Report of Investigation

Reference Material 8395

Tissue Engineering Reference Scaffold

This Reference Material (RM) is intended to provide a common tissue-engineering scaffold for measurement comparisons. A unit consists of one free-form fabricated [1] poly(ε-caprolactone) scaffold approximately 20 mm in external diameter, 5 mm in external height, 200 µm in strut diameter, 200 µm in strut spacing, and 47 % porosity.

Reference Values: Reference values for strut diameter, strut spacing, and porosity are provided in Table 1. An image of the scaffold is provided in Figure 1. Reference values are noncertified values that are the best estimate of the true value; however, the values do not meet the NIST criteria for certification [2]. The scaffolds may differ from unit to unit. The uncertainties given provide a gauge of the size of this variation.

Expiration of Reference Values: The reference values are valid within the measurement uncertainties specified until **31 December 2020**, provided that the RM is handled in accordance with the instructions given in this report (see "Instructions for Use"). The reference values are nullified if the RM is damaged, contaminated or otherwise modified.

Maintenance of Reference Values: This material is considered stable when stored at room temperature and protected from exposure to ultraviolet radiation and moisture. However, its stability has not been rigorously assessed. NIST will monitor this RM over the period of its validity. If substantive technical changes occur that affect the reference values before expiration, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the analyses was performed by F.W. Wang, M.T. Cicerone, and C.G. Simon, Jr., of the NIST Polymers Division.

A.L. Darling, L. Shor, and W. Sun fabricated the scaffolds and made optical microscopy measurements at Drexel University.

Statistical consultation for the analysis of data was provided by W.S. Liggett of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this RM were coordinated through the NIST Measurement Services Division.

INSTRUCTIONS FOR USE

The scaffold should be stored at room temperature and protected from exposure to ultraviolet radiation and moisture. The scaffold should be returned to the container after use and the container should be resealed. Do not bend the scaffolds as they may delaminate due to weak interlayer adhesive strength.

For measurements that entail an axial fluid flow though the scaffold (i.e., permeability), two surface layers immediately under one face of the scaffold may need to be removed to eliminate closed pores, if any. The layers can be shaved off with the use of a disposable microtome blade.

Eric K. Lin, Chief
Polymers Division

Gaithersburg, MD 20899
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Robert L. Watters, Jr., Chief
Measurement Services Division

When a specimen is to be cut from the poly(ε-caprolactone) scaffold, the edges of the specimen may need to be glued with a thin layer of an adhesive, for example, paraffin wax, to prevent delamination of the scaffold layers, because the scaffolds are weak in interlayer adhesive strength.

PREPARATION[1]

Scaffolds were made by a free-form fabrication process (precision extrusion deposition) [1] in a 0°/90° lay-down pattern using a research-grade poly(ε-caprolactone) polymer (number averaged relative molecular mass = 42 500; mass averaged relative molecular mass = 65 000; Sigma, Inc., St. Louis, MO). The scaffolds were fabricated by W. Sun and colleagues at Drexel University. Two heaters were used during extrusion/deposition. The feed heater was 95 °C and the tip heater was 90 °C. The extrusion tip diameter was 178 µm, the tip speed was 10 mm/s, and the flow rate was 0.017 mL/min. The targeted strut diameter was 200 µm and the targeted strut spacing was 200 µm. All scaffolds have a nominal external diameter of 20 mm and nominal external height of 5 mm.

REFERENCE VALUES

Optical Microscopy to Determine Scaffold Strut Diameter and Strut Spacing: An optical microscope was used to measure the strut spacing and the strut diameter at 5 locations in 10 scaffolds (randomly selected) for each RM.

Gravimetry to Determine Scaffold Porosity: The porosity of the scaffolds was determined by gravimetric analysis. Ten scaffolds of each type were analyzed. Calipers were used to determine the height and diameter of the scaffolds. The scaffolds were weighed to determine mass. The density of the poly(ε-caprolactone) was 1.145 g/mL as determined by the manufacturer. The following formula was used to determine porosity:

$$\text{porosity} = \{[(\pi r^2 \times h) - (m/d)]/ (\pi r^2 \times h)\}$$

where $\pi = 3.14$, r is scaffold radius (cm), h is scaffold height (cm), m is scaffold mass (g), and d is the poly(ε-caprolactone) density (g/cm^3).

Table 1. Reference Values for Strut Diameter, Strut Spacing, and Porosity of Scaffold

	Average	S.D.[a]	T.I.[b]	n	Method
Strut Diameter	220 µm	9 µm	33 µm	10	Optical Microscopy
Strut Spacing	194 µm	19 µm	68 µm	10	Optical Microscopy
Porosity	0.47	0.02	0.06	10	Gravimetry

[a] S.D. = An estimate of the standard deviation obtained from the 10 units measured [3].
[b] T.I. = 90% tolerance intervals with probability 0.99 [4]. A 90% tolerance interval contains 90% of the units of this reference material. The user should expect that a scaffold received will fall within this interval 90% of the time.

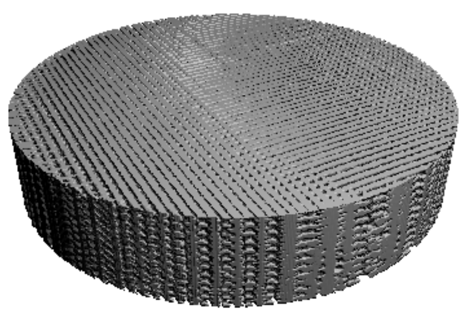

Figure 1. X-Ray Microcomputed Tomograph of Reference Scaffold RM 8395.

[1] Certain commercial equipment, instruments, or materials are identified in this report to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

REFERENCES

[1] Darling, A.L.; Sun, W.; 3D Microtomographic Characterization of Precision Extruded Poly-ε-caprolactone Scaffolds. *Journal of Biomedical Materials Research Part B: Appl. Biomater.* 70B: 311–317, 2004.

[2] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise S.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*, NIST Special Publication 260-136 Standard Reference Materials, U.S. Government Printing Office: Washington, DC (2000); available at http://ts.nist.gov/MeasurementServices/ReferenceMaterials/PUBLICATIONS.cfm.

[3] *Guide to the Expression of Uncertainty in Measurement,* ISBN 92-67-10188-9, lst Ed., ISO, Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*, NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs.

[4] NIST/SEMATECH e-Handbook of Statistical Methods (2003), http://www.itl.nist.gov/div898/handbook/.

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