H. A wound healing model using healing-impaired diabetic mice. *J. Dermatol.* 19: 673, 1992.
6. Ring, B. D., Scully, S., Davis, C. R., et al. Systemically and topically administered leptin both accelerate wound healing in diabetic ob/ob mice. *Endocrinology* 141: 446, 2000.
7. Lim, F., Karmacharya, J., and Gordon, A. Adenoviral-mediated angiopoietin-1 overexpression accelerates re-epithelialization in impaired wound healing. *Wound Repair Regen.* 9: 155, 2001.
8. Stenn, K. S., and DePalma, L. Re-epithelialization. In R. A. F. Clark and P. M. Henson (Eds.), *The Molecular and Cellular Biology of Wound Repair.* New York: Plenum Press, 1988. P. 321.
9. Olerud, J. E., Usui, M. L., Muffley, L. A., et al. Epithelial cells in the wound margins of diabetic ulcers are highly proliferative (Abstract). *J. Invest. Dermatol.* 114: 857, 2000.
10. Carter, W. G., Ryan, M. C., and Gahr, P. J. Epiligrin, a new cell adhesion ligand for integrin alpha 3 beta 1 in epithelial basement membranes. *Cell* 65: 599, 1991.
11. Lampe, P. D., Nguyen, B. P., Gil, S., et al. Cellular interaction of integrin alpha3beta1 with laminin 5 promotes gap junctional communication. *J. Cell Biol.* 143: 1735, 1998.
12. Matsuda, H., Koyama, H., Sato, H., et al. Role of nerve growth factor in cutaneous wound healing: Acceler-

ating effects in normal and healing-impaired diabetic mice. *J. Exp. Med.* 187: 297, 1998.
13. De Coninck, A., Draye, J. P., Van Strubarq, A., et al. Healing of full-thickness wounds in pigs: Effects of occlusive and non-occlusive dressings associated with a gel vehicle. *J. Dermatol. Sci.* 13: 202, 1996.
14. Dyson, M., Young, S., Pendle, C. L., Webster, D. F., and Lang, S. M. Comparison of the effects of moist and dry conditions on dermal repair. *J. Invest. Dermatol.* 91: 434, 1988.
15. Davidson, J. M. Wound repair. *J. Hand Ther.* 11: 80, 1998.
16. Coleman, D. L. Diabetes-obesity syndromes in mice. *Diabetes* 31: 1, 1982.
17. Snowden, J. M., Kennedy, D. F., and Cliff, W. J. Wound contraction: The effects of scab formation and the nature of the wound bed. *Aust. J. Exp. Biol. Med. Sci.* 60: 73, 1982.
18. Richey, K. J., Engrav, L. H., Pavlin, E. G., et al. Topical growth factors and wound contraction in the rat: Part I. Literature review and definition of the rat model. *Ann. Plast. Surg.* 23: 159, 1989.
19. Boyce, S. T., and Ham, R. G. Calcium-regulated differentiation of normal human epidermal keratinocytes in chemically defined clonal culture and serum-free serial culture. *J. Invest. Dermatol.* 81: 33s, 1983.
20. Carter, W. G., Kaur, P., Gil, S. G., Gahr, P. J., and Wayner, E. A. Distinct functions for integrins alpha 3 beta 1 in focal adhesions and alpha 6 beta 4/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: Relation to hemidesmosomes. *J. Cell Biol.* 111: 3141, 1990.
21. Atit, R. P., Crowe, M. J., Greenhalgh, D. G., Wenstrup, R. J., and Ratner, N. The Nf1 tumor suppressor regulates mouse skin wound healing, fibroblast proliferation, and collagen deposited by fibroblasts. *J. Invest. Dermatol.* 112: 835, 1999.
22. Gibran, N. S., Jang, Y. C., Isik, F. F., et al. Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. *J. Surg. Res.* 108: 122, 2002.
23. Yamamoto, T., Horikawa, N., Komuro, Y., and Hara, Y. Effect of topical application of a stable prostacyclin analogue, SM-10902 on wound healing in diabetic mice. *Eur. J. Pharmacol.* 302: 53, 1996.
24. Martson, M., Viljanto, J., Laippala, P., and Saukko, P. Cranio-caudal differences in granulation tissue formation: An experimental study in the rat. *Wound Repair Regen.* 7: 119, 1999.
25. Korula, P., Varma, S. K., and Sunderrao, S. Inhibition of wound contraction by point-to-point adherent splintage. *Plast. Reconstr. Surg.* 95: 725, 1995.
26. Cheng, C. Y., Martin, D. E., Leggett, C. G., Reece, M. C., and Reese, A. C. Fibronectin enhances healing of excised wounds in rats. *Arch. Dermatol.* 124: 221, 1988.
27. Hutson, J. M., Niall, M., Evans, D., and Fowler, R. Effect of salivary glands on wound contraction in mice. *Nature* 279: 793, 1979.
28. Wille, J. C., and Blusse van Oud Alblas, A. A comparison of four film-type dressings by their anti-microbial effect on the flora of the skin. *J. Hosp. Infect.* 14: 153, 1989.
29. Mertz, P. M., Marshall, D. A., and Eaglstein, W. H. Occlusive wound dressings to prevent bacterial invasion and wound infection. *J. Am. Acad. Dermatol.* 12: 662, 1985.