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Standard Test Method for Wound Closure Strength of Tissue Adhesives and Sealants[1]

This standard is issued under the fixed designation F2458; 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 a means for comparison of wound closure strength of tissue adhesives used to help secure the apposition of soft tissue. With the appropriate choice of substrate, it may also be used for purposes of quality control in the manufacture of medical devices used as tissue adhesives.

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

1.3 *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]
D907 Terminology of Adhesives
E4 Practices for Force Verification of Testing Machines

2.2 *Other Document:*
American Association of Tissue Banking, Standards for Tissue Banking[3]

3. Terminology

3.1 Many terms in this test method are defined in Terminology D907.

3.2 *Definitions:*

3.2.1 *tissue adhesive*—any material used as a medical device to help secure the apposition of two wound edges or opposed soft tissues.

3.2.2 *tissue sealant*—a surface coating with adequate adhesive strength to prevent leakage of body fluids.

3.2.3 *cohesive strength*—internal strength of the adhesive.

3.2.4 *adhesive strength*—the strength of the tissue adhesive/substrate interface.

3.2.5 *cohesive failure*—failure of the internal adhesive bond.

3.2.6 *adhesive failure*—failure of the adhesive/substrate bond.

3.2.7 *substrate failure*—failure of the tissue substrate.

4. Significance and Use

4.1 Materials and devices that function at least in part by adhering to living tissues are finding increasing use in surgical procedures either as adjuncts to sutures and staples, or as frank replacements for those devices in a wide variety of medical procedures. While the nature and magnitude of the forces involved varies greatly with indication and with patient specific circumstances, all uses involve to some extent the ability of the material to resist imposed mechanical forces. Therefore, the mechanical properties of the materials, and in particular the adhesive properties, are important parameters in evaluating their fitness for use. In addition, the mechanical properties of a given adhesive composition can provide a useful means of determining product consistency for quality control or as a means for determining the effects of various surface treatments on the substrate prior to use of the device.

4.2 The complexity and variety of individual applications for tissue adhesive devices, even within a single indicated use (surgical procedure, which itself may vary depending on physical site and clinical intention) is such that the results of a single tensile strength test is not suitable for determining allowable design stresses without thorough analysis and understanding of the application, adhesive behaviors, and clinical indications.

4.3 This test method may be used for comparing adhesives or bonding processes for susceptibility to fatigue, mode of failure, and environmental changes, but such comparisons must be made with great caution since different adhesives may respond differently to varying conditions.

4.4 A correlation of the test method results with actual adhesive performance in live human tissue has not been established.

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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.

[3] Available from American Association of Tissue Banks (AATB), 1320 Old Chain Bridge Rd., Suite 450, McLean, VA 22101.

5. Apparatus

5.1 *Testing Machine*—A testing machine of the constant-rate-of-crosshead-movement type and comprising essentially the following:

5.1.1 *Fixed Member*—A fixed or essentially stationary member carrying one grip.

5.1.2 *Movable Member*—A movable member carrying a second grip.

5.1.3 *Grips*—Grips for holding the test specimen between the fixed member and the movable member of the testing machine can be either the fixed or self-aligning type. Gripping pressure should be adjustable to prevent damage to the substrate and the use of sandpaper or plastic scrubbing pads between the gripping surfaces and the substrate is recommended to help prevent slippage.

5.1.3.1 Fixed grips are rigidly attached to the fixed and movable members of the testing machine. When this type of grip is used, extreme care should be taken to ensure that the test specimen is inserted and clamped so that the long axis of the test specimen coincides with the direction of pull through the centerline of the grip assembly.

5.1.3.2 Self-aligning grips are attached to the fixed and movable members of the testing machine in such a manner that they will move freely into alignment as soon as any load is applied so that the long axis of the test specimen will coincide with the direction of the applied pull through the center line of the grip assembly. The specimens should be aligned as perfectly as possible with the direction of pull so that no rotary motion that may induce slippage or damage to the sample will occur in the grips; there is a limit to the amount of misalignment self-aligning grips will accommodate.

5.1.4 *Drive Mechanism*—A drive mechanism for imparting to the movable member a uniform, controlled velocity with respect to the stationary member, with this velocity to be regulated as specified in 8.3.

5.1.5 *Load Indicator*—A suitable load-indicating mechanism capable of showing the total tensile load carried by the test specimen when held by the grips. This mechanism shall be essentially free of inertia lag at the specified rate of testing and shall indicate the load with an accuracy of ±1 % of the indicated value, or better. The accuracy of the testing machine shall be verified in accordance with Practices E4.

5.2 *Temperature-controlling Equipment*—Capable of maintaining the test temperature to ±2°C. If ambient laboratory conditions are employed, the same degree of control is required.

6. Test Substrate

6.1 *For Comparative Testing*—Either fresh or frozen split thickness porcine skin graft may be used.

6.1.1 Frozen split thickness porcine skin that has been aseptically prepared is available commercially and is preferred due to ease of use and the potential for more consistent properties. It should be thawed according to the manufacturer's instructions prior to use. Unused graft may be kept at 2 to 8°C for up to two weeks after thawing.

6.1.2 If fresh skin is chosen, it should be prepared according the method in Appendix X1.

6.2 *For Application Specific Testing*—The grips of the test machine must be able to hold the tissue without having the tissue slip or be crushed by the grips. Some tissue (liver, lung) may not be suitable for this test.

6.2.1 The strength of any adhesive is highly dependent on the test substrate or adherend. For a specific application, the preferred substrate is freshly harvested tissue from the target organ of a domestic food animal. Tissue from bovine, porcine, or ovine origin is preferred due to wide availability and the fact that relatively large samples of tissue can be harvested from a single source. Ideally, the tissue should be used within 24 h of harvest and should be kept between 5 and 10°C prior to testing if it cannot be used immediately after harvesting. Storage and handling of tissue samples should be carried out according to the guidelines set forth in Standards for Tissue Banking by the American Association of Tissue Banks. The specimens should be brought to the test temperature or other prescribed temperature (such as body temperature) prior to application of the adhesive.

6.2.2 Fixed tissue should not be used since it has been demonstrated that fixatives cause large alterations in the mechanical properties of the tissue and it is probable that the adhesive strength would be affected as well.

6.2.3 If the target organ is of a size or geometry that does not allow fabrication of test samples as shown in Fig. 1, a tissue of similar origin but larger size should be used. For example, if the intended indication is for anastomosis of small blood vessels, a larger vessel should be substituted.

6.2.4 The thickness of the tissue sample should not exceed 5 mm.

6.3 *For Quality Control Testing:*

6.3.1 For testing that is undertaken as part of a quality control process in the manufacturing of a tissue adhesive device, the use of freshly harvested tissue is highly inconvenient and may also lead to unacceptable variation in the test results, especially if the failure occurs in the adherend (substrate failure). Since the purpose of quality control testing is to demonstrate consistency in the device, substitution of a model substrate is preferred so long as it is demonstrated that the adhesive bonds to the adherend. If the test is intended to generate data on the cohesive strength of the device, any metallic or polymeric material is acceptable so long as it has been demonstrated that the adhesive bonds to the adherend and that failure is substantially cohesive (>90 % by area) and not adhesive. For adhesive quality control testing, it is recommended that test results for any substrate of non-biological or fixed tissue origin be correlated to testing previously done on fresh tissue substrates prior to acceptance of the procedure.

7. Test Specimen

7.1 The wound strength test specimen is shown in Fig. 1. Two substrate samples are required for each test specimen.

7.2 A template of the correct dimensions should be used. A sharp scalpel or similar device should be used to cut the substrate material.

7.3 Sample width should be 2.5 ± 0.1 cm.

7.4 Sample length should be 10 ± 0.2 cm.

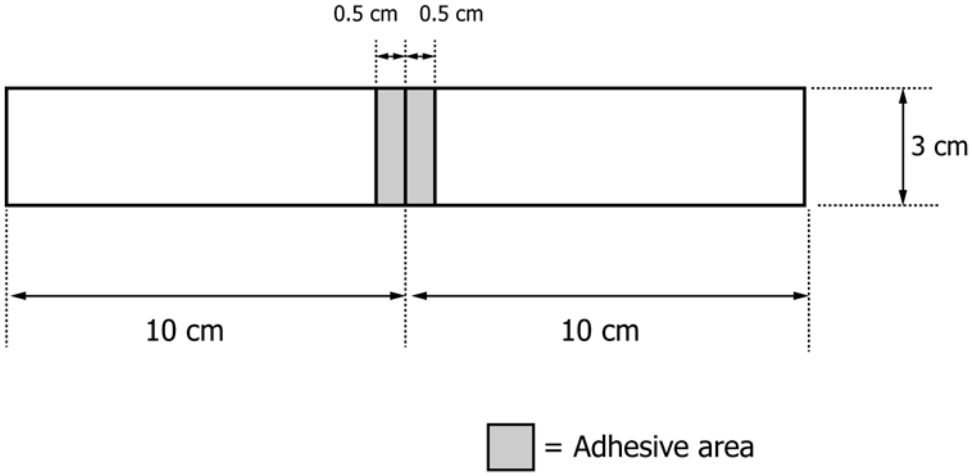

FIG. 1 Test Specimen Top View

7.5 *Number of Test Specimens*—Test at least 10 specimens of each type. Tissue substrates tend to give high variances and will require more samples to attain a reasonable estimate of the mean strength.

8. Specimen Preparation

8.1 *Tissue Preparation:*

8.1.1 Tissue substrate materials should be kept moist at all times with phosphate buffered saline (PBS).

8.1.2 The substrate will be cut to the dimensions given in Section 7 using a template and a fresh scalpel blade or a cutter fabricated to the required dimensions.

8.1.3 Wrap the tissue with gauze soaked in phosphate buffered saline (PBS), place in a plastic bag, and place them in a water bath or environmental chamber at 37°C.

8.2 *Preparation of the Adhesive Bond:*

8.2.1 Remove two tissue samples from the plastic bag and pat the surfaces of the tissue dry with fresh gauze.

8.2.2 Orient the samples as shown in Fig. 1 with the ends touching. A non-slip surface should be used to help hold the samples in place during adhesive application.

8.2.3 Prepare the adhesive and use according to the instructions for use (IFU). For topical adhesives, care should be taken to ensure that they are tested topically (as a bridge) as intended for clinical use and measures (petroleum jelly between edges and the under surface and sides) should be taken to ensure the adhesive does not seep between the wound edges and provide an interface bond. An interface bond may give highly variable results. Adhesive thickness is also an important parameter to try and control and will also lead to tensile strength variation. By keeping the adhesive within the required bond area (0.5 cm on either side of the join line), thickness may be controlled by applying a predetermined volume of adhesive.

8.3 After the adhesive has cured, measure and record the width and length of the adhesive bond to within 0.05 cm.

8.4 Re-cover the tissue with gauze soaked in PBS, replace the sample in a plastic bag, and return it to the constant temperature environment. Adhesives should be allowed to cure according to the IFU and for a minimum of 15 min.

9. Procedure

9.1 Condition the test specimens for definite periods of time under specified, controlled conditions before testing if desired. Recommended conditions for tissue adhesives intended for internal applications are 37°C in PBS. For adhesives intended for external topical use, recommended conditions are 30°C and 50 % relative humidity. For quality control testing, the recommended conditions are 23°C and 50 % relative humidity. After conditioning, it is recommended that all specimens be stabilized at the test temperature for 15 min before testing if the test temperature is different from the conditioning temperature. Tissue samples must be kept moist with PBS throughout the process to prevent shrinkage due to drying.

9.2 Place the test specimens in the grips of the testing machine so that the applied load coincides with the long axis of the specimen. The distance from the grip to the mid-line of each sample should be 5 cm, with the remaining 5 cm being firmly held with the grips. Load the specimen to failure at a constant cross-head speed of 50 mm/min.

9.3 Record the time from application to testing (cure time), force at failure (maximum force required to disrupt substrate), and the type of failure (percentage cohesive, adhesive, or substrate failure based on observation of the bond area).

10. Calculations

10.1 Calculate the median, mean, and standard deviation of the peak load at failure (Newtons (N)) for each group of samples.

11. Report

11.1 Report the following:

11.1.1 Complete identification of the adhesive tested, including type, source, date manufactured, manufacturer's lot number, and expiration date.

11.1.2 Complete identification of the substrate used, its length, width, and thickness, and any method used to clean or prepare the surface prior to bonding.

11.1.3 Amount of adhesive applied.

11.1.4 Method of adhesive application.

11.1.5 Ambient conditions at time of bonding (temperature and humidity).

11.1.6 Length and width of adhesive bond.

11.1.7 Conditioning of specimen prior to testing.

11.1.8 Maximum, minimum, median, mean, and standard deviation for the peak loads measured.

11.1.9 Number of specimens tested.

11.1.10 Type of failure. This should include estimated percentages of cohesive failure in the adhesive, apparent failure in adhesion, and failure in the adherend (substrate).

11.1.11 Test temperature employed.

12. Precision and Bias

12.1 A precision and bias statement does not exist for this test method because round robin testing has not yet been performed.

13. Keywords

13.1 adhesive strength; cohesive strength; tissue adhesive; wound closure strength

APPENDIX

(Nonmandatory Information)

X1. PROCEDURE FOR PREPARATION OF FRESH SPLIT THICKNESS PORCINE SKIN GRAFT

NOTE X1.1—The consistency of porcine skin prepared according to this method has not yet been evaluated in comparative testing with commercially available frozen porcine skin. Inconsistencies in preparation are likely to increase the variability of the test results and require a larger number of samples to achieve statistically valid results.

X1.1 Materials

X1.1.1 Fresh pig skin procured bilaterally from the flanks of the pig.

X1.1.2 Isopropyl alcohol (70 %).

X1.1.3 #20 scalpel blades with handle.

X1.1.4 Dermatome.

X1.1.5 Microtome blades.

X1.1.6 Non-sterile gauze 4×4's.

X1.1.7 Four (4) non-sterile towels.

X1.1.8 Sterile normal saline warmed to 37°C. An antibiotic-antimycotic preparation such as those used for tissue culture should be added to the saline at 10× the recommended concentration.

X1.1.9 Two (2) petri dishes.

X1.1.10 Two (2) cutting boards.

X1.1.11 Needle holder.

X1.1.12 Spray bottle.

X1.1.13 Dermatome cutting board.

X1.1.14 Two (2), 4 penny nails.

X1.1.15 Mineral oil.

X1.1.16 Forceps.

X1.1.17 20 μL micro-pipette.

X1.2 Method for Preparation

X1.2.1 Fresh, shaved pig skin harvested bilaterally from the flanks of the pig is procured from a local source and transported to the research laboratory in a cooler on ice. Each piece of skin is approximately 6 in. wide and 18 in. long.

X1.2.2 The skin is removed from the cooler and the dermal surface is cleaned with alcohol and gauze, then placed epidermal side up on a cutting board.

X1.2.3 The pig skin is then cut in 4 strips, 1.5 in. wide and 18 in. long, using a number 20 scalpel blade. The four pieces are covered with a saline soaked surgical towel to inhibit dessication of the tissue.

X1.2.4 A non-sterile surgical towel is placed onto the laboratory countertop. Several gauze 4×4's are placed on top of the surgical towel and then moistened with normal saline using a spray bottle.

X1.2.5 One of the pig skin strips is placed epidermal side down on another cutting board and the underlying fat layer is excised using a microtome blade secured in a needle holder. The fat layer is removed to the level of dermis. Once this task is completed, the skin is immediately placed epidermal side down onto the saline soaked 4×4's and the dermal surface is then covered with 4×4's moistened with normal saline. The gauze 4×4's are covered with a non-sterile surgical towel. The towel is then moistened with normal saline using the spray bottle. The microtome blade is removed and discarded and a new blade is installed. The process is completed for the remaining three pieces of skin.

X1.2.6 Once the fat layer has been removed from each of the strips of pig skin, the cutting board is washed with mild soap and dried.

X1.2.7 One piece of pig skin is placed, dermal side down, on a specifically designed dermatome cutting board. The skin is secured with one nail at each end of the skin.

X1.2.8 A dermatome with a cutting blade set at a cutting depth of 0.13 mm is used to remove the epidermal layer of pig skin. A forceps is used to remove the harvested epidermal layer during the cutting process. Immediately after completing the excision of the epidermis, the nails are removed from the two ends of the skin and the skin is placed back onto the normal saline soaked 4×4's with the freshly harvested dermal side down. The skin is then covered with additional normal saline soaked 4×4's and covered with the normal saline moistened

non-sterile surgical towel. The process is completed for the three remaining pieces of skin.

X1.2.9 The non-sterile towel and 4×4's covering the strips of skin are removed and the visible dermal layer of pig skin is wiped with a dry 4×4.

X1.2.10 The prepared skin is cut into strips of the appropriate size for the particular test being conducted.