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Scaffold Porcine Small Intestinal Submucosa

DEFINITION

Scaffold Porcine Small Intestinal Submucosa is a collagen-based scaffold, translucent and off-white in color. It is obtained from the small intestinal submucosa layer of the domestic pig (*Sus scrofa* L.). This layer has been mechanically separated from the adjoining layers of the intestine to remove the serosal, mucosal, and muscular elements. The isolated submucosa is chemically cleaned, decellularized, freeze-dried, and terminally sterilized. Scaffold Porcine Small Intestinal Submucosa also undergoes a viral inactivation; the inactivation method is validated using parvovirus, reovirus, pseudorabies virus, and leukemia retrovirus as the test viruses. By dried weight, Scaffold Porcine Small Intestinal Submucosa consists of about 70% protein, about 20% carbohydrate, and about 7% lipid. The protein component is primarily collagen type I, with minor amounts of elastin and collagen type III, collagen type IV, and collagen type VI. In addition to these components, additional extracellular matrix components, such as glycosaminoglycans and fibroblast growth factor-2, are also retained.

SPECIFIC TESTS

- **Fibroblast Growth Factor-2 Content**

Sterile PBS solution: 8.065 g/L of sodium chloride and 0.2 g/L of potassium chloride in 0.01 M sodium phosphate buffer, pH 7.4

Sample solution: Obtain a 1-cm^2 sample of Scaffold Porcine Small Intestinal Submucosa, weigh, and submerge in 400 µL of *Sterile PBS solution*. Pulverize the tissue for 90 s using a tissue grinder, intermittently checking to be sure that the tissue remains immersed in the *Sterile PBS solution* and becomes homogenized. Centrifuge at 12,000 × *g* for 5 min at 4°. Use immediately upon preparation.

Analysis: Examine duplicate aliquots of the *Sample solution* by a suitably sensitive ELISA method.[1]

System suitability: The analysis is considered valid if the ELISA kit generates a linear standard curve with the square of the correlation coefficient (r^2) NLT 0.95, and if the duplicate aliquots of the *Sample solution* yield results that are within 20% of each other.

Acceptance criteria: The average content of fibroblast growth factor-2 is NLT 10,000 pg/g of Scaffold Porcine Small Intestinal Submucosa.

- **Glycosaminoglycan Content**

1,9-Dimethylmethylene blue solution: Mix 95 mL of 0.1 M hydrochloric acid in 500 mL of water. Add 16 mg of 1,9-dimethylmethylene blue, 3.04 g of aminoacetic acid, and 2.37 g of sodium chloride. Dilute with water to 1 L, and adjust to a pH of 3.0 with sterile solutions of either 1.0 M sodium hydroxide or 1.0 M hydrochloric acid. Store in low-actinic glassware.

Sterile PBS solution: 8.065 g/L of sodium chloride and 0.2 g/L of potassium chloride in 0.01 M sodium phosphate buffer, pH 7.4

Proteinase K solution: 600 Units/mL of *Tritirachium album* proteinase K

Stock heparin standard solution: 1 mg/mL of heparin

Heparin standard curve solutions: Use *Stock heparin standard solution* to prepare three solutions containing 20, 50, and 100 µg/mL of heparin.

Sample solution: Prepare samples in duplicate. Accurately weigh about 25 mg of Scaffold Porcine Small Intestinal Submucosa and cut into small pieces (roughly 2 mm × 2 mm). Transfer to a 1.5-mL microcentrifuge tube, and add 180 µL of *Sterile PBS solution* and 20 µL of *Proteinase K solution*. Mix, and incubate the sample at 56° for 15 min; during the incubation, mix intermittently on a vortex mixer. Cool the sample to room temperature. Dilute with water to obtain a concentration of 12.5 mg/mL of digested Scaffold Porcine Small Intestinal Submucosa.

Blank solution: Water

Collagen control solution: Accurately weigh about 25 mg of a bovine collagen, type I, that contains less than 1 µg of glycosaminoglycan/mg. Transfer to a 1.5-mL microcentrifuge tube, and add 180 µL of *Sterile PBS solution* and 20 µL of *Proteinase K solution*. Mix, and incubate the sample at 56° for 15 min; during the incubation, mix intermittently on a vortex mixer. Cool the sample to room temperature. Dilute with water to obtain a concentration of 12.5 mg/mL of digested bovine collagen.

Analysis

(See *Ultraviolet-Visible Spectroscopy ⟨857⟩*.)

Add 2.5 mL of *1,9-Dimethylmethylene blue solution* to triplicate 100-µL aliquots each of *Heparin standard curve solutions*, *Sample solution*, *Blank solution*, and *Collagen control solution*. Mix on a vortex mixer for 1 s and immediately read the absorbance at 525 nm. Generate a standard curve of absorbance versus concentration using the averages of each *Heparin standard curve solution*, correcting for the blank,

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and calculate the regression line and regression coefficient. The concentration of glycosaminoglycan in the *Sample solution* and the *Collagen control solution* is determined directly from the regression line. If the absorbance of the *Sample solution* is greater than the highest *Heparin standard curve solution*, then dilute the *Sample solution* appropriately, and repeat the *Analysis* detailed in this section.

System suitability: The test is considered valid if the regression curve has a square of the correlation coefficient (r^2) NLT 0.95; the triplicate aliquots of the *Sample solution* and *Collagen control solution* yield results that are within 20% of each other, respectively; and the average glycosaminoglycan content of the *Sample solution* is statistically greater than the *Collagen control solution* using one-tailed, unequal variances, *t*-test at $\alpha = 0.05$.

Acceptance criteria: The average glycosaminoglycan content of the *Sample solution* is NLT 2 µg/mg.

- **Bioactivity**

[Note—Aseptic cell culture techniques must be used throughout the performance of this test.]

Modified RPMI-1640 culture medium: Prepare a sterile solution that contains the components included in Table 1.

Table 1

Component	Content (mg/L)
Calcium chloride	264.9
Ferric nitrate, nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate, heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic, monohydrate	125.0
Glucose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride, monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0

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Component	Content (mg/L)
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
L-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0
Sodium 1-heptanesulfonic acid	2383.0

Penicillin–streptomycin solution: 10,000 USP Penicillin Units of penicillin/mL and 10 mg of streptomycin/mL in a suitable buffer[2]

PC12 cell line culture medium: Mix 420 mL of *Modified RPMI-1640 culture medium*, 50 mL of horse serum,[3] 25 mL of fetal bovine serum,[4] and 5 mL of *Penicillin–streptomycin solution*. Sterilize by passing through a 0.22-µm filter.

Sterile PBS solution: 8.065 g/L of sodium chloride and 0.2 g/L of potassium chloride in 0.01 M sodium phosphate buffer, pH 7.4

Rat tail collagen solution: Prepare a suspension containing 0.2 mg/mL of rat tail collagen, type I, in sterile water.

Cell culture apparatus: Prepare by adding a sufficient volume of *Rat tail collagen solution* to completely cover the bottom of each well of a 12-well cell culture plate (dimension of each well is about 22–23 mm in diameter and about 17–18 mm in depth). Incubate under sterile conditions for 2 h at 37° or overnight at room temperature. Remove the *Rat tail collagen solution* by aspiration. Rinse with *Sterile PBS solution* that has been preheated to 37°.

PC12 cells: Use cultured rat pheochromocytoma cells (ATCC CRL-1721).

Cultivation of PC12 cells: Prewarm *PC12 cell line culture medium* to 37°. Add 15 mL of prewarmed *PC12 cell line culture medium* to a T-75 culture flask. Place a single vial containing the frozen *PC12 cells* in a 37° water bath with gentle agitation until they start to thaw (about 1 min). Complete the thawing procedure by slowly rotating the vial between the hands. Rinse the outside of the vial with 70% alcohol. Transfer the contents of the vial to the T-75 flask, and mix. Incubate the cells overnight at 37° in a 5% carbon dioxide atmosphere. Transfer the contents of the T-75 culture flask to a sterile centrifuge tube, centrifuge at 200 × *g* for 5 min at 37°, and discard the supernatant. Resuspend the cells in 15 mL of *PC12 cell line culture medium*, and transfer the contents back to the T-75 culture flask. Incubate the cells at 37° in a 5% carbon dioxide atmosphere for 3 days.

Cell feeding: At the end of 3 days, the cells will need to be fed for optimal growth. To feed the cells, remove a flask of cells from the incubator, tightening the cap in the process. Examine the T-75 flask under the microscope and check for microbial contamination and confluency. If there is microbial contamination, then discard the flask. If the cells appear confluent, follow the instructions below for perpetuating the PC12 cell line (see *Culture perpetuation*). Otherwise, harvest the cells from the flask by pipetting the contents of the flask across the bottom of the flask several times. Transfer the cell suspension to a sterile 50-mL centrifuge tube. Centrifuge the cells at 200 × *g* for 5 min at 37°, and discard the supernatant. Resuspend the cells in 13 mL of *PC12 cell line culture medium*, prewarmed to 37°. Transfer the cell suspension back to the T-75 flask, and mix. Loosen the cap of the flask, and return to the incubator; incubate the cells at 37° in a 5% carbon dioxide atmosphere for another 3–7 days.

Culture perpetuation: To perpetuate a line of *PC12 cells* for culture, examine under the microscope a T-75 flask containing cells and check for microbial contamination and confluency. If there is microbial contamination, discard the flask and use another. If the cells do not appear

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