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Scaffold Human Dermis

DEFINITION

Scaffold Human Dermis is derived from donated allograft human dermis that is processed to remove cells and freeze-dried to remove moisture while preserving biologic components and structure of the dermal matrix. It is biocompatible and supports remodeling by the recipient's own tissue. The matrix is composed of native human dermal architecture, consisting of collagen (mainly collagen Type I, with additional components of collagen Types III and IV), chondroitin sulfate and hyaluronic acid glycosaminoglycans, and elastin. The product can be provided in a sheet or powder form.

The donated human skin is processed in a manner that removes all cellular components, including the epidermal layer and dermal cells. The resulting product is then rendered into a particulate form (microsized), with a mean particle size of less than 100 µm, by processing with a freezer mill. Scaffold Human Dermis does not contain intact cells, cell nuclei, or chemically induced crosslinks. Human skin used to produce Scaffold Human Dermis is obtained from sources that have passed applicable donor eligibility requirements for relevant communicable diseases. Scaffold Human Dermis is manufactured using sterile solutions and equipment under aseptic conditions. The final product is inspected and tested to ensure that the product meets specifications.

SPECIFIC TESTS

• **Histological Evaluation**

Sucrose solution: Dissolve 20 g of sucrose in 100 mL of water.

Tissue preparation: Submerge a 1.4-cm or greater size piece of Scaffold Human Dermis in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Cut three 1.5-cm × 0.5-cm pieces of Scaffold Human Dermis, and place each piece from the same donor lot into a suitable histological tissue cassette for routine histology processing and paraffin embedding. Place the cassettes in 10% neutral buffered formalin[1] at room temperature for a minimum of 12 h. [Note—the cassettes may be kept at room temperature for 1 month.] From the same donor lot, cut a minimum of three 1.0-cm × 0.5-cm pieces of Scaffold Human Dermis for immunohistochemical (IHC) staining, and place in a vial containing the *Sucrose solution*. Store at between 2° and 8° for a minimum of 4 h, but the product can be held in the solution for up to 2 weeks.

Embedding and sectioning of histological samples: Place all cassettes to be processed into a suitable histological cassette basket including one cassette containing formalin-fixed normal skin to serve as a process control. The following steps can be performed using a vacuum infiltration tissue processor.[2] At room temperature (37° ± 2°), tissue samples that continue to fix in 10% neutral buffered formalin are dehydrated through graded reagent alcohol (70%, 80%, 95%, and 100%).[3] The tissue samples are cleared in a xylene substitute.[4]

The tissue is infiltrated with paraffin[5] at 60°. The following embedding procedure can be performed with the use of a histology tissue embedding unit, consisting of a thermal console, dispensing console, and cryo-console. Open the histological cassette. Partially fill embedding molds with molten paraffin (60°). While keeping the paraffin heated, position the three tissue pieces on edge in the paraffin so that cross-sections of the Scaffold Human Dermis, once they are completely embedded, can be cut. Rapidly cool the paraffin so that it partially solidifies. Completely fill the embedding mold with additional molten paraffin (60°), and cool until completely solidified.

Remove solidified paraffin blocks from embedding molds for sectioning. Sectioning of paraffin-embedded samples can be performed on a suitable microtome.[6] Clamp the tissue block into the holder of the microtome. Fill a histological water bath with fresh water. [Note—A quarter teaspoon of gelatin may be added to the water bath for section adhesion.] Maintain the water bath at 39° ± 2°. Cut sections to a thickness of 3–5 µm to form a ribbon, and gently lay the ribbon on the water bath. Place two consecutive sections on each glass microscope slide.

Embedding and sectioning of immunohistochemical samples: Fill histology embedding molds with a cryo-protectant embedding media. Transfer tissue pieces from the *Sucrose solution* to the embedding media in the mold, orient so that cross-sections can be cut, and let sit for 1–30 min. Freeze the molds with liquid nitrogen, and store at −80°. Remove the frozen blocks from the molds for sectioning. Cryo-section each block to 4–7 µm in thickness, and place the sections on glass microscope slides. Place at least two sections on each slide. Slides can be stored at between −70° and −85°.

Hematoxylin and eosin sample staining: [Note—Store all solutions at room temperature.]

1% Acid alcohol solution: Alcohol, hydrochloric acid, and water (1309:20:509)

95% Reagent alcohol solution: Alcohol and water (190:10)

80% Reagent alcohol solution: Alcohol and water (160:40)

Saturated lithium carbonate solution: Water, lithium carbonate (200:2), (v:w)

Analysis: The slide with affixed tissue (sections) is deparaffinized in three changes of xylene substitute, rehydrated in graded reagent alcohols (100% and 95%), and rinsed in tap water. The sections are stained with hematoxylin, rinsed, decolorized with *1% Acid alcohol solution*, and neutralized with *Saturated lithium carbonate solution*. The sections are counterstained with 1% alcoholic eosin Y, dehydrated through graded reagent alcohols (95% and 100%) and xylene substitute, and then coverslipped. A positive control is stained with each batch to ensure successful staining.

Acceptance criteria: Adequate staining is defined by a blue nuclear stain and a pink-red cytoplasm. Sections are assessed for tissue matrix integrity and are acceptable if they pass the following criteria: they must not show evidence of intact epidermal or dermal cells and must not show evidence of widespread collagen damage (broken, condensed, or distorted fibers) or unrecognizable papillary layer, as shown in the USP Scaffold Human Dermis Reference Photomicrographs[7] of products with acceptable appearance.

Immunohistochemical sample staining

0.5 M phosphate-buffered saline, pH 7.4 (PBS): Prepare by combining 8.50 g of sodium chloride, 0.85 g of dibasic sodium phosphate, and 0.54 g of monobasic potassium phosphate in 1 L water. Adjust the pH to 7.4 with 1.0–3.0 M sodium hydroxide. Store at room temperature.

Diluent PBS: Combine 0.250 g of bovine serum albumin, 25 mL of *PBS*, and 0.02–0.03 g of thimerosal. Store at between 2° and 8° for up to 1 year.

Primary and secondary antibodies: Perform a titer assay to determine the optimal dilution for each antibody when a new lot is used. Dilute in *Diluent PBS* as per titer assay.

Human monoclonal anti-type IV collagen:[8] 25- to 100-µL aliquots of concentrated antibody may be stored at between −70° and −85°.

Human monoclonal anti-MHC class I:[9] 100- to 200-µL aliquots of concentrated antibody may be stored at between −70° and −85°.

Human monoclonal anti-MHC class II:[10] 100- to 200-µL aliquots of concentrated antibody may be stored at between −70° and −85°.

Goat anti-mouse IgG (Fab-specific) peroxidase conjugate:[11] 100- to 200-µL aliquots of concentrated antibody may be stored at between −70° and −85°.

3,3-Diaminobenzidine staining kit or stable diaminobenzidine (DAB):[12] To make the working stain, consult kit instructions. Store the kit at between 2° and 8°. 3,3-Diaminobenzidine is light sensitive; minimum exposure to light is advisable. Stable DAB is stored at between 0° and −20°.

3% Hydrogen peroxide: Store at room temperature.

Acetone: Store at between −10° and −20°.

Analysis for monoclonal anti-MHC class I and class II: Frozen sections are obtained and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. A cocktail of *Human monoclonal anti-MHC class I*, *Human monoclonal anti-MHC class II*, and bovine serum albumin are applied to the sections. Sections are rinsed with *PBS*, and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS*, and *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives exactly the same treatment as the test samples. The negative control is the same tissue section as the positive. The difference is that one critical stain step is omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved, the stain is repeated.

Acceptance criteria: Adequate positive staining of sections will appear brown in color, focal, and cell-associated and will be clearly distinct from the background, as shown in the USP Scaffold Human Dermis Reference Photomicrographs of products with acceptable appearance for staining with MHC I and II antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

Analysis for monoclonal anti-type IV collagen: Frozen sections are obtained, and slides are fixed in *Acetone*. Endogenous peroxides are blocked with *3% Hydrogen peroxide*. *Human monoclonal anti-type IV collagen* along with bovine serum albumin are applied to the sections. Sections are rinsed with *PBS*, and the secondary antibody, *Goat anti-mouse IgG peroxidase conjugate*, is applied. Following the application of the secondary antibody, the sections are rinsed in *PBS*, and *DAB* is applied. The sections are then counterstained in hematoxylin, decolorized in *1% Acid alcohol solution*, neutralized in *Saturated lithium carbonate solution*, dried, and coverslipped.

Control slides: Normal skin samples are frozen and sectioned for control tissue. The positive control receives exactly the same treatment as the test samples. The negative control is the same tissue section as the positive. The difference is that one critical stain step is

omitted, such as the application of the primary antibodies. All other stain steps are completed in accordance with the test slides. If acceptable control stains are not achieved, the stain is repeated.

Acceptance criteria: Adequate positive stain results will reveal a brown stain in the location of the basement membrane, as shown in the USP Scaffold Human Dermis Reference Photomicrographs of products with acceptable appearance for staining with collagen type IV antibodies. Negative stain results appear clear with no brown staining; only a light blue hematoxylin counterstain will be seen.

- **Biochemical Analysis**

Sample solution: Submerge 20 mg of Scaffold Human Dermis in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Freeze the sample, and mill using a freezer mill.[13] Suspend in 10 mL of 4 M guanidine hydrochloride, 50 mM sodium acetate, 5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM *N*-ethylmaleimide (NEM), 0.2% Triton X-100, pH 5.8, and extract for 48 h at 4°, with agitation. Centrifuge for 10 min at 4° and 23,500 *g*. Remove the supernatant, and digest with 20 µL of chondroitinase ABC,[14] 1 milli-Unit/µL. Analyze for *Glycosaminoglycan (GAG) content* and *Immunochemical analysis of decorin* (below). For *Collagen content* analysis, wash the pellet three times with 10 mL of deionized water, centrifuging for 10 min at 4° and 23,500 *g* after each wash. Resuspend in a minimal amount of water, freeze at −80°, and lyophilize. Resuspend 20 mg of the lyophilized pellet in 20 mL of 0.5 M acetic acid containing 100 µg of pepsin per mg of tissue, and incubate overnight at 4° with agitation. Remove undigested material by centrifugation at 23,500 *g* for 20 min.

Collagen content

Sample buffer: Prepare as directed for *Sample Buffer 1* in *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis ⟨1056⟩, Electrophoretic Separation*.

Sample collagen solution: Add 20 µL of the supernatant from the *Sample solution* to 20 µL of *Sample buffer,* mix, and incubate at 60° for 15 min.

Control samples: Prepare suitable positive control samples (human placental collagen types I[15] and III[16]) similar to *Sample collagen solution.*

Gel electrophoresis: Load 40 µL of each of *Sample collagen solution* and the *Control samples* onto a 6% acrylamide gel, perform the electrophoretic separation, and stain with Coomassie brilliant blue as directed under *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis ⟨1056⟩*.

Acceptance criteria: The bands obtained from Scaffold Human Dermis are distinct and clearly visible at a mobility similar to that of the bands obtained from the collagen types I and III control samples. No smears corresponding to degraded collagen types I and III are found on the gel.

Glycosaminoglycan (GAG) content

Sample GAG solution: Use 15 µL of the supernatant prepared as directed for *Sample solution.*

DMMB reagent: Dissolve 16 mg of 1,9 dimethyl-methylene blue (DMMB) in 5 mL ethanol. Add 3.24 mL of formic acid and 2.94 mL of 10 M sodium hydroxide. Bring to 1000 mL with water.

50 mM Sodium acetate solution: Dissolve 1.03 g of sodium acetate in 250 mL of water.

Analysis: Add 15 µL of *Sample GAG solution* to each well of a 96-well microtiter plate. To each sample well, add 200 µL of *DMMB reagent* and 35 µL of *50 mM Sodium acetate solution*. Read the samples on a spectrophotometric microplate reader at 540 nm, using 595 nm as a reference. Perform the same procedure on a sample of bovine collagen type I[17]negative control and positive controls hyaluronic acid[18] and chondroitin sulfate,[19] using 15 µL of 3 mg/mL solutions from each of the controls.

Acceptance criteria: Scaffold Human Dermis contains glycosaminoglycans (GAG) as represented by positive control standards. GAG concentration can be determined by the preferential binding of DMMB dye to negatively charged ions such as sulfated GAGs.

Immunochemical analysis of decorin

Sample decorin solution: Submerge 100–200 mg of Scaffold Human Dermis in normal saline solution to rehydrate the tissue for a minimum of 4–8 h. Using a freezer mill as outlined above, extract the tissue in a solution of 4 M guanidine hydrochloride, 50 mM sodium acetate (pH 5.8) containing 5 mM EDTA, 0.1 mM PMSF, and 10 mM NEM for 24–48 h with agitation at 4°. Centrifuge the extracted tissue at 23,500 *g* for 10 min, and remove the supernatant.

Nitro blue tetrazolium (NBT): Dissolve 0.5 g in 10 mL of 70% dimethyl sulfoxide (DMSO). Store in the dark at room temperature.

5-Bromo-4-chloro-3-indolyl phosphate (BCIP): Dissolve 0.5 g in 10 mL of 100% DMSO. Store in the dark at room temperature.

Alkaline phosphatase buffer: 100 mM sodium chloride, 5 mM magnesium chloride, 100 mM Tris(hydroxymethyl) aminomethane hydrochloride, pH 9.5. Filter, and store at 4°.

Chromogenic substrate mixture: Mix 66 µL of *NBT* and 33 µL of *BCIP* in 10 mL of *Alkaline phosphatase buffer*.

Blotting buffer 1: 50 mM Tris buffered saline, pH 7.4, with 0.1% Tween 20 (TBST)

Blotting buffer 2: 5% blotting grade milk in TBST

Blotting buffer 3: 0.1% blotting grade milk in TBST

Analysis: Run an aliquot of *Sample decorin solution* on an 8% polyacrylamide gel at 100 V for approximately 60 min along with 100 µg of recombinant decorin[20] positive control, bovine type I collagen[21] negative control, and prestained molecular weight marker, and transfer to a polyvinylidene fluoride (PVDF) membrane according to the following procedure. In a shallow tray, soak two sheets of Whatman paper and two pieces of sponge in transfer buffer (200 mM glycine, 25 mM tris, and 20% methanol, pH 8.3). Soak the transfer membrane in methanol before soaking in the transfer buffer. Equilibrate the gel in transfer buffer for 10 min. Assemble the tank transfer apparatus according to manufacturer's instructions. Layer the following items within the apparatus in proper order: sponge, paper, gel, PVDF membrane, paper, and sponge. Clamp the apparatus closed. Connect the electrodes, and transfer the gel bands to the membrane at about 100 V at 4° for about 1 h. Using prestained protein molecular weight marker, and staining the gel after electro-blotting, allow for visual determination of transfer completion. When transfer is complete, disconnect the transfer apparatus, and carefully remove the membrane. Block the membrane for at least 1 h with *Blotting buffer 2*, cover, and shake gently for 30 min. Wash the membrane three times for 5 min each with *Blotting buffer 1*. Add rabbit anti-human decorin primary antibody,[22] diluted to 2 µg/mL in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*. Add goat anti-rabbit IgG secondary antibody conjugated[23] to alkaline phosphatase, diluted 1:3000 in *Blotting buffer 3*, to the membrane, and incubate for 1 h with gentle shaking. Wash the membrane three times for 5 min with *Blotting buffer 1*. Add 10 mL of *Chromogenic substrate mixture*, and incubate until color has developed. When the bands are clearly visible, stop the development reaction by rinsing the membrane with water.

Acceptance criteria: The decorin band obtained from Scaffold Human Dermis is the only band on the gel and is clearly visible at a mobility similar to the band obtained from the control sample recombinant decorin.

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count does not exceed 100 cfu/g, and the total combined molds and yeasts count does not exceed 10 cfu/g. Scaffold Human Dermis meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

• **Particle Size Analysis**

(See *Particulate Matter in Injections ⟨788⟩*.)

[Note—Test specific to the powder form]

Sample: 100 mg of powder form of Scaffold Human Dermis material

Analysis: Add the *Sample* to 25 mL of saline in a clean 50-mL conical tube. Rehydrate and disperse the particles by probe sonication at 20 watts for 60 s. Place an appropriate aliquot of sample suspension in an appropriate liquid particle counter (LPC) for analysis by laser light obscuration (light extinction).

Acceptance criteria: The mean particle size is NMT 100 µm.

ADDITIONAL REQUIREMENTS

• **Labeling:** The package label indicates the weight of the Scaffold Human Dermis material enclosed. The label also contains the lot number, the expiration date, the trade name, the required storage conditions, the dosage information, and manufacturer and/or distributor contact information. "Instructions for Use," which includes a summary of records used to make donor eligibility determination and necessary information for properly using the product for its intended use, is provided with each unit.

• **Packaging and Storage:** Scaffold Human Dermis is aseptically packaged and supplied in a freeze-dried configuration in a double pouch configuration. It is supplied in various sizes and thicknesses. The powder configuration is supplied in a single-use delivery syringe packaged in a hermetically sealed pouch. Scaffold Human Dermis is stored between 1° and 10° and labeled with the expiry date.

Change to read:

• ▲**USP Reference Standards ⟨11⟩**

USP Authentic Visual References RS▲ (CN 1-Aug-2020)

USP Scaffold Human Dermis Reference Photomicrographs: the samples were prepared as directed in the test for *Histological Evaluation*. These photomicrographs show the histological appearance of passing histological evaluation with no evidence of intact epidermal or dermal cells, and no evidence of collagen damage (photomicrographs 2–6) and of failed material with collagen damage and condensed papillary dermis or attached epidermal remnant (photomicrographs 7–10). Passing and failed material are compared to photomicrograph 1 showing the staining of unprocessed normal human skin with intact epidermal or dermal cells. Photomicrographs 11 and 12 are negative and positive controls for the analysis of immunostaining with MHC I and II antibodies. Photomicrographs 13 and 14 are negative and positive controls for the analysis of immunostaining with collagen type IV antibodies. For both types of staining, a passing positive stain will appear brown in color, focal, and cell-associated, whereas a failed negative stain will appear clear with no brown staining.

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[1] 10% Neutral buffered formalin can be purchased through Statlab Medical Products, Inc. Lewisville, TX.

[2] A suitable processor can be the Tissue Tek V.I.P. Model #1000 vacuum infiltration tissue processor or the Leica ASP300 tissue processor.

[3] Graded alcohols: 70%, 80%, and 95% are prepared using 100% reagent alcohol, such as that purchased from Statlab Medical Products, Inc. Lewisville, TX.

[4] Such as a citrus clearing solvent from Richard-Allan Scientific, Kalamazoo, MI.

[5] Such as Paraplast, Extra, manufactured by McCormick Scientific, St. Louis, MO.

[6] Such as Leica RM 2155 or RM 2255 Rotary Microtome.

[7] These photomicrographs are available as a CD from the USP Reference Standards collection, available to the reader through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to www.usp.org. Order item number 1535813.

[8] A suitable primary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C1926.

[9] A suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, catalog #H58A.

[10] A suitable primary antibody is available from VMRD Inc., PO Box 502, Pullman, WA 99163, catalog #TH14B.

[11] A suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103 cat #A9917.

[12] DAB can be sourced from Invitrogen/Zymed, Serotec, or Dako.

[13] Such as SPEX/CertiPrep Freezer/Mill.

[14] Suitable chondroitinase is available from Seikagaku, Tokyo, Japan, catalog #100330.

[15] Suitable type I collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C7774.

[16] Suitable type III collagen is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4407.

[17] Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave., PO Box 6482, Carlsbad, CA, 92008 catalog #100.

[18] Suitable hyaluronic acid is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #H1876.

[19] Suitable chondroitin sulfate is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, catalog #C4384.

[20] Suitable decorin is available from R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, catalog #143DE100.

[21] Suitable type I collagen is available from Invitrogen, 1600 Faraday Ave., PO Box 6482, Carlsbad, CA 92008.

[22] Suitable primary antibody is available from BioVision, 980 Linda Vista Ave., Mountain View, CA 94043, catalog #3645100.

[23] A suitable secondary antibody is available from Sigma, 3050 Spruce St., St. Louis, MO 63103, cat #A9917.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
SCAFFOLD HUMAN DERMIS	Rebecca C. Potts Associate Scientific Liaison	BIO52020 Biologics Monographs 5 - Advanced Therapies
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO52020 Biologics Monographs 5 - Advanced Therapies

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