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Standard Guide for Characterization of Hydrogels used in Regenerative Medicine[1]

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1. Scope

1.1 Hydrogels are water-swollen polymeric networks that retain water within the spaces between the macromolecules; and maintain the structural integrity of a solid due to the presence of cross-links (**1-3**).[2] They are mainly used in regenerative medicine as matrix substitutes, delivery vehicles for drugs and/or biologics, and environments for cell culture. In these applications, hydrogel efficacy may depend on the ability to: support the permeation of dissolved gases, nutrients and bioactive materials; sustain cell growth and migration; degrade; release drugs and/or biologics at an appropriate rate; and maintain their shape.

1.2 Hydrogels used in regenerative medicine can be composed of naturally derived polymers (for example, alginate, chitosan, collagen (**4, 5**)), synthetically derived polymers (for example, polyethylene glycol (PEG), polyvinyl alcohol (PVA) (**4, 5**)) or a combination of both (for example, PVA with chitosan or gelatin (**6**)). In clinical use, they can be injected or implanted into the body with or without the addition of drugs and/or biologics (**7**).

1.3 This guide provides an overview of test methods suitable for characterizing hydrogels used in regenerative medicine. Specifically, this guide describes methods to assess hydrogel biological properties, kinetics of formation, degradation and agent release, physical and chemical stability and mass transport capabilities are discussed.

1.4 The test methods described use hydrated samples with one exception: determining the water content of hydrogels requires samples to be dried. It is generally recommended that hydrogels that have been dried for this purpose are not rehydrated for further testing. This recommendation is due to the high probability that, for most hydrogel systems, the drying-rehydration process can be detrimental with possible alterations in structure.

1.5 This guide does not consider evaluation of the microstructure of hydrogels (for example, matrix morphology, macromolecule network structure and chain conformation).

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

1.7 *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:*[3]

D4516 Practice for Standardizing Reverse Osmosis Performance Data

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

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

F2027 Guide for Characterization and Testing of Raw or Starting Biomaterials 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

F2103 Guide for Characterization and Testing of Chitosan Salts 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 Tissue-Engineered Medical Products

F2214 Test Method for *In Situ* Determination of Network Parameters of Crosslinked Ultra High Molecular Weight Polyethylene (UHMWPE)

F2315 Guide for Immobilization or Encapsulation of Living Cells or Tissue in Alginate Gels

[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] The boldface numbers in parentheses refer to a list of references at the end of this standard.

[3] 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.

F2347 Guide for Characterization and Testing of Hyaluronan as Starting Materials Intended for Use in Biomedical and Tissue Engineered Medical Product Applications
F2383 Guide for Assessment of Adventitious Agents in Tissue Engineered Medical Products (TEMPs)
F2450 Guide for Assessing Microstructure of Polymeric Scaffolds for Use in Tissue-Engineered Medical Products
F2739 Guide for Quantitating Cell Viability Within Biomaterial Scaffolds

2.2 *ISO Standards:*[4]
ISO 10993 Biological Evaluation of Medical Devices
ISO 22442 Medical Devices Utilizing Animal Tissues and Their Derivatives

2.3 *ANSI/AAMI Standards:*[4]
STBK9–1 Sterilization—Part 1: Sterilization in Health Care Facilities
STBK9–2 Sterilization—Part 2: Sterilization Equipment
STBK9–3 Sterilization—Part 3: Industrial Process Control
ST72 Bacterial Endotoxin—Test Methodologies, Routine Monitoring and Alternatives to Batch Testing

2.4 *Federal Regulations:*[5]
21 CFR 210 Current Good Manufacturing Practice in Manufacturing, Processing, Packaging or Holdings of Drugs, General
21 CFR 221 Current Good Manufacturing Practice for Finished Pharmaceuticals
21 CFR 610 General Biological Products Standards
21 CFR 820 Quality System Regulation

3. Terminology

3.1 *Definitions:*

3.1.1 *adventitious agents, n*—unintentionally introduced microbiological or other infectious contaminant. In the production of tissue engineered medical products (TEMPs), these agents may be unintentionally introduced during the manufacturing process or into the final product or both.

3.1.2 *biocompatibility, n*—the ability of a foreign material to fulfill its intended function with an appropriate host organism response.

3.1.3 *conductivity, n*—property of a substance's (in this case, water and dissolved ions) ability to transmit electricity.

3.1.3.1 *Discussion*—Conductivity is the inverse of resistivity.

3.1.3.2 *Discussion*—Conductivity is measured by a conductivity meter.

3.1.3.3 *Discussion*—The units of conductivity are Siemens per metre (Sm^{-1}).

3.1.4 *hydrogel, n*—a three-dimensional network of polymer chains that retains water within the spaces between the macromolecules.

3.1.5 *loss (viscous) modulus, n*—quantitative measure of energy dissipation, defined as the ratio of stress 90° out of phase with oscillating strains to the magnitude of strain.

3.1.6 *mechanical properties, n*—those properties of a material that are associated with elastic and inelastic reaction when forces are applied and released. These properties are often described in terms of constitutive relationship between stresses, strains, and strain rates.

3.1.7 *permittivity, complex, n*—a material property deduced from the ratio of the admittance, *Yp*, of a given electrode configuration separated by that material, to the admittance of the identical electrode configuration separated by a vacuum or air for most practical purposes, *Yv*.

3.1.8 *regenerative medicine, n*—a branch of medical science that applies the principles of regenerative biology to restore or recreate the structure and function of human cells, tissues, and organs that do not regenerate adequately.

3.1.9 *relaxation modulus, n*—the modulus of a material determined using a strain-controlled (relaxation) experiment at temperature *T* and time *t*, which may also be expressed using reduced time as $E(T_{ref}, \xi)$.

3.1.10 *storage (elastic) modulus, n*—quantitative measure of elastic properties defined as the ratio of the stress, in-phase with strain, to the magnitude of the strain.

3.1.11 *tan delta, n*—ratio of the viscous (loss) modulus to the elastic (storage) modulus in a sinusoidal deformation; mathematically, the tangent of the loss angle, δ.

3.1.12 *tomography, n*—any radiologic technique that provides an image of a selected plane in an object to the relative exclusion of structures that lie outside the plane of interest.

4. Significance and Use

4.1 This guide describes methods for determining the key attributes of hydrogels used in regenerative medicine (that is, their biological properties, kinetics of formation, degradation and agent release, physical and chemical stability and mass transport capabilities). See Table 1.

5. Key Factors for Hydrogel Characterization

5.1 In regenerative medicine, hydrogels can be used with the addition of drugs or biologics, or both (for example, as drug delivery devices or for cell encapsulation (**4**)) or without (for example, as tissue scaffolds or barriers (**4**)). Although characterization of hydrogels requires consideration of the individual

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

[5] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.