Designation: F1854 – 09

Standard Test Method for Stereological Evaluation of Porous Coatings on Medical Implants[1]

This standard is issued under the fixed designation F1854; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers stereological test methods for characterizing the coating thickness, void content, and mean intercept length of various porous coatings adhering to nonporous substrates.

1.2 A method to measure void content and intercept length at distinct levels ("tissue interface gradients") through the porous coating thickness is outlined in 9.4.

1.3 The alternate sample orientation method in 8.2 is not suitable for the tissue interface gradients method in 9.4.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E3 Guide for Preparation of Metallographic Specimens

E883 Guide for Reflected–Light Photomicrography

3. Terminology

3.1 *Definitions:*

3.1.1 *field*—the portion of image of a part of the working surface upon which measurements are performed.

3.1.2 *intercept*—the point on a measurement grid line projected on a field where the line crosses from solid to void or vice versa.

3.1.3 *measurement grid lines*—a evenly spaced grid of parallel lines all of the same length.

3.1.4 *porous coating*—coating on an implant deliberately applied to contain void regions with the intent of enhancing the fixation of the implant.

3.1.5 *substrate*—the solid material to which the porous coating is attached.

3.1.6 *substrate interface*—the region where the porous coating is attached to the substrate.

3.1.7 *working surface*—the ground and polished face of the metallographic mount where the measurements are made.

3.1.8 *tissue interface*—the surface of the coating that shall have first contact with biological tissue (that is, the top of the coating).

4. Summary of Test Method

4.1 *Mean Coating Thickness*—Evenly spaced parallel grid lines are oriented perpendicular to the coating-substrate interface. For each gridline, the distance from the coating-substrate interface to the last contact with the porous coating material is measured as the coating thickness. The average of all of the coating thickness measurements obtained on a working surface is the mean coating thickness for that working surface.

4.2 *Volume Percent Void*—A regular grid of points is superimposed on a field from the working surface. The percentage of points that are in contact with void areas in the coating correlates with the volume percent of void present.

4.3 *Mean Void Intercept Length*—Measurement grid lines are oriented parallel to the substrate interface. The average length of the line segments overlaying the void space is the mean void intercept length. This is a representative measure of the scale, or size, of the pores in a porous structure.

4.4 *Tissue Interface Gradients*—The volume percent void and the mean void intercept length are characterized in three 200-μm-thick zones below the tissue interface.

5. Significance and Use

5.1 All of these test methods are recommended for elementary quantification of the morphological properties of porous coatings bonded to solid substrates.

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[1] This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.15 on Material Test Methods.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

5.2 These test methods may be useful for comparative evaluations of different coatings or different lots of the same coating.

5.3 With the exception of using the alternate mounting method, all the methods should be performed on the same working surfaces. The alternate mounting method can only be used for 9.2 and 9.3.

5.4 A statistical estimate can be made of the distributions of the mean coating thickness and the volume percent void. No estimate can be made of the distribution of intercept lengths.

5.5 There are limits to the accurate characterization of porosity, depending on spacing between the lines in the line grid (or points in the point grid) and the individual and cumulative fields used for the measurements. Increasing the size of the fields, increasing the number of fields, or decreasing the grid spacing will increase the accuracy of the measurements obtained.

5.6 This method is not suitable for ceramic coatings for which accurate coating cross sections cannot be produced using metallographic techniques.

5.7 This test method does not address characterization of coatings having a thickness of less than 300 μm.

6. Apparatus

6.1 The procedures outlined in this test method can be performed manually or using digital image analysis techniques.

6.2 *Microscope,* or other suitable device with a viewing screen, photomicrographic capability, or digital image capture capability should be used to image the sample fields of interest for these test methods.

6.3 For manual measurement, a transparent sheet, with measurement grid lines or points is superimposed on the viewing screen or photomicrograph for the measurements. The line grid (or point grid) and should consist of at least five uniformly spaced, parallel lines (or rows).

7. Metallography

7.1 The procedures outlined in this test method for characterizing porous coatings require the preparation of metallographic sections. Good metallographic preparation techniques, in accordance with Practice E3 and Guide E883, shall be used to prevent deformation of the surface of the section or creation of any other artifacts that will alter the morphology of the metallographic section. An example of an unacceptable artifact would be the absence of a portion of the porous coating, caused by its removal, thereby creating an artificial void area.

7.2 Care must be taken to ensure that the working surface is perpendicular to the substrate interface. When using the alternative mounting method shown in 8.1.2, extreme care must be taken to keep the substrate interface parallel to the final working surface.

8. Sample Working Surfaces and Fields

8.1 *Sample Orientation:*

8.1.1 *Normal Section Orientation:*

8.1.1.1 For accurate coating thickness measurements, the orientation of sample working surfaces should be approximately perpendicular to the plane of the substrate.

8.1.1.2 If the angle between the tangent to the coating-substrate interface at one edge of a field and the tangent to the substrate interface at the opposite edge of the field is greater than 2º, the substrate curvature is too large.

8.1.1.3 There is a practical limit to the magnification that can be used for measurement of the void content and mean intercept length. As magnification is increased, the number of fields should be increased to obtain a representative sample. If there are too few intercepts in the individual fields, the accuracy of the measurement could decrease.

8.1.2 *Alternative Orientation Method:*

8.1.2.1 An alternate orientation may be used for the volume percent void and mean intercept length measurements. The section should be prepared such that the working surface is parallel to the substrate interface and the measurements should be taken at a fixed distance from the substrate interface. It is recommended that the measurements be made at about 50 % of the mean coating thickness.

8.1.2.2 At least one additional section immediately adjacent to the fields used on the working surface shall also be prepared perpendicular to the working surface. This shall confirm that the substrate interface is parallel to the working surface and allow measurement of the distance from the working surface to the substrate interface.

8.1.2.3 This test method is not suitable for substrate interfaces with a radius of curvature less than 25 mm.

8.1.2.4 Since this test method also requires more aggressive porous surface removal to reach 50 % of the mean coating thickness, it may be more susceptible to creation of metallographic artifacts. Care should be exercised to ensure that the metallographic sections that are used are free of artifacts.

8.2 *Field Parameters:*

8.2.1 *Resolution:*

8.2.1.1 The magnification used for the field should be high enough to resolve all the features that need to be measured.

8.2.1.2 For most porous coatings, the magnification should be high enough that features as small as 5 μm can be easily distinguished. If digital imaging is used, the pixel size should be less than or equal to 5 μm.

8.2.2 *Field Dimensions:*

8.2.2.1 The field height must include the full thickness of the porous coating for mean coating thickness (9.1).

8.2.2.2 A good rule of thumb for an accurate measurement of mean void intercept length is that the minimum field width should be greater than or equal to 5× the resulting mean void intercept length. For example, a mean void intercept length value of 200 μm should have a measurement field width of at least 1000 μm.

8.2.2.3 It is possible to measure the mean void intercept length in a field using a series of shorter non-overlapping grid lines. This does not change the requirement for the number of fields required for the calculation. Care should be exercised using multiple short lines in a single field, because it may be possible to make the grid lines so short that the accuracy of the result is affected.

8.2.2.4 If the magnification used produces an image with a height or width smaller than that which is required, multiple images may be carefully stitched together to produce a field of sufficient height and width.

9. Procedure

9.1 *Mean Coating Thickness:*

9.1.1 An array of equally spaced parallel gridlines should be superimposed on the field perpendicular to the substrate interface, as shown in Fig. 1. The gridlines should be spaced no more than 100 μm apart. Appendix X2 includes two typical sets of gridlines each with ten equally spaced parallel lines.

9.1.2 At each gridline, the distance from the substrate interface to the last contact with a solid coating feature is measured. A measurement is only valid if the gridline is oriented 90 ± 2° to the substrate interface.

9.1.3 Coating thickness measurements should be obtained over a continuous linear distance of at least 10 mm of porous surface with no overlap between measurement sites.

9.1.4 The average of all the measurements is the mean coating thickness for that working surface. The standard deviation estimator and the 95 % confidence interval should be calculated for each working surface. The equations for calculating these values are as follows:

$$\bar{T} = \frac{1}{M \times n} \sum_{i=1}^{n} t_i \quad (1)$$

where:

t_i = the individual magnified thickness line length,
n = the number of thickness measurements,
M = the magnification, and
$\bar{T}$ = the mean coating thickness.

$$\hat{S} = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^{n} \left[\frac{t_i}{M} - \bar{T} \right]} \quad (2)$$

where:

$\hat{S}$ = the standard deviation estimator, and
CI = the confidence interval.

$$CI = 2 \times \frac{\hat{S}}{\sqrt{n}} \quad (3)$$

9.2 *Volume Percent Void:*

9.2.1 For this measurement, the field should be entirely contained between the tissue interface (see 9.4.1) and the substrate interface.

9.2.2 An array containing at least 100 regularly spaced points should be superimposed on the field, as shown in Fig. 2. The points should be spaced no more than 50 μm apart. If the void areas form a regular or periodic pattern, the use of a grid having a similar pattern should be avoided. The height of the array should be at least half the distance from the tissue interface to the substrate interface, thereby producing a value representative of an average for the entire coating thickness. Appendix X2 includes two typical arrays each with at least 100 regularly spaced points.

9.2.3 The number of points overlying void areas (Pα) on the working surface shall be counted and recorded. When using the manual method, any points falling on a boundary between a void area and solid features should be counted as one half. Any questionable points should be counted as one half.

9.2.4 The number of contact points in void area (P_α), divided by the total number of points on the grid (P_T) times 100 gives the percentage of grid points on the void for that field. This should be calculated for each grid application.

$$P_v = \frac{P_\alpha}{P_T} \times 100 \quad (4)$$

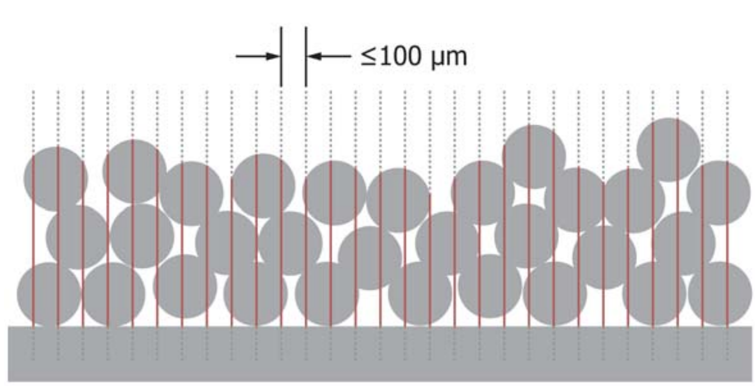
where:

P_α = the total number of counted points,
P_T = the total number of grid points, and
P_v = the volume percent void.

9.2.5 Volume percent voids should be measured over an area of the working surface totaling at least 15 mm^2 with no part of that area being measured more than once. If the coating thickness is below 500 μm, at least 3 cm of coating length must be used for the void measurement.

9.2.6 Fields to be analyzed should include as much of the coating thickness as possible.

9.2.7 These measurements may also be made with an appropriate digital image analysis system. This can be done by considering each pixel as a regularly spaced point in the array. The volume percent void for each field should be the ratio of the number of pixels representing void space to the total number of pixels in the image of the field.



NOTE 1—The solid line is the measured distance.

FIG. 1 Illustration of Coating Thickness Measurement

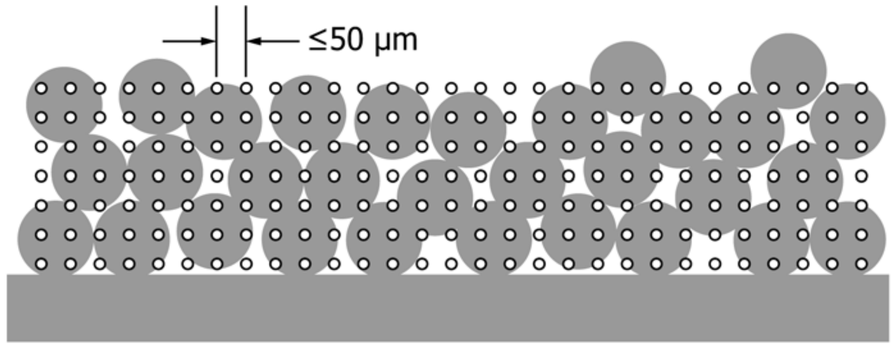


FIG. 2 Illustration of Volume Percent Void Measurement

9.2.8 The average percentage of the grid points on the voids provides an unbiased statistical estimator for the void volume percentage in the three dimensional structure. The mean void percentage ($\bar{P}_v$) for that working surface, the standard deviation estimator ($\hat{S}$) and the 95 % confidence interval (CI) should be calculated for each working surface. The equations for calculating these values are as follows:

$$\bar{P}_v = \frac{1}{n} \sum_{i=1}^{n} P_{v_i} \quad (5)$$

$$\hat{S} = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^{n} \left[P_{v_i} - \bar{P}_v \right]} \quad (6)$$

$$CI = 2 \times \frac{\hat{S}}{\sqrt{n}} \quad (7)$$

9.2.9 The volume percent void estimate is given by the following relationship:

$$V_v = P_v \quad (8)$$

9.3 *Mean Void Intercept Length:*

9.3.1 For this measurement, the field should be entirely contained between the tissue interface (see 9.4.1) and the substrate interface.

9.3.2 An array of equally spaced parallel gridlines should be superimposed on the field parallel to the substrate interface, as shown in Fig. 3. The height of the array should be at least half the distance from the tissue interface to the substrate interface, thereby producing a value representative of an average for the entire coating thickness. The gridlines should be spaced no more than 100 μm apart. Appendix X2 includes two typical sets of gridlines each with ten equally spaced parallel lines.

9.3.3 The number of times that a void region is intercepted by the test lines (N_v) is counted and recorded. There are two methods that can be used for counting.

9.3.3.1 The first method counts the number of intersections along the grid lines. Each time the grid line goes from either solid to void or void to solid is counted as one intersection. The number of intersections (n_i) is twice the number of intercepts (N_v).

$$N_v = \frac{n_1}{2} \quad (9)$$

9.3.3.2 In the second method, the crossing direction of any intersection on any line determines if it is counted as an intercept. If the beginning of the line starts on a void, count the transitions from void to solid. If that same line ends on a void, count that as a one more intercept. If the beginning of a line starts on a solid, count the transitions from solid to void. If the same line ends on a solid, there is no additional count. In this case the number of counts is the number of intercepts (N_v).

9.3.4 Mean void intercept length should be determined over a cumulative area of at least 5 mm^2 on each working surface.

9.3.5 For the alternative orientation method, the orientation of the grid should be random.

9.3.6 An estimate of the mean intercept length (L_v) can be calculated from the total length of lines (L_T), the number of intercepts (N_v), the magnification M, and the previously calculated volume percent void (V_v). Since V_v was multiplied by 100 to make it a percentage, it must be divided by 100 to be used in this equation. In addition, in automated systems where the measurements are already calibrated to the magnification, dividing by M is not required. The calculation is as follows:

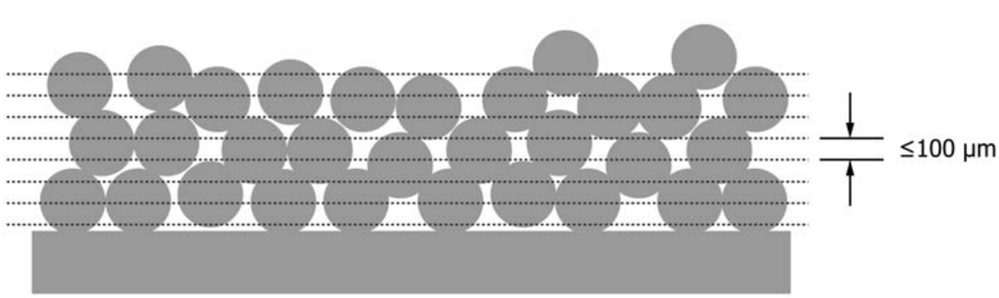


FIG. 3 Illustration of Mean Intercept Length Measurement Field

$$L_v = \frac{\frac{V_v}{100} \times \frac{L_T}{M}}{N_v} \quad (10)$$

9.3.7 For digital measurement using an image analysis system, an alternate measurement method may be used to calculate the mean void intercept length: The distance from one solid feature to another is measured everywhere a gridline crosses void space. If the region of a grid line crossing a void does not start and end at a solid coating feature (for example, it starts or ends at the edge of the field or in the middle of a void space), it should not be included. The average of all the measurements in a field is the mean void intercept length for that field (l_i). For this technique, the field cannot be broken into a set of smaller grids.

$$\bar{L}_v = \frac{1}{n}\sum_{i=1}^{n} l_i \quad (11)$$

9.4 *Tissue Interface Gradient Method:*

9.4.1 Define the tissue interface of the porous coating.

9.4.1.1 The first method to define the location of the tissue interface is a physical one. Securely attach a flat metallic surface on the porous interface of the metallographic sample prior to embedding the sample. The attached flat metal surface must show that it has not moved away from the tissue interface during the metallographic mounting process and must have an angle away from the substrate of less than 1°. Note that spring-loaded binder clips have been used to secure the attached flat metal plate to the tissues interface.

9.4.1.2 The second method is to use the average of the longest 5 % of the thickness measurements from the mean coating thickness procedure in 9.1. Use that average to draw a line, in any field examined, that distance from the substrate, parallel within 1° to the substrate, to define the tissue interface.

9.4.2 Divide the region of the porous coating immediately below the tissue interface into three zones as shown in Fig. 4. The first zone should be from 0 to 200 µm below the tissue interface second zone should be from 200 to 400 µm below the tissue interface, and the third zone should be from 400 to 600 µm below the tissue interface. If the distance from the tissue interface to the substrate is less than 600 µm but more than 500 µm, the third zone should be 400 µm from the tissue interface to the substrate.

9.4.3 In each of the three zones repeat the measurements in 9.2 (volume percent void) and 9.3 (mean void intercept length) using the zone height as the field height. Appropriate adjustment of the point and line arrays may be necessary to accommodate the decreased field height.

10. Report

10.1 A description of the object from which the sample metallographic sections are obtained and the location of those sections within that object shall be reported.

10.2 Report the chemical composition of the substrate and the coating materials.

10.3 The original morphological form of the coating material (that is, powder, wire, and so forth) shall be reported.

10.4 The number of fields of view for each measurement, the magnifications used, and the grids and lines used shall be reported.

10.5 The mean coating thickness standard deviation and confidence interval, the volume percent void standard deviation and confidence interval, and the mean void intercept length shall be reported.

10.6 If the substrate interface is curved in the plane of the working surface, an estimate of the local radius of curvature shall be reported.

10.7 If the coating tissue interface gradient method is used, the volume percent void and the mean linear intercept length, together with the distance from the substrate for each gradient zone shall be reported.

10.8 For image analysis systems that measure on digitized video images, the minimum measurement value (that is, the dimension of a single pixel) at all magnifications used in the measurements shall be reported.

11. Precision and Bias

11.1 The following factors could significantly affect the results obtained using this method:

11.1.1 The presence of structural gradients or inhomogeneities in the section can influence the precision and accuracy of the measurements. If the amount of void in the porous coating

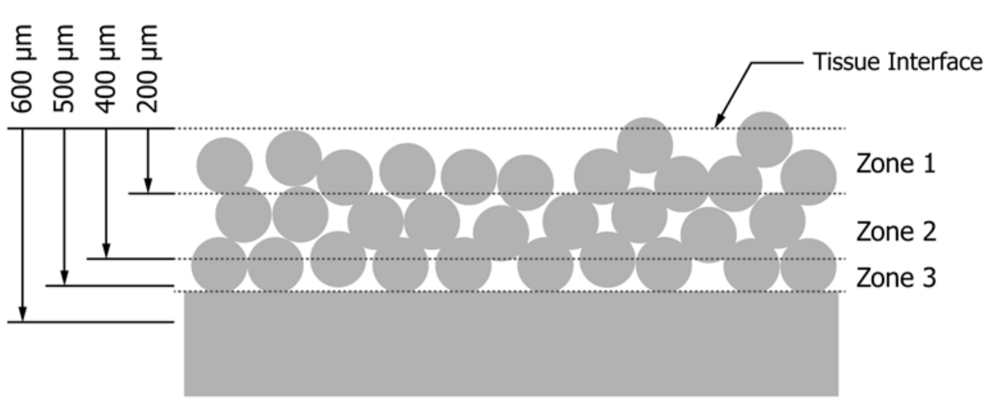


FIG. 4 Illustration of the Gradient Zones

changes with thickness, the tissue interface gradient method may be used to characterize the gradient.

11.1.2 The quality of sample preparation can influence precision and bias.

11.1.3 If the manual method is used to measure volume percent void or the estimation method is used to measure mean void intercept length, the counting of grid points and line ends at void boundaries presents an opportunity for bias in the measurements. If these techniques are used, the methods outlined in 9.2.6 and 9.3.4, respectively, shall be followed.

11.1.4 The number of fields measured, the method of field selection, and their spacing can influence the precision and accuracy. Random selection of fields and, except where noted, random orientation of grids within the field can help eliminate bias.

11.2 The results of an interlaboratory comparison, although not comprehensive enough to support statistical statements about the precision of this method, are presented in Appendix X4 for information only.

12. Keywords

12.1 coating gradiants; metallography; porosity; porous coatings; stereology; thickness

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 Porous coatings are applied to the surface of medical implants. Standardized techniques to characterize the structure of the porous coatings should be available. These techniques can be used to estimate the uniformity and repeatability of these porous structures. In their book *Practical Stereology*,[3] Russ and Dehoff describe the term mean lineal intercept measurement as a "representative measure of the scale (i.e. size) of the features in the feature set." "Mean void intercept length" is not a specific physical feature such as "pore diameter," but it is a mathematically valid measure of porosity. It is affected by the size of the porosity and its geometry. To a lesser extent it is also affected by pore interconnectivity and surface roughness. There is not another mathematically valid measure that can be made to characterize three dimensional porosity of a shape that cannot be mathematically defined.

X1.2 Porous coatings with significant gradients in the porosity from the substrate to the surface may not be accurately measurable using the test methods in 9.2 and 9.3 The alternative orientation method is not suitable for coatings with significant gradients because maintaining the orientation of the measurement surface near parallel to the substrate and precisely removing material to step through the gradient would be extremely difficult to do.

X1.3 Specimen alignment in the metallographic mount can affect measurement results. Fortunately, for most of the cross section mounts, the error that occurs from not having a perfect perpendicular cross section is minimal. If the sample mounting is not a perpendicular cross section, the coating thickness will tend to increase. However the amount of increase varies with the cosine of the angle off of perpendicular. Even if the sample is 10º off perpendicular the increase of the coating thickness is only about 1.5 %. However, the angular tolerance of the substrate is much more stringent in the tissue interface gradient method. Because of the narrow 200 μ height of the measurement zone, it would be much easier for a measurement to exceed the boundaries of the measurement zone if the tissue interface reference plane is not established with required precision.

X1.4 With the advent of automated image analysis systems, these types of measurements can be made with greater ease. Automated image analysis can be used to make these measurements, but care should be exercised to be sure that the automated techniques are comparable to the hand techniques.

[3] Russ, J. C., DeHoff, R. T., *Practical Stereology,*, 2nd Ed., Plenum Press, New York, 2000.