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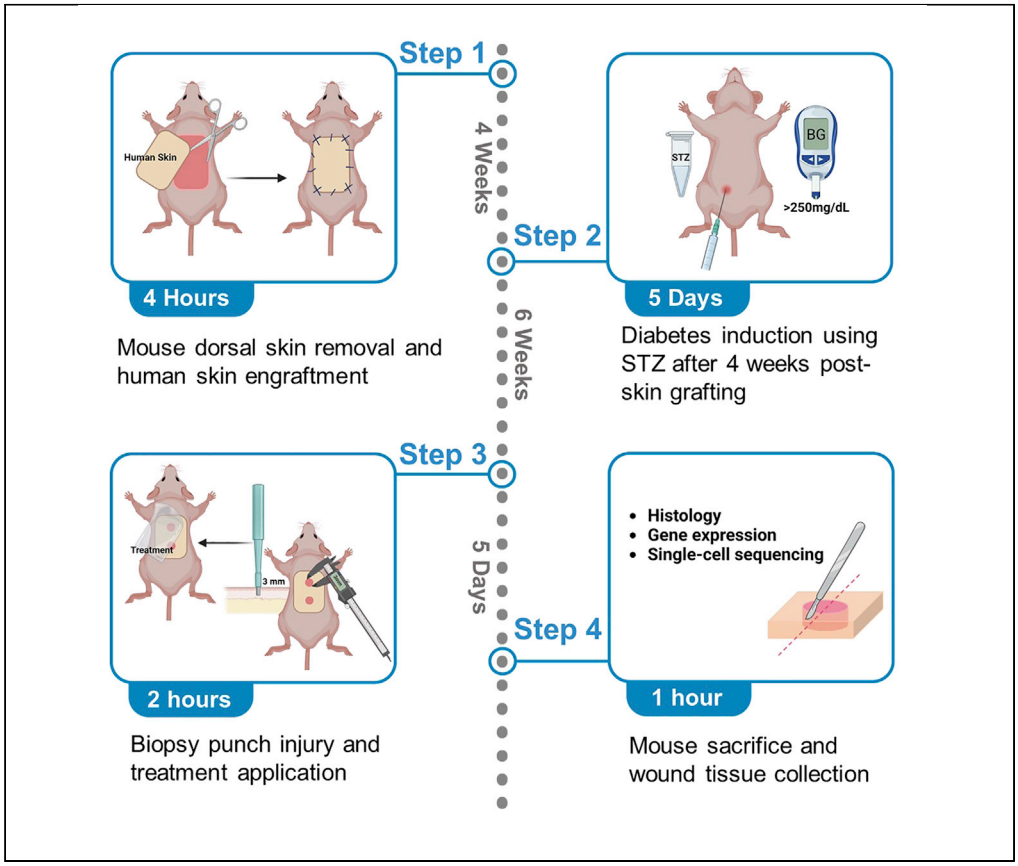
Protocol for xenotransplantation of human skin and streptozotocin diabetes induction in immunodeficient mice to study impaired wound healing



Here, we present a protocol for the integration of human skin onto the backs of diabetic immunodeficient mice, providing a versatile *in vivo* model for mimicking and studying mechanisms involved in impaired cutaneous wound healing. This protocol includes instructions for the grafting of human skin, induction of diabetes using streptozotocin and wounding/post-wounding care of immunodeficient mice, as well as suggested downstream tissue analyses. This preclinical mouse model can be used to validate the efficacy of newly developed wound dressings.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

An optimized preclinical model to study delayed cutaneous wound repair in diabetes

Step-by-step guide for transplanting human skin onto the dorsum of athymic nude mice

Procedure for inducing diabetes by using STZ and diabetic animals' post-grafting care

Application of treatments on engrafted human skin and collected wounds' analyses

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Protocol for xenotransplantation of human skin and streptozotocin diabetes induction in immunodeficient mice to study impaired wound healing

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SUMMARY

Here, we present a protocol for the integration of human skin onto the backs of diabetic immunodeficient mice, providing a versatile *in vivo* model for mimicking and studying mechanisms involved in impaired cutaneous wound healing. This protocol includes instructions for the grafting of human skin, induction of diabetes using streptozotocin and wounding/post-wounding care of immunodeficient mice, as well as suggested downstream tissue analyses. This preclinical mouse model can be used to validate the efficacy of newly developed wound dressings.

For complete details on the use and execution of this protocol, please refer to Theocharidis et al. (2022).[1]

BEFORE YOU BEGIN

The protocol below describes the specific steps for transplanting human skin onto the dorsum of athymic mice, inducing diabetes in these mice with streptozotocin (STZ), and handling diabetic mice. This wound healing model provides increased physiological relevance and can not only facilitate validation of different treatments in the context of impaired diabetic wound repair, but could also be used to study other cutaneous pathologies such as skin cancers, inflammatory skin disorders, and pressure ulcers.[2–7]

Institutional permissions

All institutional permissions for animal studies should be obtained. All animal experiments in this study were in accordance with the Institutional Animal Care and Use Committee (IACUC) at Beth Israel Deaconess Medical Center (BIDMC) under animal protocol number 062-2021. The human skin samples were obtained from IRB-approved discarded human skin from plastic surgeries from BioIVT (Westbury, NY). Their protocol is approved by WCG IRB under tracking number 20180798. Use of these samples in mice was approved under BIDMC Institutional Biosafety Committee (IBC) protocol #21-0027.

Others who plan to replicate this protocol will need appropriate approvals from their institution.

Human skin preparation

Timing: 30 min

1. Sterilize the human skin.

   Note: In this protocol, split-thickness human skin graft is recommended for better skin integration. Unlike full-thickness skin grafts, which are composed of the epidermis and full dermis layer, the split-thickness human skin includes the epidermis layer and a small section of the dermis layer. Split-thickness human skin grafts are cut with the dermatome set at 0.08 cm.

   a. Prepare 100 mL of betadine, 70% ethanol, and sterile phosphate saline buffer (PBS) in separate sterile containers under sterile conditions in a Biosafety Cabinet (BSC).
   b. Place the skin graft fully immersed in the container of Betadine and let soak for 1 min.
   c. Then, transfer to 70% Ethanol and let sit for 1 min fully immersed.
   d. Finally, transfer to PBS and let sit fully immersed for 1 min.

2. Remove fat from the skin graft.
   a. Remove the adipose layer with sterilized surgical scissors under the BSC.

   Note: A thin skin graft has a better chance of incorporating into the wound site, however it is important to be careful not to cut through the skin graft itself.

3. Cut skin into rectangular pieces with dimensions of 2 cm × 2.5 cm.

   Note: The human skin graft sizes may vary and depend on the available dorsum area of the mice and will not exceed an area of 12 cm^2.

   a. Store prepared human skin grafts in a vial of ice cold sterile PBS and keep the vial on ice.

   Note: We recommend preparing and grafting the human skin onto mice on the same day the human skin is received from surgery. However, the discarded human skin can be stored for up to 14 h at 4°Cwithout loss of viability.

Preparation on the day before grafting

Timing: 30–60 min

4. Prepare the isoflurane rodent anesthesia machine and secure the anesthetic gas mouse nose cone inside the BSC.

   Note: Isoflurane may be sustained with a concentration of 1%–2.5% during surgery. A higher concentration, 3%–4%, is given for induction of anesthesia.

   ⚠ CRITICAL: Make sure the regulator is threaded correctly and there are no leaks. The oxygen tanks need to be replaced if the pressure is less than 500psi. Remember to sanitize induction chambers with appropriate disinfectant solution after use (do not use alcohol or ammonia).

5. Set up an operation station in the BSC.
   a. Place sterile drapes next to the mouse nose cone.
   b. Set up an imaging station on the right side of BSC. Place a ruler under the camera for reference. We used a phone stand and a standard iPhone camera.
   c. Prepare Buprenorphine-SR (0.5 mg/mL) in a 1 mL sterile syringe and prepare six 25G × 5/8 needles. Prepare 30 μL per mouse.

d. Prepare alcohol swabs, povidone-Iodine preparation pads, sterile gauze, Tegaderm films, and Xeroform petrolatum dressing.
e. Prepare sterilized surgical scissors, tweezers, and 4-0 Polyglactin 910 sutures or 5-0 Monocryl.

Preparation on the day of STZ injection

Timing: 30 min

6. Prepare streptozotocin (STZ) solution in a tube with sterile water as described in the materials and equipment section.
   a. Cover the tube with tin foil to shield from light and keep on ice before injection.
7. Set up anesthesia machine as described in the previous section.
8. Prepare Buprenorphine-SR in 1 mL sterile syringe and 25G × 5/8 needles.
9. Prepare alcohol swabs, povidone-Iodine prep pads, and gauze.

⚠ CRITICAL: STZ must always be handled inside a certified chemical fume hood. Any unused STZ should be disposed as hazardous waste.

Preparation on the day before wound creation

Timing: 30–60 min

10. Set up anesthesia machine as described in previous section.
11. Set up the operation station in the BSC.
    a. Place sterile drapes next to the mouse nose cone.
    b. Set up imaging station on the right side of BSC. Place ruler under the camera for future reference.
    c. Prepare Buprenorphine-SR in 1 mL sterile syringe and 6–7 25G × 5/8 needles (20 µL/ mouse).
    d. Prepare 3 mm diameter sterile biopsy punches, alcohol swabs, povidone-Iodine prep pads, sterile gauze swabs, and Tegaderm film.
12. Prepare desired wound dressings.
13. Prepare sterile saline.

Preparation on the day before sacrifice

Timing: 30 min

14. Prepare digital camera imaging station, and calipers for measuring wounds.
15. Prepare 10% formalin solution and liquid nitrogen in appropriate liquid nitrogen container.
16. Label tin foil sections and tissue cassettes with appropriate sample names for further use.

⚠ CRITICAL: Safety glasses and chemical splash goggles are recommended during transfer and handling of liquid nitrogen to minimize injuries associated with splash.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human skin (from female human subjects age ~35–40 years old)	BioIVT	N/A
Chemicals, peptides, and recombinant proteins		
Buprenorphine-sustained release	ZooPharm, LLC	Cat# 1Z-74000-222014

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Streptozotocin (STZ)	Sigma-Aldrich	Cat# 18883-66-4
Human insulin	Humulin R	NDC 0002-8215-01 Serial No. D376262A
Critical commercial assays		
RNeasy Mini Kit	Qiagen	Ref. 74101
RT^2 First Strand Kit	Qiagen	Ref. 330404
SYBR Green ROX qPCR Master mix kit	Qiagen	Cat# NW16
Experimental models: Organisms/strains		
NU/J *Foxn1*^{nu} (Nu/J nude) Male, Age 6 weeks Homozygous for *Foxn1*^{nu}	Jackson Laboratory	Strain No. 002019
Oligonucleotides		
Col1a1 (mouse)	Qiagen	QT01055418
Col3a1(mouse)	Qiagen	QT00297094
Egf (mouse)	Qiagen	QT00151018
Tgfb1 (mouse)	Qiagen	QT00145250
Vegfa (mouse)	Qiagen	QT00160769
Fn1 (mouse)	Qiagen	QT00135758
Hgf (mouse)	Qiagen	QT00158046
Arg1 (mouse)	Qiagen	QT00134288
IL1b (mouse)	Qiagen	QT01048355
B2m (mouse)	Qiagen	QT01149547
COL1A1 (human)	Qiagen	PPH01299F
COL3A1 (human)	Qiagen	PPH00439F
EGF (human)	Qiagen	PPH00137B
TGFB1 (human)	Qiagen	QT00000728
VEGFA (human)	Qiagen	QT01010184
FN1 (human)	Qiagen	PPH00143B
HGF (human)	Qiagen	PPH00163C
GAPDH (human)	Qiagen	QT00079247
Software and algorithms		
ImageJ	Schneider et al.[8]	https://imagej.nih.gov/ij/
Other		
Blood glucose monitoring meter	Contour NEXT EZ	Item model #: 9628
Blood glucose test strips	Contour NEXT EZ	Lot: DW1EPEG05A
Sterilized surgical scissors	CYNAMED	N/A
Sterilized metal surgical tweezers	Mabis	N/A
Sterilized hemostat forceps locking tweezer clamps	Briggs	N/A
Disposable 3 mm biopsy punch	IntegraMiltex	Ref. 33-32
Isoflurane	FORANE	Serial No. 308728
Isoflurane induction chamber	N/A	N/A
BD alcohol swabs	BD	NDC/HRI3: 08290-3268-95
Povidone-iodine prep pad	PDI	REORDER No. B40600
Professional digital mini scale	TN-series	N/A
Xeroform petrolatum dressing	CURAD	Ref. CUR253590
Sterile towel drapes	Dynarex	REORDER No. 4410
Tegaderm dressing	3M	Ref. 1622W
16G needle	BD PrecisionGlide	Ref. 305197
1 mL syringe	BD	Ref. 309659
26G × 1/2 needle	BD PrecisionGlide	Re6f. 305111
25G × 5/8 needle	BD PrecisionGlide	Ref. 205122
4-0 (70 cm) Polyglactin 910 Suture coated VICRYL*Plus	ETHICON	Cat# J397H
5-0 Monocryl* (Poliglecaprone 25) suture	ETHICON	Cat# Y493G
Elastic adhesive tape	CURAD	Ref. NoN260402
Band-aid	Johnson&Johnson	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Sterile gauze sponges	Dukal	REORDER No. 6208
Sterile skin marker and fine tip with ruler	VISCOT medical	Ref. 1447XLSR-100
#15 blade scalpel	Feather	N/A
Digital calipers	Fisherbrand	Cat# 14-648-17
Betadine solution (10% Povidone-iodine)	Purdue Products	NDC 67618-150-01
DPBS (1×)	Gibco	Ref. 14190-144
Reagent alcohol	Fisher Chemical	A962P-4
Diet gel recovery	Clear H_2O	72-06-5022
Microscope slides	Fisherbrand	Cat # 22-037-246
Biological microscope (bright-field microscope)	Nikon	Model: ECLIPSE E200
Microscope Axio Imager.A2 Upright (fluorescent microscope)	ZEISS	Item # 490022-0009-000

MATERIALS AND EQUIPMENT

Human skin

Note: Typically human skin for xenografting is obtained from panniculectomy procedures. Skin with stretchmarks and/or bruising should be avoided.

Streptozotocin (STZ) Solution		
Reagent	Final concentration	Amount
STZ	15 mg/mL	22.5 mg
Sterile PBS	N/A	1.50 mL
Total	N/A	1.50 mL

Note: Prepare solution immediately before intraperitoneal (IP) injection. STZ powder should be kept at −20°C for long term storage. Keep STZ solution on ice and covered with tin foil and properly discard after use.

⚠ CRITICAL: STZ is used to induce diabetes in animal and known as carcinogen and a teratogen. Prepare STZ solution in chemical hood with proper PPE and safety training. Keep away from heat.

STEP-BY-STEP METHOD DETAILS

Excision and human skin grafting

Timing: 3 h/6 mice (for surgery)

Post-surgical human skin to mouse dorsum integration duration [4 weeks].

This step describes how to graft human skin onto the dorsum of nude mice and how to maintain the graft post-surgery (Figures 1 and 2A).

Note: To generate a humanized skin graft mouse model, nude male mice of 8–10 weeks old will be used as the recipient animals to ensure successful grafting and minimize immune rejection.

1. Anesthetize a mouse by placing it into the isoflurane induction chamber for 1–2 min. Then transfer the mouse to the nose cone that is fixed inside the BSC hood.

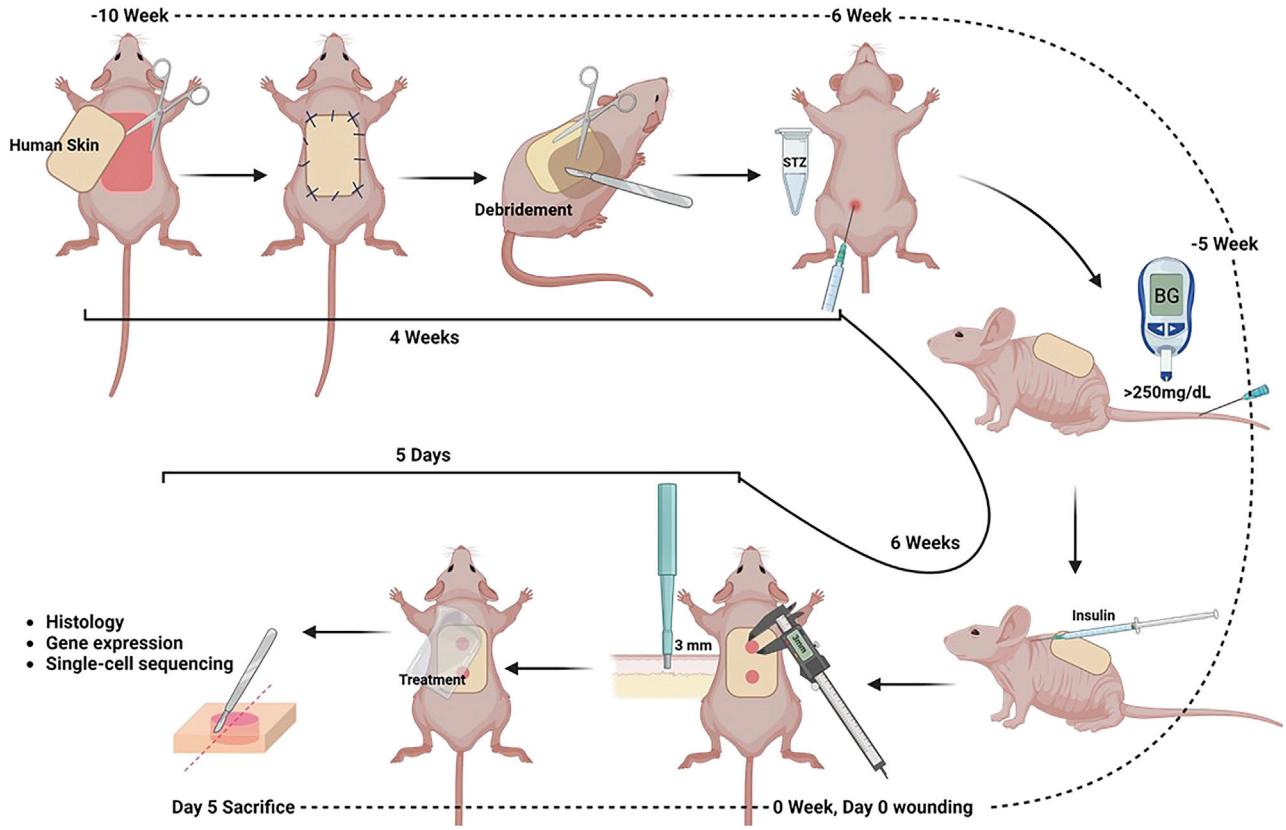

Figure 1. Schematic illustration for the xenotransplantation, STZ induction, and wounding procedures and timeline
Created with BioRender.com.

2. Pre-heat the mouse cage on a water circulating heat pad set at 37°C.
3. Disinfect the skin on the back of the mouse with Povidone-Iodine prep pad and follow with alcohol swabs. Repeat twice more.
4. Subcutaneously inject 20 μL of buprenorphine –Sustained Release (SR) with a 25G needle.
5. Use a sterile skin marker to mark the excisional area of 2 cm (width) × 2.5 cm (length).
6. Perform a full-thickness skin excision on the marked surgical site using sterile forceps and scissors.
7. Place the prepared human skin graft on the surgical site of the mouse.
8. Secure the skin graft onto the dorsum of the mouse using 5-0 Monocryl or nylon sutures.
   a. Place one stitch for each corner first.
   b. Use two stitches for each of the shorter sides.
   c. Use three stitches for each of the longer sides.

   Note: Interrupted stitches are recommended as opposed to continuous stitches to minimize the chance of the mouse tearing off the graft.

9. Perform a sterile tie-over bolus dressing technique (Figure 2).
   a. Place the non-adhesive petroleum jelly gauze (Xeroform) on top of the graft.
   b. Place a dry bandage on top of the Xeroform.
   c. Wrap self-adhesive tape around the mouse's mid-section and over Xeroform and dry bandage layers. Wrap until the wound dressing is fixed.
10. Place the mouse back into the pre-heated cage and keep the cage on the heating pad until the mouse wakes up.

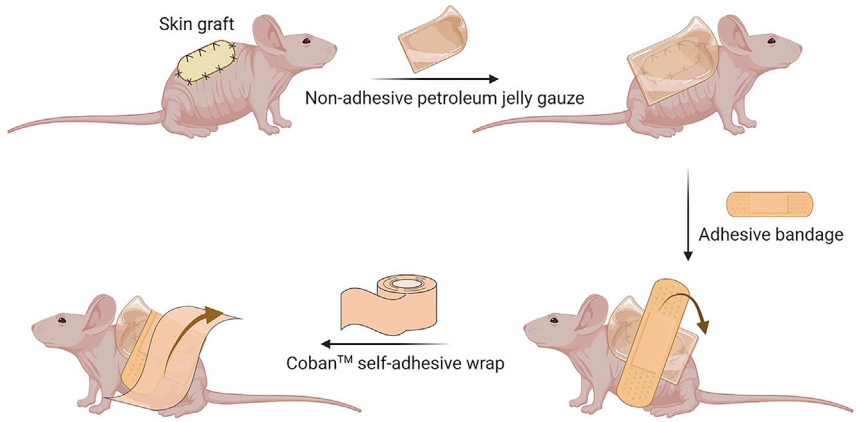

Figure 2. Schematic illustration for the sterile tie-over bolus dressing technique
Created with BioRender.com.

11. Monitor post-surgically mice every day to ensure that the dressing stays on top of the graft site. If the dressing is removed, the mouse will need to be anesthetized and the dressing retied.
12. Remove the dressing under anesthesia on the 7th day after grafting.
13. Monitor for healing, scar formation and graft acceptance/rejection every day.

Note: If at any time post-grafting we deem that the unincorporated graft is detrimental to the animal's safety regarding infection or necrosis, a serial debridement on a weekly basis can be performed under anesthesia to ensure a safe outcome for the mice: a) Subcutaneously administer 20 μL of Buprenorphine-SR; b) Debride the human skin using #15 blade scalpel.

Note: Euthanize the mouse if the skin is found to be infected.

14. Grafted mice will be left to heal for 4–6 weeks so the human skin graft can be fully integrated.
15. Grafted mice will be monitored daily by visual examination, and weekly by digital photography.
    a. Mice should be single-housed and appropriately monitored for hypothermia, weight loss, signs of distress.
    b. Be aware that restrictive bandages may cause respiratory distress. If mouse shows any sign of breathing complication, rapidly remove the bandages.
    c. If mice are hypoactive, not eating/drinking, weak, and/or are not nesting, Buprenorphine-SR should be administered to alleviate pain and Diet Gel should be provided to nurture animal.

Note: Accepted skin grafts will show signs of slight wound contraction along the human skin with re-epithelialized edges. Successful grafts have robust capillary refill without evidence of venous congestion.

STZ induction

Timing: 1 h/12 mice, 5 consecutive days (for injection)

Hyperglycemic state duration time prior to wounding: [6 weeks].

This step describes how to perform diabetes induction by administering Streptozotocin (STZ) and how to take care of diabetic nude mice (Figure 1).

Note: Research staff must notify animal resource facility supervisor before STZ induction begins.

16. Prepare STZ solution as described in materials and equipment.

Note: Always prepare STZ solution fresh.

17. Anesthetize grafted mouse by placing the mouse into the isoflurane induction chamber for 1–2 min. Then transfer the nude mouse to the nose cone that is fixed in BSC hood.

Note: We recommend injecting under anesthesia to minimize handling of the transplanted human skin.

18. Pre-heat the autoclaved mouse cage on a water circulating heat pad at 37°C.
19. Disinfect mouse back skin with Povidone-Iodine prep pad, then using alcohol swabs.
20. Subcutaneously inject 20 μL of Buprenorphine-SR with a 25G needle.
21. Draw STZ solution (15 mg/mL) into 1 mL syringe with a 25G needle and place it on the side of the BSC.

Note: We recommend drawing up 500 μL of STZ solution and keep the rest on ice.

22. Turn mouse over so that their abdomen is facing up.
23. Disinfect the abdomen skin with Povidone-Iodine prep pad, then alcohol swabs.
24. Administer STZ solution by performing intraperitoneal injection.
    a. Give a dose of 55 mg/kg (0.11 mL of a 30 g mouse) once a day for 4 consecutive days. On the 5^{th} day, give a 100 mg/kg dose.

⚠ CRITICAL: Typically, the injection site will be in the animal's lower right quadrant of the abdomen to avoid damage to the urinary bladder, cecum, and other abdominal organs. However, the injection site should be alternated between the lower left and the lower right quadrants of the abdomen to avoid repeated trauma to the same area after consecutive injections.

    b. Insert needle at a 30–40 degree angle and gently pull back plunger prior to injecting to observe contents.

Note: 1) Aspiration of green material likely indicates that the bowel has been punctured, while aspiration of yellow, liquid material may indicate the bladder has been punctured. 2) The presence of blood indicates an abdominal blood vessel has been punctured. 3) If blood, urine or gastrointestinal contents are drawn back into the hub of the needle, remove the needle from the animal. The syringe/syringe contents and needle must be discarded.

    c. Inject STZ solution.

Note: The injection volume varies in accordance with the body weight of each individual mouse.

    d. Replace the needle with a new 25G needle for next mouse injection.
25. Subcutaneously inject 300 μL–500 μL sterile saline to keep mouse hydrated.
26. Place the mouse back into the pre-heated cage and keep the cage on the heating pad until the mouse wakes up.
27. Monitor mice daily after STZ injection for any signs of pain, illness, and distress.
    a. Administer sterile saline subcutaneously if mice present weight loss of 5%–10% body weight.

⚠ CRITICAL: Closely follow the institutional guidelines on use of STZ in rodents.

28. Confirm that the mice are in diabetic state.
    a. Fast mice for 5 h on the 7th day after the last STZ injection.
    b. Prick the mouse's tail using a 25G needle and use a standard blood glucose (BG) meter to measure fasting blood glucose.
    c. A BG value that is higher than 250 mg/dL is considered diabetic.
29. Monitor mouse BG weekly.
    a. Administer insulin every other day if 3 consecutive BG measurements exceed 500 mg/dL.

Note: The dose and frequency of insulin administration (range: 0.1–1.2 U/kg daily) should be increased or decreased in response to a combination of measured BG and body weight. Mice will also be appropriately monitored for any signs of pain or distress.

30. Mice should sustain a hyperglycemic state for 6–8 weeks prior to the wounding experiment.

Wounding experiment

Timing: 2 h

This step describes how to perform a full-thickness punch biopsy injury on the grafted human skin and place selected dressing (Figure 3B).

Note: After the 6–8 weeks of sustained diabetic state, one or more wounds will be inflicted on the transplanted human skin. Wound size can range from 2 mm to 6 mm in diameter using appropriate biopsy punches. In this protocol, we use 3 mm punch biopsies to inflict two wounds per mouse.

31. Anesthetize human skin grafted mouse by placing it into the isoflurane induction chamber for 1–2 min, then transfer the nude mouse to the nose cone that is fixed in the BSC.
32. Pre-heat the autoclaved mouse cage on a water circulating heat pad at 37°C.
33. Have treatment dressing and sterilized PBS ready in the BSC.
34. Disinfect the back skin with Povidone-Iodine prep pad, then alcohol swabs.
35. Subcutaneously inject 20 μL of Buprenorphine-SR with a 25G needle prior to wounding.
36. If needed, use sterile tweezers and scissors to debride the top layer of regenerated skin to have fresh healthy skin for wounding.
37. Perform full thickness punch injuries at the center of the human grafted skin area using a biopsy punch.
38. Place the wounded mouse under digital camera set up in the BSC for imaging.
39. Measure the initial (Day 0) wound size using digital calipers and record the value for wound closure analysis.
40. Place choice of dressing immediately on the wounds.

Note: Treatment options such as patches, drug-eluting bandages,[9] or other newly developed therapeutic approaches can be applied to the wounded human skin to study healing.

41. Cover with Tegaderm, an occlusive dressing, for protection.
42. Place the mouse back into the pre-heated cage and keep the cage on the heating pad until the mouse wakes up.
43. Closely monitor mice daily for any signs of pain and/or distress, weight loss, skin wound complications, or infections until the day of sacrifice.

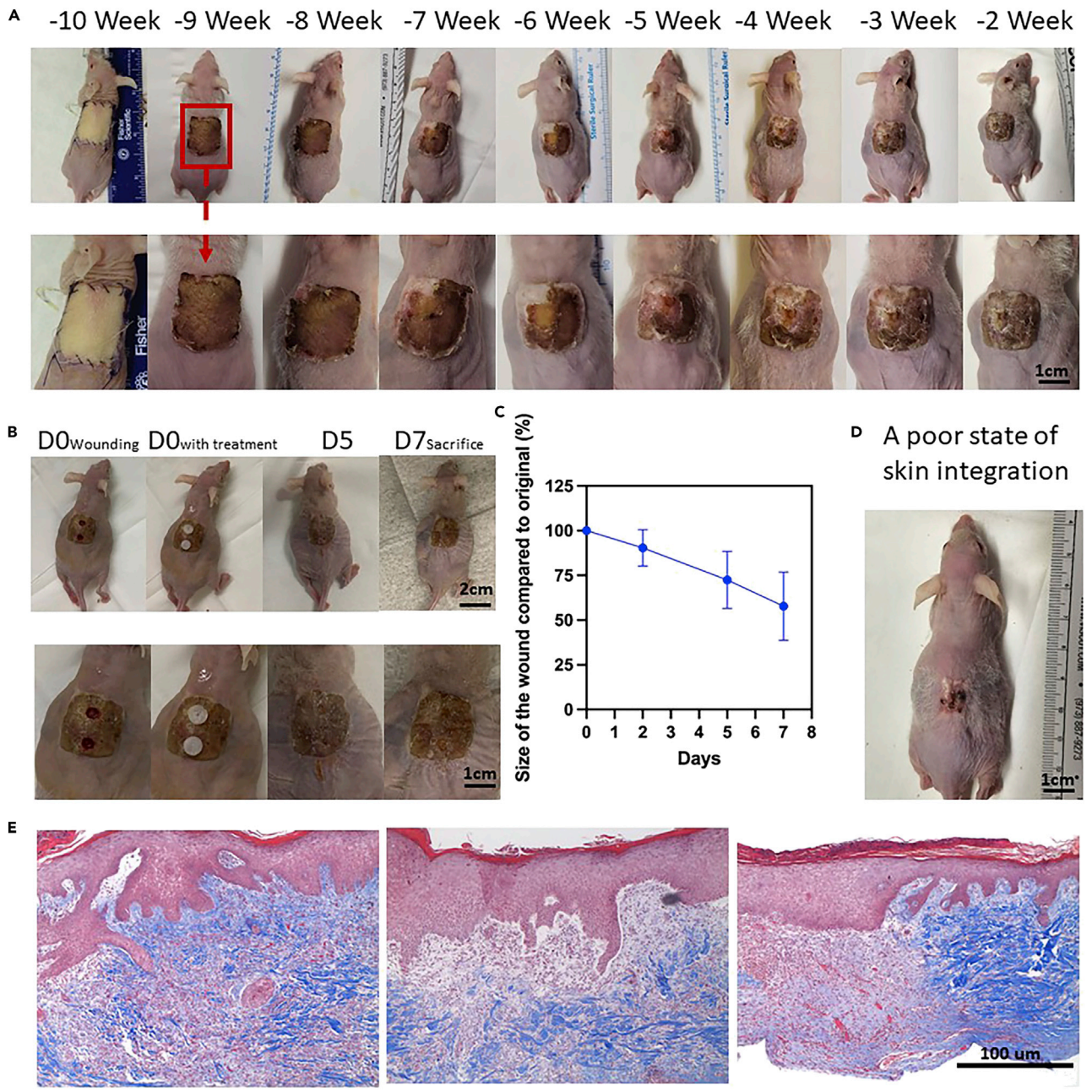

Figure 3. Representative images of xenotransplantation progress and wounding induction and quantitation

(A and B) (A) Human transplanted skin over 10 weeks, (B) representative images of post-wounding skin on days 0, 5, and 7 with an example of treatment: alginate hydrogel.

(C) The wound healing curve for alginate hydrogel treated wounds from Day0 to Day7. Data represent the mean and standard deviation values from n = 6 wounds.

(D) Representative images of skin integration in a poor state.

(E) Representative Day7 MTS histology images for the expected outcome.

Note: In this protocol, we sacrifice mice on day 5 after wounding. Due to their small wounds, we recommend sacrificing mice no later than day 5 for better results in histology and molecular analysis.

Wound tissue collection for histological and molecular analysis

Timing: 1 h

This step describes how to collect wound tissue for histological and molecular analysis.

44. Sacrifice the mice according to institutional guidelines.
    a. Euthanize mice with excess CO_2 on day 5–7.
    b. Perform cervical dislocation after euthanizing for confirmation of death.
45. Gently remove Tegaderm and wound dressing.
46. Place mouse under digital camera set up on the counter for imaging.
47. Measure wound size using digital calipers and record for wound closure quantification.
48. Excise the wound tissue with forceps and scissors including approximately 2 mm of uninjured skin around the wound.
49. Bisect the wound by using a #15 blade scalpel.
    a. Wrap one piece completely in prepared and labeled tin foil sheet and immerse into liquid nitrogen to snap-freeze.
    b. Place the other piece in prepared and labeled cassettes, then submerge the cassettes in 10% neutral buffered formalin.
50. Store snap-frozen tissues in −80°C for future molecular analysis.
    a. For gene expression, first extract RNA from snap-frozen wound tissue using mRNeasy Mini kit.
    b. Then synthesize cDNA using RT^2 First Strand kit/or miScript SYBR green PCR kit.
    c. Perform real-time PCR using RT^2 qPCR Primer assay/or miScript Primer Assay.

Note: We recommend selecting both mouse and human genes when studying wound healing on human grafted skin as the wound tissue includes both human and mouse cells.

51. Change the 10% formalin solution to 70% ethanol after cassettes have incubated in formalin for 48 h.
52. Submit the fixed tissue to histology core for paraffin-embedding and tissue cutting onto slides.

⚠ CRITICAL: Care should be taken to correctly orient the wounds during embedding, so that each microtome section includes the entire length of the wound with the tissue layers and different structures discernible.

    a. Use sections of 5 μm thickness and perform Masson's trichrome stain (MTS) and hematoxylin and eosin (H&E) staining.
        i. Quantify re-epithelialization from MTS or H&E images by measuring the length of the epithelial tongue covering the wound and dividing by the entire length of the wounds.
        ii. Quantify the thickness of the migrating epithelial tongue.
        iii. Quantify granulation tissue area using MTS images.
    b. Perform immunofluorescence staining for α-smooth muscle actin, CD31 or other markers of choice.
        i. De-paraffinize tissue section and retrieve antigens of paraffin-embedded tissue sample using buffer solutions according to the antibodies used.
        ii. Follow the general immunofluorescence stain guidelines to incubate section with primary and secondary antibodies at optimized concentrations and timings.
        iii. Obtain images of stained slides at X10 or X20 magnification with appropriate microscopy (bright-field microscope or fluorescent microscope).
        iv. Use ImageJ to quantify cells/structures positive for specific markers.

53. Quantify wound closure using both FIJI/Image J and caliper measurements (Figure 3C).

Note: Express the wound closure result as percentage healed at sacrifice compared to wound size on day 0.

EXPECTED OUTCOMES

The human skin xenografted immunodeficient nude mouse (humanized mouse) model is recognized as an important preclinical model to study different physiological conditions of the skin.[5] Our developed protocol was used for investigating mechanisms involved in impaired diabetic wound healing and enabled us to extend our observations on the therapeutic effects of a strain-programmable patch beyond standard preclinical murine and porcine models.[1] Unlike previous humanized mouse models where cultured bioengineered skin equivalents, such as fibrin containing live fibroblasts were employed,[10] the use of surgery collected human skin confers the advantage of including native human skin architecture and cell types, gene expression programs and complex signaling pathways that are involved in the healing process. This allows us to take one step closer to recapitulating the human skin functional wound healing response.

One feature that is associated with the humanized mouse model is the contraction of grafted human skin after integration. Within the first 4 weeks after human skin grafting (Figure 3A), a time frame considered as acute healing of the graft, the mouse excisional edge begins to incorporate into the human skin graft. Scabs form on the human skin graft within 2 weeks post-grafting, thus, debridement is necessary to stimulate healthy granulation tissue to grow. We have observed graft shrinking post-grafting. Nevertheless, the final skin graft was sizable enough to allow two 3 mm diameter wounds. (Figure 3B) Graft size remained unaltered after STZ induction. The size of applied wound dressing should be adjusted based on the wound size. Here, we applied blank alginate hydrogels[11] to the wounds and continuously monitored wound size on day 2, 5, and 7 post-wounding. The healing curve in Figure 3C and representative Masson's Trichrome Stain images in Figure 3E confirmed that our developed humanized mice is a practical and feasible animal model for studying diabetic wound healing. Nonetheless, human skin transplantation on the mouse dorsum has been challenging due to known potential complication, such as formation of a hypertrophic scars.[12] Figure 3D is a representative image of a poor state of skin integration, which is difficult to use for excisional wounding. However, in our experience, the split-thickness skin grafts outperformed full-thickness in terms of tissue integration and amount of available human viable skin for subsequent injury induction.

Using this model not only are researchers able to determine treatment effects on wound repair by measuring the appropriate healing curve, but they can also investigate gene and protein expression, as well as tissue morphology with histology. We performed a real time polymerase chain reaction on collected human skin grafted wounds after day 7 sacrifice and our *delta* CT values are shown in Table 1. Both human and mouse genes that are associated with cutaneous wound healing were found to be expressed on day 7. We employed the humanized mouse model to validate the strain-programmed patch developed and described in our recent publication.[1] The patch treated wounds demonstrated re-epithelialization by day 5 and had higher gene expression in human growth factors: VEGFA, FGF2, EGF and EGR1. The humanized STZ induced diabetic mouse model could support any potential investigation on diabetic wound healing, tissue engineering, and regenerative medicine. It is amenable to all different types of wound dressings or other types of therapeutic approaches.

LIMITATIONS

While the use of STZ has been shown to induce diabetes in athymic mice it is not without its complications. Intraperitoneal injections should be done by a trained researcher to avoid rupturing into vital organs. Sometimes a high dose of STZ is needed to make the animals diabetic. The animals should ideally be well hydrated prior to the injections to reduce the risk of renal and liver injury. However some animals may develop liver and kidney failure. These animals should be assessed to

Table 1. List of mouse and human genes to detect in the human skin grafted diabetic nude mice and their dCt values (mouse genes were normalized to B2m and human genes were normalized to GAPDH)

Human genes	Average dCt (target gene to B2m) with n = 8
VEGFA	3.32
TGFB1	3.29
COL1A1	−1.45
COL3A1	0.84
EGF	9.45
FN1	1.70
HGF	11.33
Mouse genes	**Average dCt (Target gene to GAPDH) with n = 8**
Arg1	5.42
Il1b	3.34
Vegfa	8.46
Tgfb1	8.48
Col1a1	5.38
Col3a1	−2.63
Egf	15.15
Fn1	3.84
Hgf	10.52

determine need for further veterinary care or euthanasia. In addition, the split-thickness human skin collected from panniculectomy procedures only reflects the feature of healthy human skin of a specific anatomical location (abdomen). Skin obtained from the lower extremity of patients with diabetes could potentially better mimic the impaired diabetic wound healing condition.

TROUBLESHOOTING

Problem 1

Premature removal of post-operative dressing by animals or production of large seroma (See step 12).

Potential solution

An ideal dressing will apply gentle pressure evenly over the skin graft while not impeding the rodent's ability to move or breathe. Without this dressing the skin graft may not adhere properly or develop a large seroma. If the mouse does not tolerate a fully circumferential dressing under anesthesia, sutures can be used to bolster the dressing. To do this you will need four 2-0 silks. Take full thickness bites at 12, 3, 6 and 9 o'clock about 1 cm from the edges of the skin graft. Cross the free ends over the dressing and tie securely to the sutures on the opposite side to bolster the dressing.

Problem 2

Small areas of necrosis on the skin edges (See step 13).

Potential solution

If the skin graft is too thick or not grafted in a timely manner it is possible the skin edges or anterior surface of the graft may show signs of necrosis. If this happens it may require gentle sharp debridement. Ideally wait 7–10 days after grafting prior to debriding the wound. Use a pair of sharp fine scissors and forceps to gently raise the necrotic tissue free. If properly adhered the basal layer of the skin graft should be viable and appropriate for use.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Aristidis Veves (aveves@bidmc.harvard.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate any unique datasets or code.

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AUTHOR CONTRIBUTIONS

Z.L., B.S., I.M., and G.T. wrote the manuscript with help from all co-authors. G.T., M.C., and E.W. developed and modified the protocol described here. All authors performed and analyzed the experimental part corresponding to this protocol. A.V. supervised the study. All authors commented on and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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