NOTICE: This standard has either been superseded and replaced by a new version or withdrawn.
Contact ASTM International (www.astm.org) for the latest information

Designation: F 2212 – 02

Standard Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)[1]

This standard is issued under the fixed designation F 2212; 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.

INTRODUCTION

Collagen-based medical devices are becoming more prevalent especially in the area of soft tissue augmentation. The use of collagen in surgery dates back to the late 1800s, with the use of catgut sutures, human cadaveric skin, and fascia. More recently, collagen has been used in hemostatic sponges, dermal equivalents, injectables for soft tissue augmentation, as a matrix for cell-based products and as a vehicle for drug delivery. It is because of the versatility of collagen in medical applications that specific characterizations should be performed as a way to compare materials.

1. Scope

1.1 This guide for characterizing collagen-containing biomaterials is intended to provide characteristics, properties, and test methods for use by producers, manufacturers, and researchers to more clearly identify the specific collagen materials used. With greater than 20 types of collagen and the different properties of each, a single document would be cumbersome. This guide will focus on the characterization of Type I collagen, which is the most abundant collagen in mammals, especially in skin and bone. Collagen isolated from these sources may contain other types of collagen, for example, Type III and Type V. This guide does not provide specific parameters for any collagen product or mix of products or the acceptability of those products for the intended use. The collagen may be from any source, including, but not limited to animal or cadaveric sources, human cell culture, or recombinant sources. The biological, immunological, or toxicological properties of the collagen may vary depending on the source material. The properties of the collagen prepared from each of the above sources must be thoroughly investigated, as the changes in the collagen properties as a function of source materials is not thoroughly understood. This guide is intended to focus on purified Type I collagen as a starting material for surgical implants and substrates for Tissue Engineered Medical Products (TEMPs); some methods may not be applicable for gelatin nor for tissue implants. This guide may serve as a template for characterization of other types of collagen.

1.2 The biological response to collagen in soft tissue has been well documented by a history of clinical use **(1,2)**[2] and laboratory studies **(3,4,5,21)**. Biocompatibility and appropriateness of use for a specific application(s) is the responsibility of the device manufacturer.

1.3 The following precautionary caveat pertains only to the test method portion, Section 5, of this guide. *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 requirements prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products[3]
F 619 Practice for Extraction of Medical Plastics[4]
F 720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test[4]
F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices[4]
F 749 Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit[4]
F 756 Practice for Assessment of Hemolytic Properties of Materials[4]
F 763 Practice for Short-Term Screening of Implant Materials[4]

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

Current edition approved Nov. 10, 2002. Published January 2003.

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

[3] *Annual Book of ASTM Standards*, Vol 11.05.

[4] *Annual Book of ASTM Standards*, Vol 13.01.

Copyright © ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States.

F 813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices[4]

F 895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity[4]

F 981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone[4]

F 1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices[4]

F 1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials[4]

F 1903 Practice for Testing for Biological Responses to Particles *In Vitro*[4]

F 1904 Practice for Testing the Biological Responses to Particles *In Vivo*[4]

F 1905 Practice for Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity[4]

F 1906 Practice for Evaluation of Immune Responses in Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation and Cell Migration[4]

F 1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Application[4]

F 2148 Practice for Evaluation of Delayed Contact Hypersensitivity Using the Murine Local Lymph Node Assay (LLNA)[4]

2.2 *ISO Standards:*[5]

ISO 10993-1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing

ISO 10993-3—Part 3 Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

ISO 10993-9—Part 9 Framework for Identification and Quantification of Potential Degradation Products

ISO 10993-10 Biological Evaluation of Medical Devices—Part 10: Tests for Irritation and Delayed-Type Hypersensitivity

ISO 10993-17—Part 17 Methods for Establishment of Allowable Limits for Leachable Substances Using Health-Based Risk Assessment

ISO 13408-1: 1998 Aseptic Processing of Health Care Products—Part 1: General Requirements

2.3 *EN (European Norm) Documents:*[6]

EN 12442-1 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk

EN 12442-2—Part 2 Controls on Sourcing, Collection and Handling

EN 12442-3—Part 3 Validation of the Elimination and/or Inactivation of Virus and Transmissible Agents

2.4 *U. S. Pharmacopeia Documents:*[7]

United States Pharmacopeia (USP), Edition XXIV (24)

USP 24/NF 19 Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

2.5 *Code of Federal Regulations:*[8]

Code of Federal Regulations, Title 21, Part 820

Federal Register Vol. 43, No. 141, Friday, July 21, 1978

Human Cells, Tissues and Cellular and Tissue-Based Products, Establishment Registration and Listing. 21 CFR Parts 207, 807, and 1271

Federal Register/Vol. 66, No. 13, Jan 19, 2001/Rules and Regulations, page 5447

Suitability Determination for Donors of Human Cell and Tissue-based Products, 21 CFR 1271 Part C, Proposed Rule

Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products, Inspection and Enforcement. Proposed Rule. Federal Register/Vol. 66, No. 5/January 8, 2001/Proposed Rules, pages 1552-1559

Guidance for Screening and Testing of Donors of Human Tissue Intended for Transplantation, Availability. Federal Register/Vol. 62, No. 145/July 29, 1997/NoticesDraft Guidance for Preclinical and Clinical Investigations of Urethral Bulking Agents used in the Treatment of Urinary Incontinence. November 29, 1995. (ODE/DRARD/ULDB), Document No. 850

Guidance for Industry and for FDA Reviewers, Medical Devices Containing Materials Derived from Animal Sources (Except for *In Vitro* Diagnostic Devices), November 6, 1998, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health

CFR 610.13(b), Rabbit Pyrogen Assay

2.6 *ICH Documents:*[9]

International Conference on Harmonization (1997) Guidance for Industry M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals 62 FR 62922

International Conference on Harmonization (1996) Guideline for Industry S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. 61 FR 18199

International Conference on Harmonization (1997) Guidance for Industry S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals 62 FR 62472

International Conference on Harmonization (1994) Guideline for Industry S5A Detection of Toxicity to Reproduction for Medicinal Products. 59 FR 48746

International Conference on Harmonization (1996) Guidance for Industry S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility. 61 FR 15360

International Conference on Harmonization (1996) Guideline for Industry S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals. 61 FR 8153

International Conference on Harmonization (1998) Guidance for Industry S1B Testing for Carcinogenicity of

iTeh Standards
(https://standards.iteh.ai)
Document Preview
ASTM F2212-02

[5] Available from International Organization for Standardization (ISO), 1 rue de Varembé, Case postale 56, CH-1211, Geneva 20, Switzerland.

[6] Available from European Committee for Standardization, CEN Management Centre 36, rue de Stassart B-1050 Brussels, Belgium.

[7] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852.

[8] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.

[9] ICH Secretariat, c/o IFPMA, 30 rue de St-Jean, P.O. Box 758, 1211 Geneva 13, Switzerland.

Pharmaceuticals. 63 FR 8983

International Conference on Harmonization (1995) Guideline for Industry S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals. 60 FR 11278

International Conference on Harmonization (1997) S1C(R) Guidance for Industry Addendum to Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limit Dose and Related Notes. 62 FR 64259

International Conference on Harmonization (ICH) Q1A ICH Harmonized Tripartite Guidance for Stability Testing of New Drug Substances and Products (September 23, 1994)

U.S. Food and Drug Administration (FDA and Committee for Proprietary Medicinal Products (CPMP), 1998 International Conference on Harmonization (ICH), Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, Consensus Guideline ICH Viral Safety Document: Step 5

2.7 *FDA Documents:*[10]

FDA Guideline on Validation of the Limulus Amebocyte Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Healthcare Products, DHHS, December 1987

U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1993 Points to Consider in the Characterization of Cell Lines Used to produce Biologicals

U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1997 Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, 94D-0259

FDA Interim Guidance for Human and Veterinary Drug Products and Biologicals, Kinetic LAL techniques, DHHS, July 15, 1991

2.8 *AAMI Documents:*[11]

ANSI/AAMI/ISO 11737-1: 1995 Sterilization of Medical Devices—Microbiological Methods—Part 1: Estimation of Bioburden on Product

ANSI/AAMI/ISO 11737-2: 1998 Sterilization of Medical Devices—Microbiological Methods—Part 2: Tests of Sterility Performed in the Validation of a Sterilization Process

AAMI TIR No. 19-1998 Guidance for ANSI/AAMI/ISO 10993-7: 1995, Biological Evaluation of Medical Devices—Part 7: Ethylene Oxide Sterilization Residuals

AAMI/ISO 14160-1998 Sterilization of Single-Use Medical Devices Incorporating Materials of Animal Origin—Validation and Routine Control of Sterilization by Liquid Chemical Sterilants

AAMI ST67/CDV-2: 1999 Sterilization of Medical Devices—Requirements for Products Labeled "Sterile"

2.9 *Other References:*

Draft Guidance for Preclinical and Clinical Investigations of Urethral Bulking Agents Used in the Treatment of Urinary Incontinence, November 29, 1995. (ODE/DRARD/ULDB), Document No. 850.[12]

3. Terminology

3.1 *Definitions:*

3.1.1 *adventitious agents*—an unintentionally introduced microbiological or other infectious contaminant. In the production of TEMPs, these agents may be unintentionally introduced into the process stream or the final product, or both.

3.1.2 *biocompatibility*—a material may be considered biocompatible if the materials perform with an appropriate host response in a specific application **(22)**.

3.1.3 *collagen*—Type I collagen is a member of a family of structural proteins found in animals. Type I collagen is part of the fibrillar group of collagens. It derives from the COL1A1 and COL1A2 genes, which express the alpha chains of the collagen. All collagens have a unique triple helical structure configuration of three polypeptide units known as alpha-chains. Proper alignment of the alpha chains of the collagen molecule requires a highly complex enzymatic and chemical interaction *in vivo*. As such, preparation of the collagen by alternate methods may result in improperly aligned alpha chains and, putatively, increase the immunogenicity of the collagen. Collagen is high in glycine, L-alanine, L-proline, and 4-hydroxyproline, low in sulfur, and contains no L-tryptophan. Natural, fibrillar Type I collagen is normally soluble in dilute acids and alkalis. When heated (for example, above approximately 40°C), collagen is denatured to single alpha chains (gelatin). At each end of the chains are short non-helical domains called telopeptides, which are removed in some collagen preparations. Through non-covalent interactions with sites on adjacent helixes, fibrillogenesis is achieved. Subsequently, non-reducible cross-links are formed. Type I collagen can be associated with Type III and Type V collagen and also with the other non-collagenous proteins like elastin and other structural molecules like glycosaminoglycans and complex lipoproteins and glycoproteins.

3.1.4 *degradation*—change in chemical, physical, or molecular structure or appearance (that is, gross morphology) of material.

3.1.5 *endotoxin*—a high molecular weight lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria that is pyrogenic in humans. Though endotoxins are pyrogens, not all pyrogens are endotoxins.

3.1.6 *microorganism*—bacteria, fungi, yeast, mold, viruses, and other infectious agents. However, it should be noted that not all microorganisms are infectious or pathogenic.

3.1.7 *solubility*—a measure of the extent to which the material can be dissolved. In the context of collagen, refers to the dissociation of the fibrillar aggregates of collagen molecules into a solution. Native Type I collagen which is soluble in dilute acids, but not soluble in neutral pH conditions is termed "insoluble" or "acid soluble," while simple aggregates of non-fibrillar collagen soluble in neutral salt solutions are

iTeh Standards (https://standards.iteh.ai) Document Preview ASTM F2212-02 https://standards.iteh.ai/catalog/standards/sist/4695208

[10] Available from the Food and Drug Administration, 5600 Fishers Ln., Rockville, MD 20857.

[11] Association for the Advancement of Medical Instrumentation, 1110 N. Glebe Rd., Suite 220, Arlington, VA 22201–4795.

[12] Available from the FDA, 5600 Fishers Ln., Rockville, MD 20857. http://www.fda.gov/cdrh/ode/oderp850.html.

termed "neutral salt soluble." Post translational surface charge modifications may alter the solubility of collagen in neutral pH condition.

3.1.8 *sterilization*—the destruction or removal of all microorganisms in or about an object, as by, chemical agents, electron beam, gamma irradiation, ultraviolet (UV) exposure, or filtration.

3.1.9 *suspension*—the dispersion of a solid through a liquid with a particle size large enough to be detected by purely optical means.

4. Significance and Use

4.1 The objective of this guide is to provide guidance in the characterization of Type I collagen as a starting material for surgical implants and substrates for TEMPs. This guide contains a listing of physical and chemical parameters that are directly related to the function of collagen. This guide can be used as an aid in the selection and characterization of the appropriate collagen starting material for the specific use. Not all tests or parameters are applicable to all uses of collagen.

4.2 The collagen covered by this guide may be used in a broad range of forms or applications, for example (but not limited to) medical devices, tissue engineered medical products (TEMPs) or cell, drug, or DNA delivery devices for implantation. The use of collagen in a practical application should be based, among other factors, on biocompatibility and physical test data. Recommendations in this guide should not be interpreted as a guarantee of clinical success in any tissue engineered medical product or drug delivery application.

4.3 The following general areas should be considered when determining if the collagen supplied satisfies requirements for use in TEMPs. These are source of collagen, chemical and physical characterization and testing, and impurities profile.

5. Chemical and Physical Characterizations

5.1 These methods are suggested assays; however, other validated assay methods may be used. Selection of assay systems will vary depending on the configuration of the collagen (that is, soluble or insoluble). The user should ensure that the method selected is reliable and commonly accepted in protein chemistry. A review of collagen materials may be found in Li, 2000 **(7)**, while a review of the collagen family of proteins may be found in **(9-13, 20)**. When selecting an appropriate test method, the user should note that impurities in highly purified collagen fibers can be as low as 1 to 2 %, while test method uncertainties can be as high as 10 to 15 % between samples. For soluble collagen, the following represents a non-inclusive list of assay systems available: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE); peptide mapping; and physico-chemical analysis. A similar list for insoluble collagen may include, but not be limited to, assay methods for hexosamine (that is, detection of glycoproteins), lipid, total sugar, desmosine (that is, elastin), and amino acid composition (that is, collagen composition profile; non-collagenous amino acids). Additionally, methods such as transmission electron microscopy may be helpful in characterizing the collagen.

5.2 The concentration of collagen should be expressed in mass/volume or mass/mass. Colorometric assays or amino acid analysis for hydroxyproline are commonly used methods to measure collagen content.

5.3 Amino acid analysis will provide information on the composition of the amino acids of collagen (that is, the amino acids must be within the range of published data for highly purified collagen preparation, generally in the form of acid soluble). The collagen must not contain amino acids that are not in collagen, such as tryptophan. Amino acid analysis is routinely performed on hydrolyzed collagens by reverse phase High Performance Liquid Chromatography (HPLC). Amino acid analysis should contain no cysteine (sulfur containing amino acid), low in tyrosine, and no tryptophan. This method can also be used for the quantification of hydroxyproline. There are other methods available for amino acid analysis.

5.4 Purity of soluble collagen can be analyzed by SDS-PAGE either on the collagen directly or after digestion of the collagen with purified bacterial collagenase to detect any remaining proteins by SDS-PAGE.

5.5 *Elastin Assay*—Elastin can be a component of the impurities in an insoluble collagen preparation. One method to assay for elastin, although other methods are available, involves the detection of desmosine **(6)**. These impurities can be detected by Western blots, ELISAs, and other types of assays.

5.6 Peptide mapping is one possible method to identify Type I collagen. The most commonly used peptide mapping method utilizes Cyanogen Bromide (CNBr) digest. The digest can be analyzed by SDS-PAGE or HPLC.

iTeh Standards (https://standards.iteh.ai) Document Preview ASTM F2212-02 https://standards.iteh.ai/catalog/standards/sist/46952080-...-astm-f2212-02

5.7 *Impurities Profile*—The term impurity relates to the presence of extraneous substances and materials in the collagen introduced during the purification process. These impurities can be detected by Western blots, ELISAs, and other types of assays. The user is also directed to Guide E 1298 for additional information. If there is a concern for the presence of processing aids or other impurities associated with the collagen, they should be addressed with the supplier. The major impurities of concern include, but are not limited to the following: endotoxins, glycosaminoglycans, elastin, lipids, improperly aligned collagen molecules, host cell contaminants, cell culture contaminants, heavy metals, bioburden, viruses, transmissible spongiform encephalopathy (TSE) agents, cross-linking, and enzymatic agents. While not considered an impurity, Type III collagen may also be associated with Type I collagen. At minimum, any protein impurity of greater than 1 % in the final collagen preparation should be identified and quantified.

5.8 *Endotoxin Content*—Endotoxin contamination is difficult to prevent because it is ubiquitous in nature, stable and small enough to pass through sterilizing filters (0.22 µm). Endotoxin tests for collagen include the gel clot, endpoint assay and the kinetic assay. The gel clot test is the simplest and easiest of the Limulus amebocyte lysate (LAL) test methods, although much less sensitive than the kinetic assay. The quantitative kinetic assay, which measures the amount of time required to reach a predetermined optical density, is the most sensitive. (Food and Drug Administration, Guideline on Validation of the Limulus Amebocyte Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Healthcare Products.) Each new lot of reagents should be validated. The endotoxin level in collagen

will ultimately be critical to its use in biomedical applications where there are regulatory limits to the amount of endotoxin that can be implanted into humans. Relevant FDA guidance for allowable levels and information regarding validation of endotoxin assays should be consulted if human trials are contemplated (Interim Guidance for Human and Veterinary Drug Products and Biologicals). The user is also directed to CFR 610.13(b) for information pertaining to the rabbit pyrogen assay.

5.9 *Heavy Metal Content by the USP Method*—This test is provided to demonstrate that the content of heavy metal impurities does not exceed a limit in the individual product specification. This method is based on <231> Heavy Metals, 1st and 6th Supplement USP-NF. Substances that typically respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. Under the specified test conditions, the limit is determined by a concomitant visual comparison of metals that are colored by sulfide ion with a control prepared from a Standard Lead Solution. Additional heavy metal contaminants may be present due to processing. If necessary, the user may detect these contaminants by various methods, not limited to, spectrographic, chromatographic, and flame atomic absorption.

5.10 *Microbiological Safety*—The presence of bacteria, yeast, and mold are also impurities that can arise in an a biological sample. User will validate sterilization and characterize effect on product. The presence of bacteria may also contribute to the presence of endotoxins. The following Microbiological Tests in USP 24 are of particular relevance: Microbial Limit Tests <61>, Sterility Tests <71>, Sterilization and sterility assurance of compendial articles <12211>, and the Biological Tests and Assays: Bacterial Endotoxins Tests <85>. The user should also consider other relevant standards, such as, but not limited to, Association for the Advancement of Medical Instrumentation (AAMI) standards and international standards, of which the following are examples: ANSI/AAMI/ISO 11737-1: 1995; ANSI/AAMI/ISO 11737-2: 1998; and ISO 13408-1: 1998. The collagen is first dissolved in a sterile, aqueous solution, then filtered using sterile techniques through a 0.45 μm membrane filter. The filters are subsequently incubated on Tryptic Soya Agar to determine the presence of bacteria, and on Sabouraud Dextrose Agar to determine the presence of yeast and mold. If collagen products are intended to serve as a barrier to microorganisms, this function will need to be validated with specific experiments.

5.11 Carbohydrate analysis of collagens can be carried out by classical gas-liquid chromatographic methods or spectrophotometric methods. If novel source, then glycosylation of proteins may need to be considered.

5.12 Trypsin Susceptibility will detect that portion of collagen that has been denatured from the purification steps such as acid and base treatment, solvent treatment, and so forth. Trypsin will digest that portion of the collagen and can be measured by assaying the hydroxyproline content of the supernatant. Triple helical collagen is resistant to digestion by most proteases. Susceptibility to trypsin or other appropriate proteases is determined by exposing the collagen to the enzyme and assaying the digest for degradation. There are several methods for this test.

5.13 Differential Scanning Calorimetry (DSC) determines dissociation temperature of collagens in fibrils, as well as detecting microfibrils and denatured collagen at lower melting temperatures.

5.14 Viscosity is more applicable to gels or suspensions but may be useful with collagen configured in forms such as, but not limited to, pastes or films **(8)**. Viscosity of collagen-based materials depends on a number of factors, but not limited to the following: solution or dispersion/suspension, concentration, temperature, operating condition, and so forth. It is not feasible to determine the viscosity of films. This is a routine test performed with a viscometer (not a u-tube). The user must clearly state the conditions of the test.

5.15 Transmission electron microscopy may be used to show the quality of collagen fibers. Unraveling or changes in banding will be obvious.

5.16 DNA sequence data on recombinant or transgenic source cells: Verify sequence data for expression gene, that is, COL1A1 or Col1A2.

5.17 The collagen material shall have specifications for an extensive set of chemical and physical properties such as, but not limited to, those listed in Table 1. The table represents methods which may or may not be appropriate for characterizing a particular collagen sample. Not all the methods listed

iTeh Standards
(https://standards.iteh.ai)
Document Preview
ASTM F2212-02

TABLE 1 Characterization Methods for Type I Collagen

Characterization Method	Applicable to
Chemical	
Appearance	Soluble or Insoluble
Concentration	Soluble or Insoluble
Purity	Soluble or Insoluble
Amino acid analysis	Soluble or Insoluble
Peptide mapping	Soluble or Insoluble
Impurities profile, includes Heavy Metal Analysis	Soluble or Insoluble
Carbohydrate analysis	Soluble or Insoluble
Trypsin resistance	Soluble or Insoluble
pH of implantable	Soluble or Insoluble
Additives (cross-linkers, lubricants, drugs, sterilents)	Soluble or Insoluble
Physical	
Shrink Temperature (DSC)	Insoluble
Viscosity	Mainly soluble
TEM	Insoluble
SDS-PAGE	Soluble or Insoluble
Moisture Content (5 to 20 %), dependent on storage environment	Insoluble
Electron Micrograph (native banded 640 Å structure for fibrils)	Insoluble
Biochemical	
Endotoxin level	Soluble or Insoluble
Bioburden	Soluble or Insoluble
% Type I collagen/Total Protein	Soluble or Insoluble
% Other Types Collagen and List of which Types present	Soluble or Insoluble
Total DNA (ppm or %)	Soluble or Insoluble
Total Lipid	Soluble or Insoluble
% native collagen (by trypsin resistance, circular Dichroism)	Soluble or Insoluble

Abbreviation in Table:
DSC = Differential Scanning Calorimetry
TEM = Transmission Electron Microscopy
SDS-PAGE = Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

may be required to characterize the sample and the specificity and sensitivity vary among the methods listed. The user should be familiar with the limitations of the appropriate test methods.

6. Product Development Considerations

6.1 *Storage Conditions/Shelf Life Stability of Collagen*—For collagen, the most relevant stability-indicating parameters are those related to the functionality of the polymer. Dependent upon what function the collagen will have in the final formulation, parameters such as viscosity (apparent and intrinsic) and biological activity may also be considered. Storage conditions are of importance, especially for collagen solutions. International Conference on Harmonization (ICH) guidance documents should be consulted for information on stability testing of pharmaceuticals (that is, ICH Q1A ICH Harmonized Tripartite Guideline for Stability Testing of New Drug Substances and Products).

6.2 *Sterilization Method, if Applicable, and Effects of Sterilization on Product*—The user should verify that the sterilization method does not adversely effect the collagen end product. Collagen can be sterilized by gamma irradiation (with subsequent degradation of the collagen) or by ethylene oxide with potential residuals, or prepared using aseptic processing steps. Solutions of collagen may be (*1*) filter sterilized if the viscosity of the collagen solution permits; (*2*) gamma-irradiated with a resulting loss in viscosity (molecular weight). Selection of the method of sterilization will depend upon the viscosity or molecular weight needs of the final application. Use of ethylene oxide will also require testing for residuals for ethylene oxide. The reader should refer to the relevant standards regarding the sterilization of healthcare products by radiation, steam and ethylene oxide gas, such as AAMI TIR No. 19-1998; AAMI/ISO 14160-1998; and AAMI ST67/CDV-2: 1999.

6.3 *Sourcing*—The criteria to consider for safe sourcing include appropriate human or animal donor selection and the tissue collection procedures to assure that the source material is unlikely to contain TSE infectivity. Additional information can be obtained from the following documents: EN 12442-1, EN 12442-2, EN 12442-3; 21 CFR Parts 207, 807, 820, and 1271 Part C; Federal Register Vol. 43; Federal Register Vol. 62; Federal Register Vol. 66, No. 5, January 8, 2001, Pages 1552-59; Federal Register, Vol. 66, No. 13, January 19, 2001, Page 5447; ISO 13408, 1998. Additional documents may be available. The user should verify the most current version of the document. The collagen can be isolated from tissues or cell cultures by any method, including, but not limited to extraction by dilute acids or dilute salt solutions or by enzymatic digestion of the tissue **(14-17)**. The user should be aware that even though Type III collagen is less abundant, it is often associated with Type I, except in bones and tendons. Type V collagen is also associated with Type I.

6.4 *Viral and Transmissible Spongiform Encephalopathy (TSE) Agent Inactivation*—Viruses and TSE agents can be introduced into a product as a result of raw materials sourcing or through adventitious means. Appropriate measures should be taken so that the resultant product is free from viruses and TSE agents. For further guidance on viral or TSE clearance, or both, the user is directed to the references throughout this guide as well as USP 24/NF 19 <1050>, and other pertinent references, as appropriate. Additional information may be found in the following FDA Guidance Document, FDA points to consider and International Conference on Harmonization (ICH) documents: Guidance for Industry and for FDA Reviewers: Medical Devices Containing Materials Derived from Animal Sources (Except for *In Vitro* Diagnostic Devices); U.S. Food and Drug Administration (FDA and Committee for Proprietary Medicinal Products (CPMP), 1998; "International Conference on Harmonization (ICH), Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin." Consensus Guideline ICH Viral Safety Document: Step 5; U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1993; "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals; U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER), 1997; "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use." 94D-0259.

6.4.1 *Viral Clearance*—To reduce or eliminate potential infectivity, the user should exclude sources of raw materials from humans or animals likely to be infected with known viral pathogens. Viral clearance methods can include, but not be limited to methods such as, high or low pH, urea treatment, chemical treatments, and filtration. However, even these harsh treatments may not ensure complete viral inactivation. Viral clearance should be demonstrated by an appropriately validated viral clearance study protocol. The user should verify that the viral clearance procedure is compatible with the starting material or the configured end product. For human tissue sources for manufacturing into collagen, the observance of Good Tissue Practices should be considered.

6.4.2 *TSE Clearance*—Due diligence should be made to the sourcing of raw materials, process design to remove potential TSE agents and treatments to inactivate TSE agents for those products which can withstand the harsh treatments required to inactivate TSE agents. The user is referred to the "Meeting Report, International Workshop on Clearance of TSE Agents from Blood Products and Implanted Tissues," **(23)** and the FDA Guidance Document "Guidance for Industry and for FDA Reviewers: Medical Devices Containing Materials Derived from Animal Sources (Except for *In Vitro* Diagnostic Devices)" for additional guidance on recommended practices for sourcing and for TSE clearance. Technology is under development for quantitation of TSE agents in biological materials. The following references are cited as examples of two of the many methods for detecting TSE agents **(18,19)**. The user should be aware that although detection of the protease resistant form of the ubiquitous prion protein in a tissue generally indicates that it contains the transmissible agent and is not suitable for preparing collagen for human or animal implantation, the converse is not necessarily true. Therefore, a negative test for the protease resistant prion alone may not be sufficient to assure that the source material is safe for producing collagen.

6.4.3 *Source Documentation*—Guidance for viral inactivation validation and CBER guidances.

iTeh Standards (https://standards.iteh.ai) Document Preview ASTM F2212-02