



Standard Guide for Characterization of Hydrogels used in Regenerative Medicine¹

This standard is issued under the fixed designation F2900; 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 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.

Current edition approved March 15, 2011. Published March 2011. DOI: 10.1520/F2900-11.

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.

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>.

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, Y_p , 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, Y_v .

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

TABLE 1 Key Factors and Attributes to be Considered in Hydrogel Characterization

NOTE 1—Example standards with relevant content are also indicated.

Attribute	Key Factors for Hydrogel Characterization			
	Biological Properties (Sections 5 and 6)	Kinetics (Section 7)	Physical and Chemical Stability (Section 8)	Mass Transport (Section 9)
	Biocompatibility (ISO 10993, F895)	Gelling time (F2315)	Environmental stability (D4516)	Cell migration (F2315)
	Adventitious agents (F2383 , ST72, ISO 22442, 21 CFR 210, 21 CFR 221, 21 CFR 610, 21 CFR 820)	Swelling rate (ISO 10993, F2214)	Mechanical properties (F2150)	Transport of nutrients and waste (F2450)
	...	Matrix degradation (F2150)	Cell encapsulation (F2315)	Release rate of bioactive agents (F2450)

composition and application the hydrogel will be used for, there are common generic requirements that can be summarised as follows:

They need to be biocompatible (**8**).

Their mechanical properties should enable them to be compatible with their intended clinical use.

They should be capable of swelling and potentially degrading at a rate that meets the needs of the intended clinical use (**9**, **10**).

They should be sufficiently permeable to promote and maintain cell viability, nutrient and waste product transport, or release therapeutic agents, or combination thereof.

Hydrogels, when combined with drugs or biologics, including any seeded or encapsulated cells, should not negatively alter the functional characteristics of the biologic or the drug through physical or biological interactions arising from the presence of hydrogels.

Ease of handling and delivery must also be considered since it is of importance for clinical use (**4**).

Variability in the composition of the starting polymer material used in hydrogels impacts the hydrogel final properties. It is therefore necessary to characterize the starting material, particularly for polymers derived from natural sources due to the inherent variability of their composition. Guidance on characterization of starting biomaterials for TEMP's can be found in Guides **F2027**, **F2064**, **F2103**, and **F2347**. Further, when hydrogels used in regenerative medicine are prepared under broad manufacturing conditions, the effect of variable co-agent concentrations and the effect of variable manufacturing conditions (for example, pH, temperature, ionic strength) on the final hydrogel properties should be considered and measured as appropriate.

5.2 The degree of hydrogel crosslinking is an important parameter affecting hydrogel physical properties and performance. Correlation between the stoichiometric and effective degree of crosslinking in the final hydrogel is a direct indication of the extent of reaction. In some cases determination of crosslinking is not trivial and it is therefore recommended that an estimation of percent post-gelation extractables should be performed. This will serve as a direct indicator for the crosslinking efficiency and will provide information on potentially harmful leachables.

5.3 Sterilization processes can affect the properties of the hydrogel or any active or inactive added components, or both (for example, drug, excipient, and so forth), all of which need to be taken into account when considering characterization

data. In order to appropriately assess hydrogel characterization data, it is recommended that a sterilization summary is provided along with the sample. Further guidance on sterilization strategies can be found in Guide **F2150**, STBK9–1, STBK9–2, and STBK9–3. It is noted, however, that a particular sterilization method may not be applicable to a certain type of hydrogel. In this case, provision of non-sterile hydrogels in a medium that contains antimicrobial agents or producing hydrogels under aseptic conditions may need to be considered with additional controls such as bioburden reduction and sterility testing.

5.4 It may be possible to assess the hydrogel response to *in vivo* conditions through the use of suitable *ex vivo* models. Factors that such models should take into consideration include: tissue-specific mechanical loading; tissue-specific metabolic activity; tissue-specific pH; relevant chemistry to the site/condition of implantation; temperature; oxygen content; and tissue-specific cell types present. For example, the testing of ionically crosslinked hydrogels which are susceptible to ion exchange during implantation (for example, Ca^{2+} crosslinked alginates) should be done in media resembling the physiological environment at least in terms of electrolytic content and osmolarity and the relevant chemistry such as divalent cation delivery. For temperature-sensitive hydrogels, the hydrogel gelling temperature is key to assess *in vivo* performance, and tests should be carried out at 37°C. For gels implanted in interstitial fluid, fat tissues, and so forth, in addition to maintaining a physiologically relevant electrolytic balance, testing may need to be done in serum, or at least in the presence of proteins and lipids, and so forth.

5.5 Consideration should be given to hydrogel stability during storage and transportation. It may be necessary to maintain hydrogels in a controlled environment with factors such as temperature and pH regulated.

5.6 To adequately assess the suitability of a candidate hydrogel for use in regenerative medicine it is necessary to consider the key factors identified in Table 1. Each factor can be assessed through measurement of different hydrogel properties that will be discussed below. In all cases measured quantities should be reported in the relevant SI units.

6. Biological Properties

6.1 *Biocompatibility*—The biocompatibility of the hydrogel product shall be established. Currently **Documents**