Designation: ~~F1854 – 09~~ F1854 – 15

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")~~("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.~~

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

1.4 *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:*~~*Definitions of Terms Specific to This Standard:*

3.1.1 *field*—the ~~portion of~~ image of a ~~part~~portion 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~~an 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.~~

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

3.1.8 *working surface*—the ground and polished face of the metallographic mount where the images of the fields are captured.

4. Summary of Test Method

4.1 *Mean Coating Thickness*—Evenly spaced parallel grid lines are oriented perpendicular to the coating-substrate ~~interface.~~ interface on a field. For each gridline, the distance from the coating-substrate interface to the last contact with the porous coating

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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.

material is measured as the coating thickness. The average of all of the coating thickness measurements obtained ~~on a working surface~~ from all the measured fields is the mean coating thickness for that ~~working surface.~~ coating.

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~~ is the volume percent of void ~~present.~~present in that field.

4.3 *Mean Void Intercept Length*—Measurement grid lines are oriented parallel to the substrate ~~interface.~~ interface in a field. The average length of the line segments overlaying the void space is the mean void intercept ~~length.~~ length for that field. 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~~Volume Percent Void and the ~~mean void intercept length~~Mean Void Intercept Length are characterized in three 200-µm-thick zones below the ~~tissue interface.~~Tissue Interface in each field.

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.

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~~ All the methods should be performed on the same ~~working surfaces. The alternate mounting method can only be used for~~ set of images of fields.~~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~~may be suitable for ceramic coatings ~~for which~~if an accurate coating cross ~~sections cannot be produced using~~ section can be produced. Producing an accurate ceramic coating cross section may require other techniques than standard metallographic techniques.

5.7 ~~This test method does not address characterization of~~ For coatings having a mean thickness ~~of~~ less than 300 ~~µm.~~microns, it is not recommended to attempt to determine the volume percent void or the mean intercept length.

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~~Guides 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~~shall 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 2°, 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.

8.2.1.3 For digital images the pixel size in µm and the image field size in pixels shall be included with the images.

8.2.2 *Field Dimensions:*

8.2.2.1 The field height ~~must include the full thickness of~~ of the images shall be large enough to include all the features of any portion of the porous coating for ~~mean coating thickness~~ (Mean Coating Thickness determination (section 9.1).

8.2.2.2 A good rule of thumb for an accurate measurement of ~~mean void intercept length~~Mean Void Intercept Length is that the minimum field width should be greater than or equal to ~~5×~~ 5 times the resulting ~~mean void intercept length.~~Mean Void Intercept Length. For example, a ~~mean void intercept length~~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~~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~~coating field length required for the calculation. Care should be exercised using multiple short lines in a single field, because it ~~may be~~ is 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.

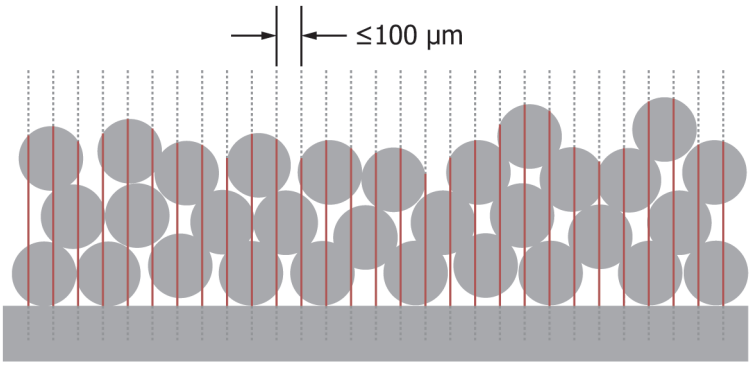
8.2.2.5 All four types of measurements shall be performed over a minimum coating area of 15 mm^2 with no area being measured more than once. For thinner coatings that require higher magnifications to allow reasonable measurements the minimum total measured field length shall be 20 mm with no part of that length being measured more than once.

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~~90° ± 2° to the substrate interface.



NOTE 1—The solid line is the measured distance.

FIG. 1 Illustration of Coating Thickness Measurement

9.1.2.1 The surface of the substrate is usually rough due to the processing involved in applying the coating. If the surface is too rough, a line indicating a subjective average substrate interface shall be made on the each field. Thickness measurements shall then be made from this line.

9.1.2.2 If a subjective average interface line is used, the same line should be used for the positioning of the other measurements in this standard.

9.1.3 Coating thickness measurements should be obtained over a ~~continuous~~ linear distance of at least ~~10~~20 mm of porous surface with no overlap between measurement sites.

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

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

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.

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

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

where:

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

$$CI = 2 \times \frac{\hat{S}}{\sqrt{n}} \tag{3}$$

9.1.5 Define the Tissue Interface of the porous coating.

9.1.5.1 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 shall have an angle away from the substrate of less than 1°.

NOTE 1—Spring-loaded binder clips have been used to secure the attached flat metal plate to the tissues interface.

9.1.5.2 The second method is to use the average of the longest 5% of the thickness measurements from the Mean Coating Thickness procedure in section 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.

9.1.6 For rough surface coatings, such as plasma spray coatings intended to create a roughened surface if the spacing of the thickness lines is too large it can be affect the repeatability of the thickness measurement. If the mean of the thickness plus the standard deviation of the thickness is more than one half of the standard deviation of the thickness below the value of the tissue interface, then the line spacing should be decreased and the thickness re-measured. For such coatings, it may be better to start with the distance between the thickness measurement lines at 33 µm or less.

9.2 *Volume Percent Void:*

9.2.1 For this measurement, the field should be entirely contained between the ~~tissue interface (see~~Tissue Interface (section 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~~ include the coating from 10% to 90% of the tissue interface ~~to the substrate interface, thereby producing a value representative of an average for the entire coating thickness.~~ value. The same grid positioned at 10% of the tissue interface away from the substrate should be used for all fields. 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)~~(P_α) on the ~~working surface~~ fields 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.

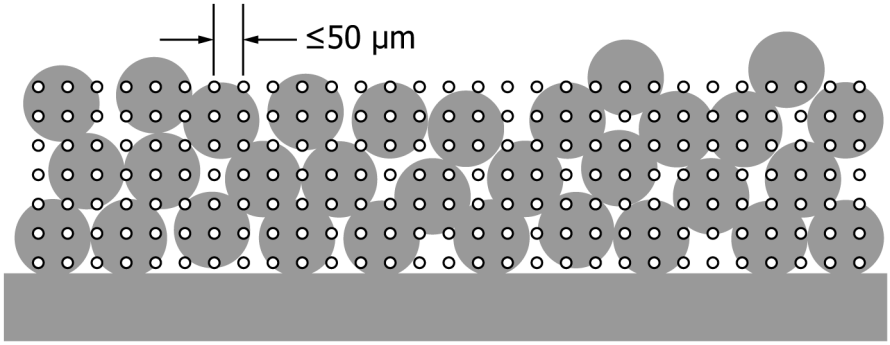


FIG. 2 Illustration of Volume Percent Void Measurement

9.2.4 The number of contact points in void ~~area~~areas (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.

$$P_v = \frac{P_\alpha}{P_T} \times 100 \tag{4}$$

~~where:~~

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

$$P_v = \frac{P_\alpha}{P_T} \times 100 \tag{4}$$

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.~~

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

9.2.6 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.

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

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

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

$$CI = 2 \times \frac{\hat{S}}{\sqrt{n}} \tag{7}$$

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

$$\hat{S} = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^{n} \left[P_{v_i} - \bar{P}_v\right]^2} \tag{6}$$

$$CI = 2 \times \frac{\hat{S}}{\sqrt{n}} \tag{7}$$

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

$$V_v = P_v \tag{8}$$

9.3 *Mean Void Intercept Length:*

9.3.1 For this measurement, the field should be entirely contained between the ~~tissue interface (see~~Tissue Interface (section 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~~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).

$$N_v = \frac{n_1}{2} \tag{9}$$

9.3.3.2 In the second ~~method,~~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~~(N_v).

9.3.4 Mean ~~void intercept length~~Void Intercept Length should be determined over a cumulative area of at least ~~5~~15 mm^2 on each working ~~surface.~~surface for thick coatings and 20 mm of length for thinner coatings.

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

9.3.5 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:

$$L_v = \frac{\frac{V_v}{100} \times \frac{L_T}{M}}{N_v} \tag{10}$$

$$L_v = \frac{\frac{V_v}{100} \times \frac{L_T}{M}}{N_v} \tag{10}$$

9.3.6 For digital measurement using an image analysis system, an alternate measurement method may be used to calculate the ~~mean void intercept length:~~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,~~ (e.g., it starts or ends at the edge of the field or in the middle of a void space), it ~~should~~shall 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.

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

9.4 *Tissue Interface Gradient Method:*

~~9.4.1 Define the tissue interface of the porous coating.~~

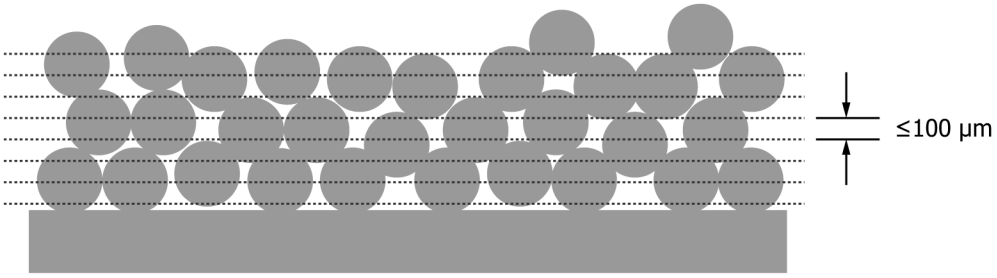


FIG. 3 Illustration of Mean Intercept Length Measurement Field