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Standard Guide for Testing and Characterization of Alginate Foam Scaffolds Used in Tissue-Engineered Medical Products (TEMPs)[1]

This standard is issued under the fixed designation F3274; 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 Consistent functionality of scaffolds used in TEMPs can be made more predictable by monitoring relevant characteristics related to physical and biological properties. This guide may be used in the selection of suitable test methods of dried ionically gelled alginate foam scaffolds that may be a component of a medical device or considered a medical device itself.

1.2 This guide provides information relevant for the physical testing of alginate foam scaffolds such as mechanical properties, hydration properties, pore structure, and scaffold degradation. In addition, issues related to biological properties such as elemental impurities, bacterial bioburden, bacterial endotoxins, sterility, and biocompatibility are outlined.

1.3 This guide is intended to be used in conjunction with appropriate characterization and evaluation of any raw or starting materials utilized for fabrication of the alginate foam, such as described in Guides F2027 and F2064.

1.4 This guide addresses alginate foam scaffolds with and without bioactive agents or biological activity. This guide does not address the characterization or release profiles of any biomolecules, cells, drugs, or bioactive agents that are used in combination with the scaffold.

1.5 Only SI units are used in this standard.

1.6 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D638 Test Method for Tensile Properties of Plastics

D1621 Test Method for Compressive Properties of Rigid Cellular Plastics

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

F1635 Test Method for ***in vitro*** Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants

F2025 Practice for Gravimetric Measurement of Polymeric Components for Wear Assessment

F2027 Guide for Characterization and Testing of Raw or Starting Materials for Tissue-Engineered Medical Products

F2064 Guide for Characterization and Testing of Alginates as Starting Materials Intended for Use in Biomedical and Tissue Engineered Medical Product Applications

F2150 Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products

F2312 Terminology Relating to Tissue Engineered Medical Products

F2450 Guide for Assessing Microstructure of Polymeric Scaffolds for Use in Tissue-Engineered Medical Products

F2739 Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds

2.2 *ISO Standards:*[3]

ISO 527 Plastics—Determination of tensile properties—Part 1: General principles

ISO 10993 Biological evaluation of medical devices

ISO 11135 Sterilization of health-care products—Ethylene oxide—Requirements for the development, validation and routine control of a sterilization process for medical devices

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.42 on Biomaterials and Biomolecules for TEMPs.

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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 National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

2.3 *Other Referenced Document:*

AAMI ST72 Bacterial endotoxins—Test methods, routine monitoring, and alternatives to batch testing[4]

3. Terminology

3.1 *Definitions:*

3.1.1 *foam, n*—a solid structure that contains a large number of inherent or induced channels and open spaces (that is, pores; see Terminology F2312).

4. Summary of Guide

4.1 Alginate foam scaffolds are being used in the formulation of tissue-engineered medical products. Such scaffolds are often intended to be implanted in animals and humans. Factors such as foam integrity, strength, adhesion, biocompatibility, pore structure, and degradation will be critical for scaffold performance.

4.2 Several methods are available to produce alginate foam scaffolds, including porogen leaching, gas-foaming, freeze-drying, and phase-separation techniques. For example, pores in alginate foam may be introduced by the incorporation of air into a liquid alginate solution (wet foam) that is subsequently dried (dry foam), or pores in alginate foam may be introduced by the removal of ice crystals from a frozen solution of alginate through the use of freeze-drying techniques.

4.3 This guide summarizes physical, mechanical, and biological properties that are important for use of alginate foam scaffolds in TEMPs.

5. Significance and Use

5.1 This guide is a guideline for the characterizing and testing of alginate foam scaffolds used in tissue-engineered medical products. Alginate foam scaffolds can be used in a number of tissue engineering and regenerative medicine applications such as anti-adhesion, internal wound healing, and guided tissue regeneration. In addition, alginate foam can be used as a matrix for cell-based cell therapies and for the release of bioactive agents.

6. Physical Properties

6.1 Physical properties of TEMPs in the format of an ionically gelled alginate foam can be tuned and optimized to meet the requirements for its intended use. The major controllable characteristics are related to mechanical properties, hydration properties, pore structure, and degradation and will be presented in this section together with suggestions for test methods. The foam can be manufactured to be cut to size or in a size appropriate for surgical implantation. The dimensions and mechanical properties should allow for easy implantation and positioning and have an appropriate degradation profile. There may be additional relevant characterization procedures not covered by this guide that are specific to the designated end use of the foam.

6.2 *Mechanical Properties:*

6.2.1 Controlled mechanical properties of a foam scaffold are crucial for most applications. The foam must withstand forces exerted during insertion related to the selected method without rupture. When using a trochar, the foams typically must tolerate higher tensile stress and strain compared to foams implanted during open surgery.

6.2.2 *Tensile Properties*—Tensile properties of dry foams and rehydrated foams can be characterized as specified in Test Method D638 and ISO 527. The dumbbell-shaped test specimens are recommended to reduce the likelihood of rupture at the grips/clamps. Determination of tensile force at break (N), stress at break (Pa), strain at break (% elongation), and elasticity (Pa) will be relevant measures for these foams. If the intended use of the implanted foam is to provide mechanical support of a tissue or device, the foam must be hydrated before characterization in a model physiological solution. The mechanical properties of ionically gelled alginate structures are influenced by ions in the surrounding solution. To ensure relevant measures, it is important that the solution contains the biologically relevant concentration of non-gelling monovalent ions such as sodium ions (Na^+), gelling calcium ions (Ca^{2+}), and calcium-chelating phosphate ions (PO_4^{3-}) of approximately 154 mM, 1.3 mM, and 0.8 mM, respectively. Beware that simulated body fluids (SBF), cell culture media, phosphate buffered salt solutions (PBS), and water vary considerably in composition. During a defined time period the foam shall be added to a known amount of liquid versus volume or weight, or alternatively soaked in excess amounts. The selected strain rate may influence the results when characterizing these viscoelastic materials. Also monitor the temperature of the solution and environment.

6.2.3 *Compressive Properties*—The bulk elasticity of dry and rehydrated foams can be determined as specified in Test Method D1621. This may be relevant when aiming to match the compliance of the tissue at the implantation site. This elasticity will, however, not be comparable with, for example, local elasticities sensed by infiltrating cells.

6.2.4 *Suture Pull-Out Strength*—There are currently no recognized standard methods for determination of suture pull-out strength. Development of a method is recommended based on the specific application related to suture pattern, type, and depth, and expected forces exerted at the implantation site.

6.3 *Hydration Properties:*

6.3.1 Different aspects of the hydration properties of alginate foams can be considered and are presented below. The hydration properties depend on the swelling properties of the alginate in the pore wall and capillary bound liquid filling the pores of the foam. The swelling properties of alginate are dependent on the concentration of ions (as discussed in 6.2.2), in addition to pH, time, and temperature which should be monitored.

6.3.2 *Hydration Rate*—The interconnectivity of the pores of the foam is the most important characteristic that controls the hydration rate of the foam. Fast hydration rates are typically required for foams used when the aim is to absorb and retain body fluids such as blood during surgery. Slower hydration rates may, for example, be preferred for foams used as delivery vehicles for sustained release of bioactive agents. Different

[4] AAMI ST72 available at https://standards.aami.org/higherlogic/ws/public/download/12530/AAMI%20CDV1%20ST72%20public%20review%20draft.pdf.

visual methods can be considered for evaluation of this property. Examples include placing a foam with a defined area onto a liquid surface and determining the time needed for the foam to be fully hydrated, or by placing a known volume of liquid onto a foam and determining the time for it to be fully absorbed. A more quantitative method would be to measure the weight of a foam in excess liquid at different time points as described in 6.2.2.

6.3.3 *Absorption Capacity*—A measure of the amount of liquid that can be retained by the foam. Absorption capacity is often expressed as mass uptake of liquid per mass of dry material, area, volume, or combinations thereof. After the dry material is weighed, it shall be placed in a relevant model solution of greater volume than the dry material at a relevant temperature and kept therein for a defined time to allow for absorption. Active or passive drainage is recommended before weighing the wet foam. This can be achieved by lifting the foam using forceps and letting excess liquid drip off for a predefined time before weighing, or by placing the foam onto an absorbent material such as a filter paper to wick away capillary bound liquid. The latter technique is recommended for weaker foam structures.

6.3.4 *Dimensional Changes*—Dimensions of a dry foam may change upon hydration. Depending on the method of manufacture, it can expand or shrink in one, two, or all three directions (x, y, z, or combinations thereof). This property is of special importance when the intention of the foam is to fill a defect and to be used as a guide for regeneration of new tissue. Depending on the size of the scaffold, the dimensions can be determined via calipers or microscope.

6.4 *Pore Structure:*

6.4.1 The pore structure of the foam is herein related to the open space in the foam, and the scaffolds are considered macroporous. Macroporous scaffolds for tissue engineering were developed to mimic the extracellular matrix (ECM) and allow for cell infiltration and blood vessel in-growth. The increased surface area compared to non-macroporous structures may be varied by the pore size and porosity.

6.4.2 *Pore Size*—The pore size typically varies from 5 µm to 500 µm. The average pore size and the width of the pore size distribution will depend on the selection of manufacturing technique and conditions during preparation. Pore size, distribution, and other characteristics can be evaluated using the methods outlined in Guide F2450 for the appropriate pore size of the alginate foam TEMP or as outlined below.

6.4.2.1 Alginate foam pore size may not be determinable using standard light microscopy. Perfusion of the scaffold with a radio-opaque contrast agent will allow determination of pore size via micro-CT imaging. In addition, analysis of micropores inherent in the material can be performed using atomic force microscopy (AFM), scanning electron microscopy (SEM), or other imaging techniques. Determination of pore structure should be performed in a manner consistent with the implantation site, that is, if under pressure or tensile load, as this can distort the pore size of the material.

6.4.3 *Porosity*—The interconnectivity of the pores in the alginate foam can be evaluated using methods described in Guides F2150 and F2450.

6.5 *Degradation:*

6.5.1 Altering the chemical makeup of the alginate foam scaffold through oxidation, amidiation, sulfation, or esterification will affect degradation once implanted and should be considered when determining the kinetics of scaffold degradation. Many of these modifications facilitate degradation via hydrolysis. Guide F2150 and Test Method F1635 provide factors to consider when evaluating the *in vivo* degradation rate of alginate foams when modified with these groups. *In vivo* degradation of unmodified alginate foams occurs via release of divalent ions into surrounding tissues due to exchange reactions with monovalent cations that leads to alginate gel dissolution. The presence of cells during formation of alginate gels may also increase stability via receptor-ligand interactions.

6.5.2 *In vitro Degradation Assessment Methods*—The degradation properties of alginate foam scaffolds can be evaluated using methods identified in Test Method F1635 and Practice F2025. Quantification of released uronic acids by colorimetric analysis or by or gravimetric measurements over time may provide an estimate of the *in vivo* degradation rate.

7. Biological Properties

7.1 *Impurities:*

7.1.1 Guidance on the impurity profile relative to alginate as a starting material in the formulation of an alginate foam scaffold can be found in Guide F2064.

7.1.2 *Elemental Impurities*—Elemental impurities can come from several sources such as contaminants in starting materials, contaminants from processing equipment, and those added via chemical synthesis, typically from metal catalysts. Limits on elemental impurities are selected based on toxicity. Platinum group elements typically used as catalysts (Ru, Rh, Pd, Os, Ir, Pt) have been added to Cd, As, Hg, Cu, and Mo in addition to Cr, Ni, and V.

7.1.3 *Bacterial Bioburden*—The microbiological safety of an alginate foam scaffold will be affected by the presence of bacteria, yeast, and mold. Bacterial bioburden may be associated with the alginate starting material, other components, or in the process/production of the alginate foam. In principle, an alginate foam scaffold intended for implantation must be sterile; however, the presence of bacteria in any of the starting materials or introduced during processing and prior to terminal sterilization may also contribute to the presence of endotoxins (see 7.1.4).

7.1.4 *Bacterial Endotoxins*—Endotoxin contamination may arise from endotoxins present in the alginate starting material, other components used to formulate the foam, or from the process/production of the foam scaffold itself. A relevant method to detect bacterial endotoxins should be chosen and validated for detecting endotoxins in the alginate foam scaffold. AAMI ST72 provides guidance on test methods for endotoxins. In some cases, it will be necessary to dissolve the foam in order to ensure complete detection of bacterial endotoxins residing within the alginate foam scaffold. If dissolving the foam, it is preferred that the solvent used be the same as the solvent for production of the foam scaffolds. The solvent should be tested individually for endotoxin presence in