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Generation of a Full-Thickness Human Skin Equivalent on an Immunodeficient Mouse

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Abstract

Human skin equivalents composed of epidermal cells and fibroblasts are important for modeling human epidermal development, testing new therapeutics, and designing novel treatment strategies for human skin diseases. Here, we describe a procedure for the generation of an *in vivo* full-thickness human skin equivalent on an immunodeficient mouse using a grafting chamber system. The protocol involves mixing human epidermal cells and fibroblasts in a silicone grafting chamber that is surgically inserted onto the muscle fascia of a recipient immunodeficient mouse. Following the removal of the silicone chamber, the graft area is exposed to air to induce stratification of developing epidermis, resulting in the reconstitution of full-thickness human skin tissue on a live mouse. This grafting system provides a straightforward approach to study human skin diseases in an animal model and has been previously used to determine the ability of both mouse and human primary epidermal cells and cells derived from pluripotent stem cells to regenerate functional skin *in vivo*.

Keywords

Xenograft; Human skin graft; Mouse model; Grafting chamber; Keratinocytes; Epidermal cells; Fibroblasts

1. Introduction

The skin is a complex organ that serves to protect the body from external insults, dehydration, and infection while also aiding with thermoregulation and sensory functions (1,2). Despite extensive research, many basic science questions related to human skin development and mechanisms underlying human skin diseases remain unanswered. This is primarily due to significant differences in the structure of the skin between humans and

rodent models commonly used for studying the skin. Therefore, the ability to generate *in vitro* and *in vivo* human skin equivalents with functional epidermal and dermal functions is critical for studying human skin development and skin diseases, as well as developing novel therapies for many human skin conditions (3). While both *in vitro* and *in vivo* human skin equivalents are widely used in research, *in vitro* skin models often fail to undergo complete maturation in culture (4). Therefore, skin equivalents generated *in vivo* may allow for a more faithful recapitulation of human skin structure and disease phenotypes (5–7). Such *in vivo* human skin graft models involve either the direct transplantation of human skin onto the backs of immunodeficient mice (5,6) or the reconstitution of human skin within grafting chambers implanted on the backs of immunodeficient mice from a suspension of skin cells that includes keratinocytes and fibroblasts (3,7).

This chapter provides a detailed protocol for the reconstitution of human skin on the back of a NOD-SCID mouse using cultured primary human epidermal cells (also termed as keratinocytes) and fibroblasts in the grafting chamber system (8). Primary human skin cell lines suitable for grafting may be obtained either directly from biorepositories, such as ATCC or Lonza, or derived from patient skin samples. In addition, keratinocytes and fibroblasts differentiated from human pluripotent stem cells have been successfully used in a similar grafting chamber assay, providing an alternative source of cells for human skin modeling (7). While fibroblasts and keratinocytes are main cell types necessary for the successful generation of full-thickness human skin equivalents in the chamber system, other skin cell types may also be included, such as melanocytes, dermal papilla cells, and endothelial cells, increasing the complexity of the structure of skin equivalents for disease modeling and basic science research.

NOD-SCID mice used in the provided protocol possess a homozygous *Prkdc^{scid}* (SCID) mutation that results in a severe combined immune deficiency (9), making these mice ideal as human cell recipients due to a low risk of graft rejection. Interestingly, while attempting the grafting-chamber assay on a more severely immunocompromised NOD-SCID gamma (NSG) mouse strain (10), we repeatedly failed to achieve human skin reconstitution. Therefore, the NSG strain may not be well suited for the described grafting procedure. The use of other mouse strains, such as *Foxn1^{nu}* (nudes), in this protocol requires further study.

2. Materials

2.1 Cell lines and Culture

1. Primary keratinocytes (5×10^6 per graft) and fibroblasts (5×10^6 per graft). The number of cells per graft may need to be optimized if the cells are derived from pluripotent stem cells.
2. EpiLife™ medium with 60 μ M calcium (Thermo Fisher Scientific) supplemented with 1x EpiLife™ Defined Growth Supplement (EDGS) (Thermo Fisher Scientific) and 1x HyClone Antibiotic/Antimycotic (Fisher Scientific).
3. Human dermal fibroblast medium (HDF-M): supplement Gibco™ Minimum Essential Medium (MEM) with 10% raw US origin qualified Gibco™ Fetal Bovine Serum (FBS), 1x MEM Non-Essential Amino Acids Solution (MEM-

NEAA), 1x GlutaMAX™ supplement, 55 µM Gibco™ 2-mercaptoethanol (all from Thermo Fisher Scientific), and 1x HyClone Antibiotic/Antimycotic (Fisher Scientific).

4. Eagle's Minimum Essential Medium (EMEM) (ATCC).
5. Bovine Collagen Solution, Type I, 3 mg/mL (Advanced BioMatrix).
6. Trypsin-EDTA (0.05%), phenol red (Thermo Fisher Scientific).
7. Accutase™ (STEMCELL Technologies).
8. 1x Gibco™ DPBS without calcium and magnesium (Fisher Scientific).
9. Chelexed FBS prepared as previously described (8,11).
10. 10 cm tissue culture treated culture dishes (Corning Incorporated).
11. 15 mL conical tubes (Corning Incorporated).
12. Whatman® general purpose filter paper (Millipore).

2.1 Grafting Procedure

1. Silicone grafting chambers produced by Qure Medical, 1810 Renaissance Boulevard, Sturtevant, WI, 53177, (262) 417-1307, <http://www.quire-med.com>, according to the following dimensions: 12 mm inner diameter (ID), 20 mm outer diameter (OD), 10 mm tall (Part# 12-24, upper chamber). Chambers are made of Momentive LSR 2650, two-component liquid silicone rubber for injection molding processes (Fig. 1). The chambers are reusable and autoclavable. Manually punch three holes in the dome of the chamber using a 2 mm biopsy punch to allow for gas exchange during the time the chamber is in place under the skin.
2. NOD.CB17-Prkdc^{scid}/J mice as recipients for grafting (The Jackson Laboratory). Obtain an IACUC approval before performing surgery.
3. Surgical tools: forceps, scissors (blunt edge), WAHL 9861-900 Pocket Pro Universal Trimmer, PDI Prevantics antiseptic skin swabsticks (Fisher Scientific), Reflex brand 9 mm wound clip system and clip remover (Roboz).

2.3 Graft Harvesting

1. 10% formalin, buffered (Fisher Scientific).
2. 100% ethanol (EtOH), diluted to 70%

2.4 Equipment

1. Biological safety cabinet.
2. 37°C water bath.
3. 37°C/5% CO₂ humidified tissue culture incubator.
4. Centrifuge (room temperature).

5. Animal cage heating pad.

3. Methods

To generate one full-thickness graft in vivo, 5×10^6 of primary human keratinocytes (approximately four 10 cm culture dishes) and 5×10^6 primary human fibroblasts (approximately four 10 cm culture dishes) will need to be grafted in a chamber. It is critical not to let keratinocytes and fibroblasts exceed 70–80% confluency. All cell culture-related procedures are to be performed in a biological safety cabinet using aseptic techniques.

3.1 Culturing Keratinocytes

3.1.1. Coating Tissue Culture Dishes with Collagen

1. Dilute bovine collagen stock solution (3 mg/mL) to a final working concentration of 30 µg/mL in 1x DPBS. For each 10 cm dish, resuspend 50 µL of the stock in 5 mL of 1x DPBS in a 15 mL conical tube. Pipette to mix.
2. Add 5 mL of diluted collagen solution to a 10 cm tissue culture dish.
3. Rock back and forth to ensure complete coverage.
4. Place in 37°/5% CO₂ incubator for 1 h.

3.1.2. Thawing and Maintaining Keratinocytes

1. Allow EpiLife medium to equilibrate to room temperature.
2. Add 9 mL of EpiLife medium to a collagen-coated 10 cm dish, and set aside in a biological safety cabinet.
3. Remove the vial with approximately 5×10^5 frozen keratinocytes from a liquid nitrogen storage tank (see Note 1). Thaw quickly in a 37°C water bath (approximately 1 min), taking care to avoid contamination by keeping the cap above the water level.
4. Spray the entire vial containing thawed keratinocytes with 70% ethanol, and proceed under strict aseptic conditions in a biohazard safety cabinet.
5. Pipette 5 mL of EpiLife into a 15 mL conical tube, and transfer the entire content of the vial containing thawed keratinocytes into this conical tube. Pipette to mix.
6. Spin the cells down at 150 x g for 5 min.
7. Gently resuspend the pellet in 1 ml of EpiLife medium, and transfer the entire cell suspension dropwise to the 10 cm dish filled with the pre-equilibrated EpiLife medium from step 2 above.
8. Incubate the dish overnight in a 37°C/5% CO₂ tissue culture incubator to allow the cells to attach.
9. The next day, aspirate the medium without disturbing the keratinocyte monolayer, and replace with 10 mL of fresh, pre-warmed EpiLife. Continue

changing EpiLife medium every other day until cells reach approximately 70–80% confluency, at which point the cells need to be passaged (*see* Note 2).

3.1.3. Keratinocyte Subculture

1. Allow Accutase™ detachment solution and EpiLife medium to equilibrate to room temperature.
2. Add 10 mL of EpiLife medium to a new collagen-coated 10 cm dish, and set aside in a biological safety cabinet. Prepare up to four plates to obtain a sufficient number of keratinocytes for one graft.
3. Take the dish with keratinocytes from the tissue culture incubator, and aspirate spent media without disturbing the cell monolayer.
4. Add 5 mL of DPBS, gently rocking the dish to wash the cells; aspirate the DPBS.
5. Add 3 mL of Accutase™ detachment solution, and incubate at 37°C for 4–8 min (*see* Note 3).
6. After incubation, add 3 mL of EpiLife medium to neutralize Accutase™.
7. Tilt the dish, and gently pipette to wash the surface of the dish, removing cells from the dish surface and into suspension. Avoid forming bubbles.
8. Transfer the dissociated cells into a new, sterile 15 mL conical tube.
9. Centrifuge cells down at 150 x g for 5 min.
10. Resuspend cells in fresh EpiLife medium and count cells using a hemacytometer.
11. Transfer approximately 3×10^5 keratinocytes to each collagen-coated 10 cm dish with pre-equilibrated EpiLife from step 2 above.
12. Place the dishes with freshly seeded keratinocytes into the incubator, and leave undisturbed for at least 24 h while cells attach to the dish.
13. Change medium by aspirating spent medium and adding 10 mL of fresh, pre-warmed EpiLife medium every other day until cells are ready for passaging or grafting (70–80% confluency).

3.2 Culturing Fibroblasts

3.2.1. Thawing and Maintaining Fibroblasts

1. Allow HDF-M to equilibrate to room temperature.
2. Add 9 mL of HDF-M to a 10 cm tissue culture dish, and set aside in a biological safety cabinet.
3. Remove the vial with approximately 5×10^5 frozen fibroblasts from a liquid nitrogen storage tank (*see* Note 1). Thaw quickly in a 37°C water bath (approximately 1 min), taking care to avoid contamination by keeping the cap above the water level.

4. Spray the entire vial containing thawed fibroblasts with 70% ethanol, and proceed under strict aseptic conditions in a biohazard safety cabinet.
5. Pipette 5 mL of HDF-M into a 15 mL conical tube, and transfer the entire content of the vial containing thawed fibroblasts into this conical tube. Pipette to mix.
6. Spin the cells down at 200 x g for 5 min.
7. Gently resuspend the pellet in 1 mL of HDF-M, and transfer the entire cell suspension dropwise to the 10 cm dish filled with the pre-equilibrated HDF-M medium from step 2 above.
8. Incubate the dish overnight in a 37°C/ 5% CO₂ incubator to allow the cells to attach.
9. The next day, aspirate the medium without disturbing the fibroblasts monolayer, and replace with 10 mL of fresh, pre-warmed HDF-M. Continue changing HDF-M every other day until cells reach approximately 70–80% confluency, at which point the cells need to be passaged (*see Note 2*).

3.2.2. Fibroblast Subculture

1. Allow Trypsin-EDTA detachment solution and HDF-M to equilibrate to room temperature.
2. Add 10 mL of HDF-M to a new 10 cm dish, and set aside in a biological safety cabinet. Prepare up to four plates to obtain a sufficient number of fibroblasts for one graft.
3. Take the dish with fibroblasts from the incubator, and aspirate spent media from cells without disturbing the monolayer.
4. Add 5 mL of DPBS, gently rocking the dish to wash the cells. Aspirate the DPBS.
5. Add 3 mL of Trypsin-EDTA detachment solution. Gently rock the plate to ensure even, complete coverage of the solution over the cells, and then aspirate the excess fluid off the monolayer.
6. Incubate cells at 37°C for 1 min. Observe cells under a microscope. If the cells have rounded, gently tap the sides of the dish to encourage detachment.
7. Add 3 mL of HDF-M to neutralize Trypsin-EDTA.
8. Gently pipette up and down to detach the cells from the dish surface, taking care to avoid bubble formation.
9. Move the cell suspension into a new, sterile 15 mL conical tube.
10. Centrifuge the cells down at 200 x g for 5 min.
11. Resuspend cells in pre-warmed HDF-M, and count cells using a hemacytometer. Transfer approximately 3×10^5 fibroblasts to each 10 cm dish with pre-

equilibrated HDF-M from step 2 above. Place the dishes with freshly seeded fibroblasts into the tissue culture incubator, and leave undisturbed for at least 24 h while cells attach to the dish.

12. Change medium by aspirating spent medium and adding 10 mL of fresh, pre-warmed HDF-M every other day until cells are ready for passaging or grafting (70–80% confluency).

3.3 Harvesting Cells for Grafting

Prepare a sufficient number of plates with keratinocytes and fibroblasts at 70–80% confluency.

3.3.1 Collecting Keratinocytes

1. Allow Accutase™ detachment solution and EpiLife medium to equilibrate to room temperature.
2. Take the dishes with keratinocytes from the tissue culture incubator and aspirate spent media without disturbing the cell monolayer.
3. Add 5 mL of DPBS, gently rocking the dish to wash the cells; aspirate the DPBS.
4. Add 3 mL of Accutase™ detachment solution per dish, and incubate for 4–8 min at 37°C (*see* Note 3).
5. Add 3 mL of EpiLife medium to Accutase™ detachment solution to neutralize.
6. Tilt the dish, and gently pipette up and down to wash the surface of the dish to detach and put cells into suspension. Avoid forming bubbles.
7. Transfer the dissociated cells into a new, sterile 15 mL conical tube.
8. Count cells using a hemacytometer, and aliquot the appropriate volume needed to achieve 5×10^6 cells into a new, sterile conical tube. For multiple grafts, prepare multiple 5×10^6 aliquots.
9. Centrifuge the cells down at 150 x g for 5 min.
10. Resuspend keratinocyte pellet in 300 µL of cold EMEM supplemented with 10% chelexed FBS (*see* Note 4).
11. Transfer the slurry to a new, sterile 1.5 mL microcentrifuge tube.
12. Place the tube on ice while preparing fibroblasts.

3.3.2 Collecting Fibroblasts

1. Allow Trypsin-EDTA detachment solution and HDF-M to equilibrate to room temperature.
2. Aspirate spent media from fibroblasts without disturbing the monolayer.
3. Gently rinse each dish with 5 mL of DPBS and aspirate.

4. Add 3 mL of Trypsin-EDTA detachment solution to each dish of fibroblasts. Gently rock the plates to ensure even, complete coverage of the solution over the cells, and then aspirate the excess fluid off the monolayer.
5. Incubate cells at 37°C for 1 min. Observe cells under a microscope. If the cells have rounded, gently tap the sides of the dish to encourage detachment.
6. Add 3 mL of HDF-M per dish to neutralize Trypsin-EDTA solution.
7. Gently pipette to wash cells from dish surface, taking care to avoid bubbles.
8. Move cell suspension into a new, sterile 15 mL conical tube.
9. Centrifuge at 200 x g for 5 min.
10. Resuspend the cells in 300 µL of cold EMEM supplemented with 10% chelexed FBS.
11. Add the fibroblast slurry to the microcentrifuge tube containing the keratinocyte slurry to create a 600 µL aliquot of the combined keratinocyte/fibroblast slurry.
12. Place the tube with the keratinocyte/fibroblast mix back onto ice. The cell slurry must remain on ice until it is ready to be transferred into the silicone grafting chamber.

3.4 Chamber Insertion

Perform surgery in a biological safety cabinet using aseptic techniques in adherence to animal protocol and IACUC regulations.

1. Inject mouse intraperitoneally with prepared anesthetic solution in adherence to animal protocol.
2. Trim the nails on the hind legs of the anesthetized mouse using nail clippers or scissors to reduce possible chamber damage or removal caused by scratching.
3. Shave the back of the anesthetized mouse with whisker clippers (alternatively, waxing can be used for hair removal). Remove as much hair as possible from behind the shoulders, just past the ears (Fig. 2a).
4. Wipe the shaved (or waxed) back skin with a sterilization swab.
5. Using forceps, pull up the skin over the flat part of the back, and cut the skin with curved scissors to create a hole with a circumference similar to the dome of the silicone grafting chamber, approximately 1 cm (Fig 2b-c).
6. Dip forceps or blunt-tipped scissors in sterile EMEM to lubricate. Free a rim of the skin surrounding the hole (approximately 5 mm) by lifting the edges of the hole with forceps and running another pair of moistened forceps or scissors around the edge of the hole, loosening the skin from the underlying muscle fascia (Fig. 2d).
7. Add a drop of sterile EMEM onto the exposed muscle fascia to lubricate the wound.

8. Using forceps, pull up the skin near the hole, creating a slit-like opening. With forceps in the other hand, pick up a grafting chamber.
9. Insert one side of the chamber rim under the skin on one side of the slit (Fig. 2e).
10. With forceps, gently pull the skin over the chamber rim on the other side of the slit until the entire lower rim of the chamber is seated securely under the skin (Fig. 2f).
11. Tug extremely gently on the chamber to ensure secure placement.
12. If the hole is too large or the skin becomes too stretched, the dome will not stay firmly in place. To correct this, use one or two 9 mm wound clips to secure the skin to the chamber rim to prevent the chamber from dislodging from place (Fig. 2g).
13. Move the mouse into a new clean cage that is resting on a heating pad (*see Note 5*).
14. After placing the mouse in the new cage on a heating pad, gently mix the cell slurry pellet with a sterile 1 mL tip attached to a sterile P1000 pipetman. Pull the entire slurry into the pipette tip (avoid air bubbles), and carefully eject the entire 600 μ L of combined keratinocyte/fibroblast slurry into the chamber through a hole in the silicone chamber (Fig. 2h, i). With secure and proper chamber placement, cell slurry/media should be visible in the upper portion of the silicone chamber (Fig. 2i).
15. Do not disturb the mouse during recovery, allowing the cells to settle down onto the back of the mouse.
16. Monitor the mouse until its full recovery from anesthesia.
17. Administer pain relief medication over the next 48 h.
18. Monitor mouse daily for the next six days until chamber removal on day 7.

3.5. Chamber Removal

1. On day 7 post grafting, anesthetize the recipient mouse (*see Note 6*).
2. Once the mouse is anesthetized, carefully loosen the skin around the lower rim of the chamber with sterile forceps (Fig. 2j).
3. With forceps or clean, gloved fingers, gently squeeze the silicone chamber and carefully pull it out from under the skin, revealing a monolayer of cells in the wound (Fig. 2k, *see Note 7*). During removal, if the edge of the wound remains attached to the silicone chamber, run another pair of forceps along the silicone lip of the chamber to free the skin. Once removed, the silicone chamber can be cleaned, sterilized, and reused in future experiments.
4. Place the mouse into a clean cage (with no wire feeder) that has been resting on a heating pad, and monitor until recovery (*see Note 8*).

5. Leave the mouse in a clean cage without the wire feeder for the duration of the healing (approximately 3–4 weeks after surgery).
6. The wound will scab and begin healing (Fig. 2l).

3.6. Graft Harvesting

Monitor mice daily until engrafted area is no longer scabbed and therefore ready for harvesting (4–5 weeks post grafting).

1. Euthanize mice according to approved animal care facility protocol.
2. Gently shave the engrafted area and surrounding skin using the chamber insertion procedure above, taking care not to injure the engrafted or surrounding skin (Fig. 2m).
3. The engrafted area should be visible and easily identified. Mark its border with a dotted line using the fine-point tip of a marker. Also, mark a dashed line where excisions will be made to remove the skin from the animal's back, as shown in Fig. 2n.
4. With forceps, pinch and raise the skin about a centimeter away from the caudal edge of the graft (Fig. 2o). Snip with scissors. Extend this cut, creating a transverse cut along the caudal edge of the engrafted area along the dashed line made in **step 3**.
5. Gently lift the skin with forceps. From one edge of the transverse cut, turn scissors, and extend another cut toward the cranial edge of the engrafted area along the dashed line made in step 3 (*see Note 9*).
6. From the remaining edge of the transverse cut, extend another cut toward the cranial edge of the engrafted area parallel to the cut made in step 5.
7. Once the three cuts surrounding $\frac{3}{4}$ of the engrafted area are completed, use forceps, and gently begin to lift the skin flap (Fig. 2p). Carefully, using another set of forceps or sharp scissors, break any adhesions between the skin and muscle fascia (Fig. 2q, *see Note 10*).
8. Place a small piece of Whatman® paper under the skin flap. Avoid letting the paper sit on the back of the mouse, as it will stick to the moist muscle fascia. Unroll skin and flatten it across the paper (Fig. 2r).
9. Cut transversally across the skin flap, liberating the paper and skin from the mouse's back. The paper can then be marked to denote the orientation of the skin graft. For example, use H and T to indicate which edge of the graft was toward the head (cranial) side and tail (caudal) side, respectively (Fig. 2s).
10. Transfer the skin and paper into a 15 mL conical tube containing 10% buffered formalin (Fig. 2t, *see Note 11*). Incubate at 4°C overnight to allow fixative to permeate the tissue.
11. The next day, transfer the tissue into a conical tube filled with 70% EtOH for long-term storage.

12. Fixed tissue can be paraffin embedded and sectioned for further histological analysis.
13. The presence of human engrafted skin equivalent can be validated by a standard histological examination of tissue sections stained with hematoxylin and eosin (H&E). Human engrafted skin typically displays the thicker stratum corneum and epidermis compared to adjacent mouse skin (Fig. 3). Further validation can be performed by a standard immunofluorescence analysis using human-specific antibodies, such as the commercially available anti-Vimentin (ab16700) or anti-Mitochondria (ab92824) antibodies from Abcam.

4. Notes

1. To improve the engraftment of fibroblasts and keratinocytes, use the earliest passage available, preferably no later than passage 6.
2. It is critical not to allow keratinocytes to reach 100% confluency. Otherwise, the cells will terminally differentiate and fail to engraft.
3. When using Accutase™ to detach keratinocytes, examine the cells after 4 min of incubation. Most cells will start detaching and acquiring a round morphology. If there are still many cells adhering to the dish, tap gently, and place the dish back into the incubator for additional 2 min. By this time, nearly all cells will detach. If not, tap and move the dish back to the incubator, and examine the cells every minute until all cells detach to avoid prolonged exposure of the cells to the detachment solution.
4. When adding FBS to EMEM media prior to grafting, use calcium-reduced chelexed FBS (8,11). The high calcium concentration in regular FBS will stimulate the terminal differentiation of keratinocytes, ultimately causing the failure of the graft.
5. If animal cages have a wire feeder, remove the feeder, as it may disturb the chamber if the mouse is permitted to feed from it. Instead, put chow into a Petri dish, and place the dish directly on the bottom of the cage. It is also advisable to supply some moistened chow, as the mice may not be inclined to drink enough water during recovery.
6. When removing the chamber, a lower dose of anesthetic is sufficient, as chamber removal is much less invasive than insertion.
7. When removing the chamber, it is critical not to disturb the monolayer of cells inside the dome of the chamber. When removed properly, the mouse's skin will remain open in a circular shape, and a monolayer of human cells should be visible in the center of the wound (Fig. 2k).
8. After chamber removal, it is still crucial not to have a wire feeder in the animal cage. If the wire feeder remains, the animal may disturb or injure the open wound while feeding.

9. When making incisions to harvest the graft, the skin may roll in upon itself. Use forceps to keep skin relatively flat while making cuts, carefully unrolling the tissue if necessary. It is extremely important to be mindful of the engrafted area to avoid injuring the tissue.
10. During graft harvest, use extreme caution to leave the engrafted area undisturbed while breaking any adhesions.
11. This protocol uses formalin as the recommended fixative for engrafted skin equivalents. However, other fixatives are permissible depending on the requirements of downstream applications.

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Abbreviations

H&E	Hematoxylin and eosin
ID	Inner diameter
OD	Outer diameter
NOD-SCID	Nonobese diabetic/severe combined immunodeficiency

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Fig 1.

Dimensions of silicone grafting chambers. Silicone grafting chambers are produced from liquid silicone rubber with the inner diameter (ID) of 12 mm, the outer diameter (OD) of 20 mm, and the height of 10 mm. Three manually punched holes in the dome of the chamber allow for gas exchange during the time the chamber is in place under the skin

Fig 2.

Systematic visual representation of the grafting procedure and tissue harvesting. Chamber insertion and grafting of keratinocytes and fibroblasts on day 1 (**a-i**). Chamber removal occurs 7 days after insertion (**j-k**). The wound will scab between day 7 and day 15 (**l**). The engrafted area can be harvested at approximately 4–5 weeks post transplantation (**m-t**)

Fig 3. Confirmatory histological examination of a human skin xenograft. A graft area is sectioned and stained with H&E. The dashed line separates engrafted human skin equivalent (right) from recipient mouse skin (left). Note the thicker stratum corneum and epidermis in the human skin graft. Scale bar, 100 μ m