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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 formalin¹ 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 paraffin⁵ 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 Photomicrographs⁷ 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