Designation: F3089 – 14

Standard Guide for Characterization and Standardization of Polymerizable Collagen-Based Products and Associated Collagen-Cell Interactions[1]

This standard is issued under the fixed designation F3089; 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

The collagen family of proteins represents the major structural and mechanical component of the *in-vivo* extracellular matrix of human tissues and organs. Type I collagen is the most abundant and as such, it is an ideal candidate for medical materials, tissue-engineered medical products, delivery of therapeutic cells/molecules, and *in-vitro* cell/tissue culture applications. Furthermore, it is now evident that specific collagen material properties, including microstructure, mechanical integrity (stiffness), cell adhesion, and biodegradation are major determinants of the interfacial properties between cells and collagen-based materials, including guidance of fundamental cell behaviors that contribute to recapitulation and/or restoration of tissue structure and function. Advanced understanding of collagen self-assembly, as occurs *in vivo* and *in vitro*, is contributing to a rapid expansion of commercial and laboratory-produced collagen formulations that polymerize (self-assemble) or exhibit solution to gel (matrix) transition. Most recent developments have focused on collagen polymer formulations with tunable features to support the rational design of collagen materials for improved tissue integration and guidance of cell fate. Unfortunately, the term "collagen" is applied generally to describe various collagen types and formulations (soluble, insoluble, monomeric, atelocollagen) that vary significantly in their molecular compositions, self-assembly capacity and properties, and ability to interact with cells. As such, the need exists for an expanded set of characterization and standardization strategies to facilitate comparison, safety and efficiency testing, and translation of the next generation collagen polymer formulations and associated self-assembled collagen-based materials produced with these formulations.

1. Scope

1.1 This guide for characterizing polymerizable collagens is intended to provide characteristics, properties, test methods, and standardization approaches for use by producers, manufacturers, and researchers to identify specific collagen polymer formulations and associated self-assembled collagen-based products produced with these formulations. This guide will focus on the characterization of polymer forms of Type I collagen, which is the most abundant collagen in mammalian connective tissues and organs, including skin, bone, tendon, and blood vessels. Type I collagen may be derived from a variety of sources including, but not limited to, animal or cadaveric tissues, cell culture, recombinant, and chemical synthesis. This guide is intended to focus on purified Type I collagen polymers as a starting material for wound and hemostatic dressings, surgical implants, substrates for tissue-engineered medical products (TEMPs), delivery vehicles for therapeutic cells or molecules, and 3D *in-vitro* tissue systems for basic research, drug development, and toxicity testing. Polymerizable or self-assembly implies that the collagen composition exhibits spontaneous macromolecular assembly from its components in the absence of the addition of exogenous factors including cross-linking agents. Self-assembling collagen polymers may include, but are not limited to: *(1)* tissue-derived atelocollagens, monomers, and oligomers; *(2)* collagen proteins and peptides produced using recombinant technology; and *(3)* chemically synthesized collagen mimetic peptides. It should be noted that the format of associated self-assembled collagen-based products also will vary and may include injectable solutions that polymerize in situ as well as preformed sheets, particles, spheres, fibers, sponges, matrices/gels, coatings, films, and other forms. This guide may serve as a

[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 May 1, 2014. Published June 2014. DOI: 10.1520/F3089-14.

template for characterization and standardization of other fibrillar collagen types that demonstrate polymerization or self-assembly.

1.2 The ability of self-assembled collagen materials to guide cellular responses through provision of cellular adhesion and proteolytic domains as well as physical constraints (for example, structural, cell-matrix traction force) has been well documented through extensive clinical (**1, 2**)[2] and basic research studies (**3, 4**). The biocompatibility and appropriateness of use for a specific application(s) is the responsibility of the product manufacturer.

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

1.4 **Warning—***Mercury has been designated by the Environmental Protection Agency (EPA) and many state agencies as a hazardous material that can cause central nervous system, kidney, and liver damage. Mercury, or its vapor, may be hazardous to health and corrosive to materials. Caution should be taken when handling mercury and mercury-containing products. See the applicable product Material Safety Data Sheet (MSDS) for details and the EPA website (http://www.epa.gov/mercury/faq.htm) for additional information. Users should be aware that selling mercury or mercury-containing products, or both, in your state may be prohibited by state law.*

1.5 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 limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products

F619 Practice for Extraction of Medical Plastics

F720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test

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

F749 Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit

F756 Practice for Assessment of Hemolytic Properties of Materials

F763 Practice for Short-Term Screening of Implant Materials

F813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone

F1251 Terminology Relating to Polymeric Biomaterials in Medical and Surgical Devices (Withdrawn 2012)[4]

F1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials

F1903 Practice for Testing For Biological Responses to Particles ***In Vitro***

F1904 Practice for Testing the Biological Responses to Particles ***in vivo***

F1905 Practice For Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity (Withdrawn 2011)[4]

F1906 Practice for Evaluation of Immune Responses In Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration (Withdrawn 2011)[4]

F1983 Practice for Assessment of Compatibility of Absorbable/Resorbable Biomaterials for Implant Applications

F2148 Practice for Evaluation of Delayed Contact Hypersensitivity Using the Murine Local Lymph Node Assay (LLNA)

2.2 *ISO Standards:*[5]

ISO 10993–1 Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing with a Risk Management Process

ISO 10993–3 Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

ISO 10993–9 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 Methods for Establishment of Allowable Limits for Leachable Substances Using Health-Based Risk Assessment

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

ISO 14971 Medical Devices—Application of Risk Management to Medical Devices

ISO 22442–1 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 1: Analysis and Management of Risk

ISO 22442–2 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 2: Controls on Sourcing, Collection, and Handling

ISO 22442–3 Animal Tissues and their Derivatives Utilized in the Manufacture of Medical Devices—Part 3: Validation and the Elimination and/or Inactivation of Virus and Transmissable Agents

[2] The boldface numbers in parentheses refer to the 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.

[4] The last approved version of this historical standard is referenced on www.astm.org.

[5] Available from International Organization for Standardization (ISO), 1, ch. de la Voie-Creuse, CP 56, CH-1211 Geneva 20, Switzerland, http://www.iso.org.

2.3 *U.S. and European Pharmacopeia Documents:*[6]

United States Pharmacopeia (USP), Edition XXX (30)

USP 30/NF 19 Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

European Pharmacopeia 5.0

2.4 *Code of Federal Regulations:*[7]

21 CFR 312 Investigational New Drug Application

21 CFR Part 820 Quality System Regulation

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

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

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

Federal Register, Vol. 72 No. 8, Jan. 12, 2007, pp. 1581–1619, Proposed Rule: Use of Materials Derived from Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants

21 CFR Part 1271, Part C Suitability Determination for Donors of Human Cell and Tissue-based Products, 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, pp. 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/Notices 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

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

2.5 *ICH Documents:*[8]

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

ICH S2A Guideline for Industry S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals 61 FR 18199 (1996)

ICH S2B Guidance for Industry S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals 62 FR 62472 (1997)

ICH S5A Guideline for Industry S5A Detection of Toxicity to Reproduction for Medicinal Products 59 FR 48746 (1994)

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

ICH S1A Guideline for Industry S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals 61 FR 8153 (1996)

ICH S1B Guidance for Industry S1B Testing for Carcinogenicity of Pharmaceuticals 63 FR 8983 (1998)

ICH S1C Guideline for Industry S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals 60 FR 11278 (1995)

ICH 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 (1997)

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

2.6 *FDA Documents:*[9]

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

FDA Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers, DHHS, June 2012

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

2.7 *AAMI Documents:*[10]

ANSI/AAMI/ISO 11737-1: 2006 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

[6] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

[7] 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.

[8] Available from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Secretariat, c/o IFPMA, 15 ch. Louis-Dunant, P.O. Box 195, 1211 Geneva 20, Switzerland, http://www.ich.org.

[9] Available from Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993-0002, http://www.fda.gov.

[10] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

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"

2.8 *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[11]

Council Directive 93/42/EEC, with Respect to Medical Devices Using Tissues of Animal Origin[12]

Commission Directive 2003/32/EC, with Respect to Medical Devices Manufactured Using Tissues of Animal Origin[12]

EMEA/410/01-rev.2, Committee for Proprietary Medical Products, Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medical Products[13]

The European Agency for the Evaluation of Medicinal Products, (EMA), Committee for Proprietary Medicinal Products (CPMP) Guidance Document for Decision Trees for the Selection of Sterilization Methods (CPMP/QWP/054/98 corr 2000) and Annex to Note for Guidance on Development Pharmaceutics (CPMP/QWP/155/96)[14]

3. Terminology

3.1 *Definitions:*

3.1.1 *adventitious agents, n*—an unintentionally introduced microbiological or other infectious contaminant.

3.1.2 *atelocollagen, n*—triple helical molecule in which the telopeptide regions have been partially or completely removed from tropocollagen (see Fig. 1). Such preparations are typically

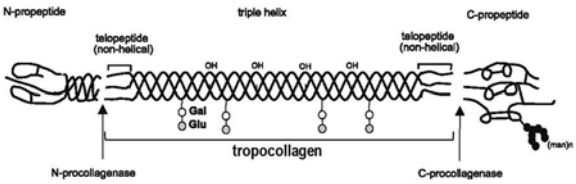

FIG. 1 Schematic of Procollagen Molecule and Associated Propeptide, Telopeptide, and Triple Helical Regions. Enzymatic Removal of Amino- and Carboxy-terminal Propeptide Ends of Procollagen Molecule by Procollagenases Yields Tropocollagen.

the outcome of enzyme-based (for example, pepsin) collagen extraction procedures from tissues.

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

[12] Available from Office for Official Publications of the European Communities—European Law, 2, rue Mercier, L-2985, Luxembourg, http://eur-lex.europa.eu/en/'index.htm.

[13] Available from European Medicines Agency (EMEA), 7 Westferry Circus, Canary Wharf, London E14 4HB, U.K., http://www.eudora.org/emea.html, and http://www.emea.europa.eu/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf.

[14] Available from European Medicines Agency (EMEA), 7 Westferry Circus, Canary Wharf, London E14 4HB, U.K., http://www.eudora.org/emea.html, and http://www.emea.europa.eu/pdfs/human/qwp/005498en.pdf.

3.1.3 *collagen, n*—a family of at least 20 genetically different secreted proteins that serve a predominantly structural function and possess a unique triple helical structure configuration of three polypeptide units known as alpha chains.

3.1.4 *collagen mimetic peptides, n*—specific amino acid sequences representing the triple helical portion of collagen, often $–(Pro–Hyp–Gly)_x–$, forms a triple helix conformation that resembles that found in natural collagens.

3.1.5 *collagen polymer, n*—purified Type I collagen formulation that demonstrates the capacity to self-assemble or polymerize into higher order structures (macromolecular assemblies) in absence of exogenous agents such as cross-linkers.

3.1.6 *diffusion, n*—the random thermal motion of atoms, molecules, clusters of atoms, etc., in gases, liquids, and some solids.

3.1.7 *endotoxin, n*—pyrogenic high molar mass lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria.

3.1.7.1 *Discussion*—Although endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a Limulus Amebocyte Lysate (LAL) test (USP<85> Bacterial Endotoxin Tests).

3.1.8 *fibrillogenesis, n*—the process of tropocollagen monomers assembling into mature fibrils and associated fibril-network structures.

3.1.9 *gel, n*—the three-dimensional network structure arising from intermolecular polymer chain interactions.

3.1.9.1 *Discussion*—Such chain interactions may be covalent, ionic, hydrogen bond, or hydrophobic in nature.

3.1.10 *mechanotransduction, n*—process by which cells convert mechanical stimuli into a chemical response.

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

3.1.12 *permeability, n*—a measure of the ability of porous materials to transmit fluids; the rate of flow of a liquid through a porous material.

3.1.13 *procollagen, n*—collagen molecule comprising three hydroxylated prototcollagen (alpha) chains; amino- and carboxy-terminal propeptide ends are intact (Fig. 1).

3.1.14 *propeptides, n*—amino- and carboxy-terminal nontriple-helical domains of individual collagen protocollagen (alpha) chains that direct triple-helix folding and formation of procollagen molecules (Fig. 1); propeptide removal is required for collagen fibrillogenesis and self-assembly.

3.1.15 *protocollagen, n*—single collagen alpha polypeptide chain as produced by ribosomes.

3.1.16 *recombinant collagen protein/peptide, n*—collagen or collagen-like polypeptide produced by recombinant methods, such as by expression of a nucleotide sequence encoding the protein or peptide in a microorganism, insect, plant, or animal host. Such compositions often comprise Gly-X-Y triplets where Gly is the amino acid glycine and X

and Y can be the same or different, are often proline or hydroxyproline, but can be any known amino acid.

3.1.17 *self-assembly, n*—the process by which a complex macromolecule (as collagen) or a supramolecular system (as a virus) spontaneously assembles itself from its components.

3.1.18 *solution, n*—a type of homogenous mixture in which atoms, ions, or molecules (the solute) are distributed uniformly throughout another substance (the solvent) and which does not separate upon standing.

3.1.19 *sterilization, n*—the destruction or removal of all microorganisms in or about an object (for example, by chemical agents, electron beam, gamma irradiation, or filtration).

3.1.20 *stiffness, n*—a general term describing the extent to which a material resists deformation in response to an applied force; specific measures of stiffness depend upon the material loading format (for example, tension, compression, shear, bending).

3.1.21 *suspension, n*—the dispersion of a solid through a liquid with a particle size large enough to be detected by purely optical means and which separates or settles upon standing.

3.1.22 *telopeptide, n*—amino- and carboxy-terminal nontriple-helical domains of tropocollagen strands known to be important to fibrillogenesis and intermolecular cross-link formation (Fig. 1).

3.1.23 *tropocollagen, n*—collagen molecule comprising three alpha chains with amino- and carboxy-terminal propeptide ends removed (Fig. 1); carboxy- and amino-terminal non-helical telopeptide ends are intact; able to undergo self-assembly into fibrillar matrix.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *adhesion, n*—steady or firm attachment; in the context of collagen, adhesion refers to the ability of cells to physically attach or bind to collagen molecules and macromolecular assemblies of collagen via cell surface proteins like integrins.

3.2.2 *degradation, n*—change in chemical, physical, or molecular structure or appearance (that is, gross morphology) of material; degradation of collagen under physiologic conditions involves site-specific cleavage within the central triple helical region by proteolytic enzymes known as collagenases. Collagenases are members of the larger family of proteases known as matrix metalloproteases.

3.2.3 *matrix, n*—loose meshwork within which cells are embedded or arrangement of connected things. In the context of collagen, matrix refers to a composite material comprised of an insoluble collagen-fibril network or amorphous nanostructure surrounded by an interstitial fluid phase.

3.2.4 *monomer, n*—individual tropocollagen molecule (Fig. 1).

3.2.5 *oligomer, n*—two or more tropocollagen molecules covalently attached by a naturally occurring intermolecular cross-link.

3.2.6 *solubility, n*—a measure of the extent to which a material can be dissolved; in the context of collagen polymers, solubility refers to collagen molecules (partial, full, or multiples) or peptides in a solution; further qualification of solubility may include “acid-soluble” and “neutral salt-soluble” which describes compositions that are soluble in dilute acids and neutral salt solutions, respectively.

4. Significance and Use

4.1 The objective of this document is to provide guidance in the production, characterization, testing, and standardization of: *(a)* collagen polymers as a starting material for surgical implants, substrates for tissue-engineered medical products (TEMPs), vehicles for therapeutic cells and molecules, and 3D *in-vitro* tissue systems for basic research, drug development, and toxicity testing; and *(b)* self-assembled collagen-based materials produced with collagen polymer formulations. This guide can be used as an aid in the selection, characterization, and standardization of the appropriate collagen polymer starting material as well as associated self-assembled collagen-based products for a 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 applications, forms, or medical products, for example (but not limited to) wound and hemostatic dressings, surgical implants or injectables, hybrid medical devices, tissue-engineered medical products (TEMPs), injectable or implantable delivery vehicles for therapeutic cells, molecules, and drugs, and 3D *in-vitro* tissue systems or models for basic research, drug development, and toxicity testing. The practical application of the collagen polymers and associated self-assembled collagen-based materials should be based, among other factors, on biocompatibility, application-specific performance measures, as well as chemical, physical, and biological test data. Recommendations in this guide should not be interpreted as a guarantee of success for any research or medical application.

4.3 The following general areas should be considered when determining if the collagen supplied satisfies requirements for use in the above mentioned medical and research applications: source of collagen polymer, impurities profile, and comprehensive chemical, physical, and biological characterization and testing.

4.4 The following documents or other relevant guidances from appropriate regulatory bodies relating to the production, regulation, and regulatory approval of devices, biologics, drugs, and combination products should be considered when determining if the collagen supplied satisfies requirements for use in medical and research products, including TEMPs, therapeutic delivery vehicles, and 3D *in-vitro* tissue systems:

FDA CFR:
21 CFR 3: Product Jurisdiction:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=3
21 CFR 58: Good Laboratory Practice for Nonclinical Laboratory Studies:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58

FDA/CDRH CFR and Guidances:
21 CFR Part 803: Medical Device Reporting:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=803
21 CFR 812: Investigational Device Exemptions:

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=812
21 CFR 814: Premarket Approval of Medical Devices:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=814
21 CFR 820: Quality System Regulation:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=820
Design Control Guidance for Medical Device Manufacturers:
http://www.fda.gov/cdrh/comp/designgd.pdf
Preproduction Quality Assurance Planning Recommendations for Medical Device Manufacturers (FDA 90-4236):
http://www.fda.gov/cdrh/manual/appende.html
The Review and Inspection of Premarket Approval Applications under the Bioresearch Monitoring Program—Draft Guidance for Industry and FDA Staff:
http://www.fda.gov/cdrh/comp/guidance/1602.pdf

FDA/CDRH Search Engines:
CDRH Guidance Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfggp/search.cfm
CDRH Premarket Approval (PMA) Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm
CDRH 510(k) Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm
CDRH Recognized STANDARDS Search Engine:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

FDA/CBER CFR and Guidances:
21 CFR 312: Investigational New Drug Application:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=312
21 CFR 314: Applications for FDA Approval to Market a New Drug:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=31
21 CFR 610: General Biological Products Standards:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=610
21 CFR 1271: Human Cells, Tissues and Cellular and Tissue-Based Products:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271
Cellular & Gene Therapy Guidances and Other Publications:
http://www.fda.gov/cber/genetherapy/gtpubs.htm
Human Tissue Guidances and Other Publications:
http://www.fda.gov/cber/tissue/docs.htm
CBER Product Approval Information:
http://www.fda.gov/cber/efoi/approve.htm
21 CFR 600, 601 BLA Regulations:
http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfrv7_07.html
21 CFR 210, 211 GMP Regulations:
http://www.access.gpo.gov/nara/cfr/waisidx_07/21cfr210_07.html

5. Chemical, Physical, and Biological Characterizations of Collagen Polymers and Associated Self-assembled Collagen-based Products

5.1 *General Comments*—These methods represent suggested chemical, physical, and biological assays or analyses; however, other validated assays and analyses may be used (**5**). Method selection will vary, depending on the formulation and source of the collagen (for example, tissue-derived molecular collagen or collagen peptides produced synthetically). The user should ensure that the method selected is reliable and commonly accepted for protein, polymer, biological, and biomaterial analyses. In addition, the test should have appropriate dynamic range, detection limits, and sensitivity.

5.2 *Collagen Concentration*—Collagen concentration should be expressed in mass/volume or mass/mass. Calibrated colorometric assays for collagen, such as Sirius red, or amino acid analysis for hydroxyproline, are commonly used methods to measure collagen content.

5.3 *Viscosity*—Viscosity of collagen polymer formulations depends on a number of factors which may include, but are not limited to, the following: solution or dispersion/suspension, concentration, molecular composition, molecular size, temperature, operating condition, and so forth. Viscosity measurements are routinely performed with a viscometer or rheometer. The user must clearly state the conditions of the test. Determinations of intrinsic viscosity can be used in calculation of average polymer molecular weight.

5.4 *Purity of Collagen Polymer Formulations*—Collagen polymer formulations should be highly purified solutions with impurity levels lower than 2 % by mass. Formulations can be analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), either on the collagen polymer directly or after specific enzymatic (bacterial collagenase, trypsin) or chemical (cyanogen bromide (CNBr)) cleavage reactions to analyze cleavage products. The following represents a non-inclusive list of chemical analyses available: SDS-PAGE, peptide mapping, and amino-terminal sequencing. Assay methods for specific non-collagenous impurities such as 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) may also be included.

5.5 *Collagen Type Composition*—Tissues commonly used to isolate Type I collagen typically contain other collagen types since co-assemblies of different collagen types are commonplace. Collagen Type I is the predominant collagen type found in the majority of connective tissues and organs, including skin, bone, tendon, cornea, and the interstitial extracellular matrix. Type II collagen is found primarily in cartilage, while Type IV collagen is a major component of basement membranes. The collagen type composition is an important determinant of the polymerization capacity and properties of collagen formulations. Since it is well established that other collagens, such as Type III and Type V, affect Type I self-assembly kinetics and products, the levels of these should be evaluated and controlled for manufacturing consistency. Collagen type composition is usually determined via western blot or ELISA analysis and requires the use of type-specific antibodies. Validation of antibody specificity, as well as the test procedure, using suitable standards, should be conducted prior to analysis. A risk assessment should be performed on the potential for other collagens in the product. If the presence of other collagens is likely, an assessment should be completed for collagens that have the potential to generate undesired responses. The extent of analysis required will depend upon the risk of other collagen types being present as impurities in a particular collagen product.

5.6 *Elastin Assay*—Elastin can be a component of the impurities in tissue-derived collagen polymer preparations. One method to assay for elastin involves the detection of desmosine (**6**). Western blot, enzyme-linked immunosorbent assay (ELISA), and other types of analyses also may be used.

5.7 *Amino Acid Analysis*—Amino acid analysis provides information on the composition of the amino acids of the collagen polymer. Tissue-derived molecular collagen formulations should have an amino acid profile that falls within the range of published data for highly purified collagen preparations, generally in the acid-soluble form. Amino acid analysis is routinely performed on hydrolyzed collagens by reverse phase high performance liquid chromatography (HPLC). This method can be used to quantify hydroxyproline, tyrosine, tryptophan, and cysteine. Because tyrosine residues are only present within the nontriple-helical telopeptide ends, their content may be used as an indicator of telopeptide integrity. There are other methods available for amino acid analysis.

5.8 *Peptide Mapping*—Peptide mapping is one possible method to identify oligomer and collagen type composition of tissue-derived collagen polymer formulations. The most commonly used peptide mapping method for collagen utilizes CNBr digestion. The digest can be analyzed by SDS-PAGE, HPLC, mass spectroscopy (MS), matrix-assisted laser desorption/ionization (MALDI), or other analysis methods.

5.9 *DNA Sequence Data on Recombinant or Transgenic Source Cells*—Verify sequence data for expression product, that is, COL1A1, COL1A2 or collagen-related protein or peptide.

5.10 *Carbohydrate Analysis*—Carbohydrate analysis of collagen polymers can be carried out using established gas-liquid chromatographic methods or spectrophotometric methods. Novel sources of collagen (for example, animal or recombinant) may result in a different glycosylation pattern and/or sugars that differ from human collagen. If a novel source of collagen is used and the carbohydrate pattern is unknown there may be a potential risk of autoimmune disease may be present. In this case, a risk assessment should be performed based on an analysis of the sugars present on the collagen. If necessary, the full glycosylation properties of the collagen should be determined and an assessment of the autoimmunity potential performed.

5.11 *Intermolecular Cross-link Composition*—Extraction and isolation of purified collagen from tissues may yield atelocollagen, monomers, oligomers (at least 2 monomers covalently attached by a naturally-occurring intermolecular cross-link), molecular aggregates, or combinations thereof depending upon the specific procedure employed. Oligomers and molecular aggregates have been routinely minimized or eliminated from collagen polymer preparations via enzymatic digestion, secondary purification strategies, or use of young or experimentally induced lathyritic animals where tissue intermolecular cross-link content is decreased. Experimental induction of lathyrism routinely involves administration of beta-aminopropionitrile (BAPN), a potent inhibitor of the tropocollagen cross-linking enzyme lysyl oxidase. Since the molecular integrity and composition of collagen polymers, including the intermolecular cross-link composition, play an important role in their self-assembly kinetics and capacity, molecular characterization of tissue-derived collagens should include intermolecular cross-link analysis. A number of analytical methods have been developed for intermolecular cross-link quantification. These methods are applied to tissues routinely and to purified collagen preparations less frequently. For example, a cation-exchange HPLC method which allows rapid, sensitive quantification of immature (reducible, divalent) and mature (non-reducible, trivalent) cross-links, which can be applied to collagen or tissue hydrolysates has been described. Borohydride reduced intermediate, divalent cross-links, including dehydrohydryoxylysinohydroxynorleuicine (reduced form: dihydroxylysinonorleucine, DHLNL), dehydrohydroxylysinonorleucine (reduced form:hydroxylysinonorleucine, HLNL), and dehydrolysinonorleucine (Δ-HLNL) (reduced form lysinonorleucine, LNL) and the mature, non-reducible, trivalent cross-link histidinohydroxylysinonorleucine (HHL) are detected by post-column o-phthalaldehyde derivatization. The other mature, non-reducible, trivalent cross-links pyridinoline and deoxypyridinoline are detected based on natural fluorescence. Most recently, multiple reaction monitoring, such as liquid chromatography (LC)/mass spectroscopy (MS), has also been performed. Here the effluent from the LC column is introduced into the first-stage MS and specific cross-link components characterized by their mass. These species are further fragmented into ions with masses characteristic of particular cross-links and detected in a second-stage MS. Chromatographic methods have not been developed for pyrrole detection. Pyrrole crosslinks are routinely quantified by colorimetric detection. The content of the various intermolecular cross-links is routinely expressed as mol/mol collagen.

5.12 *Molecular Mass Analysis*—The molecular mass of collagen polymer formations may vary widely from about 1,000 g/mol for collagen mimetic peptides to over 300,000 g/mol for tissue-derived monomers and oligomers. MS and dynamic light scattering (DLS) are commonly employed for molecular mass analysis of small-sized (<100,000 g/mol) collagen peptides and proteins. Since the large size of tissue-derived collagens (100,000 g/mol or greater) poses significant challenges to MS analysis, these polymers are routinely analyzed using alternative methods including SDS-PAGE, size exclusion chromatography, or viscosity measurements.

5.13 *Circular Dichroism*—Circular dichroism, which measures differential absorption of left and right circularly polarized light, has been used extensively for structural characterization of collagen proteins and peptides. It is commonly used to characterize the secondary structure (helical) content of collagen polymers. This detection method has also been applied to monitor thermal transitions of collagen polymers and collagen-based products.

5.14 *Differential Scanning Calorimetry (DSC)*—DSC determines dissociation temperature of collagen polymers and self-assembled collagen materials. Thermal properties of collagens provide information on transitions in the structural state, thereby providing information on initial primary (chemistry) sequence, structural state, and purity of samples.

5.15 *Trypsin Susceptibility*—Trypsin susceptibility will detect that portion of collagen polymers that has been denatured during 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.

5.16 *Impurities Profile*—The term impurity relates to the presence of extraneous substances and materials within the Type I collagen polymer solutions. These impurities can be detected by western blot, ELISA, gas chromatograph (GC)-MS, and other types of assays. The user is also directed to Guide E1298 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, donor DNA, donor cell components, cell culture components, heavy metals, bioburden, viruses, transmissible spongiform encephalopathy (TSE) agents, enzymatic agents, and agents used in preparation, processing, extraction, or solubilization (for example, acids, surfactants, solvents, and so forth).

5.17 *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 polymers and associated self-assembled collagen-based materials 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, Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers and <85> Bacterial Endotoxins Test). Each new lot of reagents should meet acceptance criteria established by appropriate qualification or validation studies (for investigational or licensed/cleared products, respectively). The endotoxin level in collagen will 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.

5.18 *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 that may include, but are not limited to, spectrographic, chromatographic, and flame atomic absorption techniques.

5.19 *Microbiological Safety*—Bacteria, viruses, and fungi are also contaminants that can arise in a biological sample. The user will validate sterilization and characterize its effect on the product. The presence of bacteria may also contribute to the presence of endotoxins. The following Microbiological Tests in USP 30 are of particular relevance: Microbial Limit Tests <61>, Sterility Tests <71>, Sterilization and sterility assurance of compendial articles <12211>, the Biological Tests and Assays: Bacterial Endotoxins Tests <85>, and viral validation studies <1050>. 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: 2006; ANSI/AAMI/ISO 11737-2: 1998; and ISO 13408–1. 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 Soy Agar to determine the presence of bacteria, and on Sabouraud Dextrose Agar to determine the presence of yeast and mold. If self-assembled collagen products are intended to serve as a barrier to microorganisms, this function will need to be validated with specific experiments. Refer to 6.4.1 within this document for additional information.

5.20 *Polymerization/Self-Assembly Capacity*—Collagen preparations often differ in their capacity to self-assemble or polymerize into supramolecular structures. In fact, collagens that polymerize may yield different assembly products and assembly kinetics, which may affect their appropriateness for specific applications. Assembly kinetic parameters have routinely been defined by monitoring time-dependent changes in turbidity or viscoelastic properties (for example, shear storage modulus) using a spectrophotometer or rheometer, respectively. Such analyses routinely yield sigmoidal-shaped polymerization curves from which kinetic parameters, including lag time, rate of linear growth phase, and polymerization half-time can be quantified. It is important to note that no specific information regarding assembled microstructure can be derived from spectrophotometric (turbidity) data. Image-based methods, such as time-lapse confocal reflection microscopy, have also been applied.

5.21 *Standardization of Collagen Polymer Formulations*—To ensure a high level of manufacturing consistency and low lot-to-lot variability in the functional properties of collagen polymers and associated self-assembled materials, standardization or quality control methods should be established for polymerization capacity. In some instances, polymerization capacity has been standardized by the quantified relationship between viscoelastic properties of self-assembled materials (shear storage modulus; matrix stiffness) as a function of the collagen concentration of the polymerization reaction. Such standardization strategies have been found

to improve predictability and reproducibility of *in-vitro* cell and *in-vivo* host responses to self-assembled collagen materials.

5.22 *Nano- and Micro-structure Analyses of Self-assembled Collagen Materials*—A number of imaging technologies and analysis tools have been applied for visualization and quantification of structural features of self-assembled collagen materials at multiple size scales. Electron microscopy uses a beam of electrons to form an image of the specimen with nanometer-scale resolution. Both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) provide high-resolution, two-dimensional (2D) images representing the specimen surface topography. Serial-section TEM and serial block face-SEM have been applied for 3D analyses. Both TEM and SEM imaging modalities require extensive specimen processing including fixation, dehydration, and drying. In contrast, cryo-SEM involves freezing of specimens under vacuum followed by sectioning. While still frozen the specimen is sputter-coated and transferred onto the SEM cryo-stage for imaging. Compared to conventional SEM, CryoSEM better preserves the native detail of the collagen material and induces fewer structural artifacts. Electron microscopy images are routinely used for defining microstructure parameters for fibril-based materials including porosity, fibril area fraction, fibril diameter, and fibril D-period. The D-period is defined by the characteristic gap and overlap repeats (for example, 67nm) along the fibril length which results from the staggered packing of collagen molecules. Electron microscopy has also been used for defining size and shape of various nanostructures, including microflorettes, spherical aggregates, and nanofibers formed from collagen mimetic peptides. Confocal and multiphoton microscopy provides imaging technologies that allow collection of 3D microstructure details of self-assembled collagen materials without extensive specimen processing or staining. Since collagen fibrils differ in their refractive index from their surroundings, laser-scanning confocal microscopy in reflection mode allows collection of reflected or back-scattered light from the collagen microstructure in fully hydrated specimens, thereby minimizing artifacts associated with extensive specimen processing. Time-lapse confocal reflection imaging during collagen polymerization also provides useful information regarding kinetics and molecular mechanisms of self-assembly. On the other hand, the non-centrosymmetric (not symmetric with respect to a central point), triple-helical structure inherent to collagen produces a nonlinear, second order polarization of light. Second-harmonic generation (SHG) produces scattered light that is polarized along the helix axis and half the wavelength of the incident multiphoton excitation. Since collagen possesses intrinsic fluorophores, its autofluorescence can also be visualized using confocal and multiphoton microscopy. However, autofluorescence intensity is typically relatively low thereby compromising image quality and resolution. For fixed specimens, collagen-specific antibodies and probes have been shown to improve signal-to-noise and image quality of collagen microstructures. The collection of 3D microstructure information supports more comprehensive, quantitative microstructure descriptions, including fibril density (fibril volume fraction), fibril diameter, extent of interfibril branching, and total and average fibril length. Atomic force microscopy (AFM) imaging is routinely used for visualization of the nanoscale topography of collagen materials in a dry or hydrated state. Because measurements require interaction of the AFM tip with surfaces, its applicability is limited to 2D contexts. Roughness and diameter (height) of fibrils are routinely measured from these images. AFM is also applied for visualization and quantification of differences in fibril D-period.

5.23 *Viscoelastic/Mechanical Properties of Self-assembly Collagen Materials*—The mechanical or viscoelastic properties of collagen materials have been shown to be a critical determinant of fundamental cell behavior and cell-induced material remodeling properties. Definition of mechanical behavior of collagen materials has been performed using a number of testing formats including shear, oscillatory shear, compression and extension (uniaxial or multiaxial). Creep, stress-relaxation, and dynamic mechanical tests are often used to measure time-dependent mechanical or viscoelastic properties. The mechanical behavior of collagen materials is not only dependent upon the test geometry and format, but also the loading regimes (strain rate, frequency of dynamic loading) and material conditions during loading (temperature, medium). As such mechanical testing parameters and conditions should be specified. Material properties measurements commonly reported include modulus, failure stress, failure strain, and Poisson's ratio. A number of other conventional mechanical tests may be applied including suture pull out, ball burst, and micro- and nano-scale indentation. While testing set-ups routinely provide mechanical information at only a single size scale, various systems and approaches to support collection of multi-scale mechanical behavior of collagen materials have been developed recently.

5.24 *Transport Properties of Self-assembled Collagen Materials*—Fluid and solute transport of polymerized collagen materials or engineered tissue constructs plays a critical role in guiding fundamental cell behavior, tissue morphogenesis and repair, maintenance of tissue structure-function, and tissue pathologies (for example, cancer). It does so by modulating the cell microenvironment through *(1)* nutrient delivery and waste removal, *(2)* spatiotemporal distribution of soluble factors, and *(3)* physical cues (for example, shear force). In addition, material permeability and diffusivity have been recognized as important design considerations for biomaterial-based delivery of therapeutic cells, drugs, or molecules. Permeability quantitatively defines the ease of fluid flow under an applied pressure gradient through porous materials. Experimental approaches used routinely for measuring permeability involve application of a pressure gradient to collagen materials with defined dimensions and quantification of average flow rate. Darcy's law is then applied to calculate permeability. Diffusivity quantifies the random motion of solutes driven in response to concentration gradients. Two of the most widely used methods for quantifying diffusivity include fluorescent recovery after photobleaching (FRAP) and integrative optical imaging (IOI). FRAP involves uniform distribution of a dilute fluorescent tracer, of various sizes, within the construct. A well defined

concentration gradient is then induced by laser-induced photobleaching. The time-dependent recovery of the tracer concentration profile is analyzed to yield the diffusion coefficient. Alternatively, IOI involves creation of a point source of fluorescent tracers via microinjection followed by image-based measurement of their temporal and spatial distribution.

5.25 *Collagenase Degradation of Self-assembled Collagen Materials*—Collagen degradation of self-assembled collagen materials is routinely determined from time-dependent release of hydroxyproline following treatment with bacterial collagenase. Alternatively, collagenase-dependent changes in macroscopic appearance or specific mechanical properties (stress relaxation rate, shear storage, and loss moduli) of collagen materials may be monitored.

5.26 *Collagen-Cell Interactions*—It is now well established that the cell response to its collagen-based extracellular microenvironment *in vivo* is modulated by both biochemical and biophysical cues. The inherent cell signaling capacity of collagen, in large part, is driven by its ability to *(a)* support integrin-mediated cell adhesion, *(b)* impose physical and mechanical constraints through structural and material properties, and *(c)* participate in cell-induced dynamic remodeling (biodegradation, growth factor binding, and microstructure deformation). Since collagen polymers and associated self-assembled collagen materials may differ dramatically in molecular composition, microstructure, and physical properties, their ability to interact and guide fundamental cell responses should be measured. Several approaches to determine the interaction and biological response of cells to collagen materials have been established. Cells may be readily entrapped within the self-assembled collagen network by suspending them in the collagen polymer solution prior to polymerization. Alternatively, they may be seeded onto the surface of collagen materials after self-assembly. Cell response parameters including cell morphology, area, and volume are visualized and quantified using various 2D and 3D imaging methods. The organization of cytoskeletal elements, especially actin, is used to define cell-matrix adhesion and associated cell-matrix traction forces. Antibody blocking experiments can be used to determine which cell surface receptors are being used to engage collagen, such as integrins or discoidin domain receptors (DDRs). Various methods have been developed for quantification of cell-induced traction forces from a cell population or at the individual cell level. These include time-dependent changes in free-floating tissue construct size, force exerted on culture-force monitor system, and strain/deformation induced within the collagen network by individual cell. Other cell response parameters routinely monitored include viability, proliferation, apoptosis, and migration. Other functional measures of cell-collagen interactions applicable to stem and progenitor cell populations include lineage-specific differentiation, expansion of specific stem/progenitor cells, or tissue morphogenesis (de novo vessel formation by endothelial progenitor cells). The above mentioned functional measurements of collagen-cell interaction (biological activity) may also serve as useful methods for standardization and quality control.

5.27 *Characterization Methods for Type I Collagen Polymers and Associated Self-Assembled Collagen Products* (see Table 1)—The collagen polymer and associated self-assembled collagen products shall have specifications for an extensive set of chemical/biochemical, physical, and biological 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 formulation. Not all the methods listed may be required to characterize the sample, and the specificity and sensitivity vary among the methods listed.

TABLE 1 Characterization Methods for Type I Collagen Polymers and Associated Self-Assembled Collagen Products

Characterization Method	Applicable To
Chemical/Biochemical	
Appearance	Collagen Polymer
Concentration	Collagen Polymer
Viscosity	Collagen Polymer
Purity Analysis, including collagen types other than Type I, elastin, glycosaminglycans, non-collagenous proteins, lipids, nucleic acids	Collagen Polymer
Amino Acid Analysis; Sequence Analysis; Peptide Mapping	Collagen Polymer
Carbohydrate Analysis	Collagen Polymer
Intermolecular Crosslink Analysis	Collagen Polymer
Molecular Mass, Average Polymer Molecular Weight	Collagen Polymer
Telopeptide Integrity, Helical Content	Collagen Polymer
Thermal Dissociation	Collagen Polymer
Trypsin Susceptibility	Collagen Polymer
Impurities Profile, including heavy metal analysis, endotoxin, bioburden	Collagen Polymer
pH	Collagen Polymer; Self-assembled Product
Additives (drugs, molecules, sterilants)	Collagen Polymer; Self-assembled Product
Polymerization Kinetics	Collagen Polymer
Polymerization Capacity	Collagen Polymer
Physical	
Nano-/Micro-structure	Self-assembled Product
Transport Properties (Permeability, Diffusivity)	Self-assembled Product
Mechanical/Viscoelastic Properties	Self-assembled Product
Collagenase Degradability	Self-assembled Product
Shrink/Melting Temperature	Self-assembled Product
Biological	
Collagen-Cell Interactions, including biocompatibility and fundamental cell responses (morphology, cell-matrix traction forces, proliferation, apoptosis, differentiation, morphogenesis, migration)	Cell-Seeded Collagen Product

The user should be familiar with the capabilities and limitations of the appropriate test methods.

6. Product Development Considerations

6.1 *Storage Conditions/Shelf Life Stability of Collagen Polymers and Associated Self-assembled Collagen-based Materials*—Optimal storage conditions and shelf life stability will be dependent upon the physical form of the collagen (for example, solution, lyophilized). The preferred mode of storage for tissue-derived lyophilized collagen polymers is at 4°C in the presence of a desiccating agent. Tissue-derived collagen polymers are highly stable in lyophilized form with a typical shelf life beyond 1 year. Acidic solutions of tissue-derived collagen polymers also should be stored at 4°C; however, tWu, Peidonghey are stable for less time in this form, typically 4 to 6 months. Repeat freeze/thawing of tissue-derived collagen polymers in either form should be avoided as it may induce denaturation. When performing storage and shelf life testing for collagen polymers and associated self-assembled collagen-based materials, the most relevant stability-indicating parameters are those related to the functionality of the polymer formulation or material. Depending upon what function is required of the final collagen formulation, parameters such as viscosity (apparent and intrinsic), polymerization kinetics, polymerization capacity, microstructure, physical properties, and biological activity, along with other parameters deemed relevant, may also be considered. International Conference on Harmonization (ICH) guidance documents should be consulted for information on stability testing of pharmaceuticals (that is, ICH Q1A(R2)).

6.2 *Sterilization Method, if Applicable, and Effects of Sterilization on the Product*—The user should verify that the sterilization method does not adversely affect the collagen polymer or associated self-assembled collagen-based material. Collagen can be sterilized by various methods, for example (but not limited to) gamma irradiation, electron beam, or by ethylene oxide, or prepared using aseptic processing steps. Potential degradation of the collagen or sterilization residuals should be evaluated to determine the impact on the product. Solutions of collagen may be *(1)* filter-sterilized if the viscosity of the collagen solution permits; or *(2)* gamma-irradiated. Any changes in viscosity may reflect an alteration of the molecular mass and should be evaluated. The method of sterilization is dictated primarily by the effect on the product effectiveness. The method chosen must be validated to determine the effectiveness of sterilization. The reader should refer to the most current version of the relevant standards regarding the sterilization of healthcare products by radiation, ethylene oxide gas, or other methods, such as AAMI TIR No. 19-1998; AAMI/ISO 14160-1998; and AAMI ST67/CDV-2: 1999; The European Agency for the Evaluation of Medicinal Products, (EMEA), Committee for Proprietary Medicinal Products (CPMP) Guidance Document for Decision Trees for the Selection of Sterilization Methods (CPMP/QWP/054/98 corr 2000), and Annex to Note for guidance on Development Pharmaceutics (CPMP/QWP/155/96).

6.3 *Sourcing*—The criteria to consider for safe sourcing include appropriate human or animal donor selection and tissue collection procedures to assure that the source material is unlikely to contain TSE infectivity. Additional information can be obtained from the following documents: ISO 22442–1, ISO 22442–2, ISO 22442–3; 21 CFR Parts 207, 807, and 1271, 21 CFR Part 820, and 21 CFR Part 1271, Part C; Federal Register Vol. 43; Federal Register Vol. 62; Federal Register Vol. 66, No. 5, January 8, 2001, pp. 1552–1559; Federal Register, Vol. 66, No. 13, January 19, 2001, p. 5447; ISO 13408–1. Council Directive 93/42/EEC with respect to medical products using tissues of animal origin; Commission Directive 2003/32/EC with respect to medical products manufactured using tissues of animal origin; EMEA/410/01-rev.2: Committee for Proprietary Medical Products, Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medical products. Additional documents may be available. The user should use the most current version of all documents.

6.3.1 For further information, the user is referred to Appendix X2, Sourcing Issues; X2.1, Tissue for collagen or collagen-containing medical devices; X2.2, Requirements for a closed herd; and X2.3, Documentation.

6.3.2 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 (**7-10**). The user should be aware that even though Type III collagen is less abundant, it is often associated with Type I collagen, except in bones and tendons. Type V collagen is also associated with Type I collagen.

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 30/NF 19 <1050>, and other pertinent references, as appropriate. Additional information may be found in the following FDA Guidance 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. The European Pharmacopoeia 5.0 has a monograph describing methods to minimize TSE risks (5.2.8 Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products).

6.4.1 *Viral Clearance*—The sources of starting or raw materials from humans or animals should be screened for known viral pathogens to reduce or eliminate the potential infectivity. For material of animal origin to be considered, see also ISO 22442–1. Viral clearance methods can include, but not be limited to, methods such as detergent treatment, high or low pH, urea treatment, other chemical treatments, and filtration or other purification methods. However, even these harsh treatments may not ensure complete viral inactivation. Viral clearance should be demonstrated by an appropriately validated viral clearance study protocol (see USP 30/NF19 <1050>). 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 Sourcing Issues and TSE Clearance*—Careful attention should be given 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," (**11**), 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)," and also ISO 22442–3 (for material of animal origin) 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 (**12, 13**). 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. See also the following Reference Sections for additional information: Section 2.1 (Guide E1298); Section 2.2 (ISO 22442–1, ISO 22442–2, and ISO 22442–3); Section 2.4 (USP 30/NF 19); Section 2.6 (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); and Refs (**14, 15**). Additional resources may be available, as this list is not comprehensive. The user should use the current version of all documents.

7. Safety and Toxicology Aspects of Collagen

7.1 The safety of collagen in biomedical and pharmaceutical applications and in TEMPs should be established according to current guidelines such as ISO 10993 and Practice F748. Suppliers of collagen may have such documentation on file. Preclinical safety studies specific to the clinical application under consideration must be done in accordance with applicable investigational new drug (IND; 21 CFR 312) or investigational device exemption (IDE) regulations.

7.1.1 A database generated to support the safety of collagen-containing pharmaceuticals should reflect consideration of the proposed clinical route of administration and product formulation, although it may be appropriate for certain studies to involve a route of administration or formulation which differs from the clinical situation. Guidance on the need for, timing, and conduct of the nonclinical toxicology studies is available in the ICH (International Conference on Harmonization) guidelines on the respective topics. Such studies may include, but are not limited to: acute toxicology testing, repeated dose toxicology testing with a treatment regimen and duration that is relevant to the proposed clinical use (ICH M3), hypersensitivity testing, and genetic toxicology testing (ISO 10993–3, ICH S2A, and ICH S2B). Additional studies that may be relevant to a proposed pharmaceutical use include reproductive/developmental toxicology testing (ISO 10993–3, ICH S5A and ICH S5B) and carcinogenicity testing (ISO 10993–3, ICH S1A, ICH S1B, ICH S1C, and ICH S1C(R)). Additional testing may be specific to the route of administration, for example, application or injection site irritation, ocular irritation, dermal carcinogenicity testing, or studies of photoirritation and photo co-carcinogenicity potential. Other testing may be appropriate, depending on the results of early studies and the intended clinical use of the product. For instance, the user may consider the following, among other documents: Practice F2148, or Draft Guidance for Preclinical and Clinical Investigations of Urethral Bulking Agents Used in the Treatment of Urinary Incontinence. Specific guidance on the development or marketing of drug products, biologics, or medical devices in the United States may be obtained by contacting the Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, or the Center for Devices and Radiological Health, respectively, of the U.S. Food and Drug Administration.

7.2 *Biocompatibility:*

7.2.1 There are few interspecies differences in the structure of Type I collagen. The extensive similarity in primary and higher-order structure of Type I collagen across species may explain why animal-sourced collagen is acceptable as a material for human implantation (**16**). With regard to biocompatibility, when new applications, modifications or changes in physical form of a material are being considered, then the recommendations and test methods of the following standards should be considered: Practices F619, F748, F749, F756, F763, F813, F981, F1439, F1903, F1904, F1905, F1906 and F1983, as well as Test Method F895, Terminology F1251, and ISO 10993–1, ISO 10993–9, Part 9, ISO 10993–17, ISO 22442–1, and ISO 22442–3.

7.3 *Immunogenicity*—Immunogenicity of collagen may vary depending on the source material (that is, extracted versus recombinant collagen), though, for reasons noted in the preceding paragraph, immune reactions due to species differences

alone are uncommon (**17**). Products from different manufacturers may vary in properties such as, but not limited to, fiber quality and percentage hydroxyproline. The user should be aware that differences in collagen structure and chemistry may result in potential variability to the immunological responses. The manufacturer should ascertain to what degree modifications of the structure of native collagen (for example, by chemical crosslinking or methods not limited to biomimetics) may modify (enhance or reduce) immunogenicity when implanted *in vivo*. When new applications, modifications or changes in physical form of a material are being considered, then the immunogenicity testing should include the following standards for testing potential skin sensitizers: Practice F720 and ISO 10993–10.

7.4 Collagen for use in biomedical and pharmaceutical applications including Tissue-Engineered Medical Products (TEMPs) should ideally be documented in a Device or Drug Master File to which end users may obtain a letter of cross reference from suppliers of collagen. Such a Master File should be submitted to the US FDA and to other regulatory authorities, both national and international. ISO 14971 should also be referenced when appropriate.

8. Keywords

8.1 biomaterials; characterization; collagen-cell interactions; collagen polymer; self-assembly; tunable

APPENDIXES

(Nonmandatory Information)

X1. BACKGROUND

X1.1 Background

X1.1.1 Below is a brief overview of the natural polymer collagen, including its classification, molecular structure, hierarchical assembly, and biosynthesis. When describing the processes of collagen biosynthesis and assembly, emphasis is placed on intermolecular cross-link formation and its role in tissue-specific structure and function. Additional details on collagen biosynthesis, collagen intermolecular cross-links, and collagen self-assembly may be found in (**18-21**). For reviews on design and function of collagen mimetic peptides as well as recombinant collagen technology refer to (**22-24**).

X1.2 Overview/Introduction

X1.2.1 The vertebrate collagen superfamily represents secreted extracellular matrix proteins that serve as major determinants of *in-vivo* tissue structure and function. More than twenty genetically different family members have been identified to date. A common feature of collagen molecules is that they are composed of three polypeptide chains of left-handed helices that form a right-handed triple helix. Triple helix formation is facilitated by the repeating amino acid triplet sequence, Gly-X-Y, where X and Y are often but not always proline and hydroxyproline, respectively. It is this primary structure feature that provides the basis for the design and development of self-assembling collagen mimetic peptides.

X1.3 Collagen Classification

X1.3.1 The various collagen types are designated by Roman numerals (for example, collagen Type I), the associated polypeptide chains (or subunits) as alpha chains with Arabic numbers (for example, alpha2(I)), and the genes by COL[Arabic numeral]A[Arabic numeral] (for example, COL1A2). Classification of the different collagen types is based on protein domain structures, macromolecular assemblies, and exon structures of the corresponding genes. Six different sub-families are currently identified (**25**):

X1.3.2 *Fibrillar Collagens*—This group contains collagens Type I, II, III, V, and XI, important for the mechanical support of multicellular organisms. These collagens (formed by either homotrimers [Types II and III] or alpha chain heterotrimers) participate in the formation of staggered fibrils of varying diameter. The central triple helical domain (≈ 100,000 g/mol size) usually contains 300 repeats of the Gly-X-Y triplet, forming long, rigid structures. After extracellular processing, the single collagen molecules aggregate to fibrils. The extent of processing may be important in determining the diameter of the fibril.

X1.3.3 *FACIT Collagens*—The fibril-associated collagens with interrupted triple helices (FACIT) collagen group is comprised of collagen Types IX, XII, XIV, XVI, XIX, and XX. Collagen IX has been shown to be associated with Collagen II and XI fibrils. Collagen Types XII and XIV have been associated with Collagen I fibrils. FACIT collagens contain large non-collagenous domains connecting relatively short triple-helical domains. Their associations with fibrils are thought to be important in the regulation of fibril diameter and the connection of fibrils with other extracellular molecules.

X1.3.4 *Short Chain Collagens*—Collagen Types VIII and X contain short triple helical domains of 50,000 to 60,000 g/mol flanked by globular domains. Collagen Type VIII forms the backbone of the hexagonal network in Descemet's membrane, Type X is found in hypertrophic cartilage matrix. Both are thought to provide open structures resisting compressive forces.

X1.3.5 *Basement Membrane Collagens*—Collagen Type IV forms the network structures found in basement membranes. Triple helical domains are frequently interrupted by non-collagenous domains, allowing for flexibility within the rod-like molecules. The triple helices interact at the N- and C-termini, forming a three-dimensional network; collagen Type IV also binds directly to other basement membrane

molecules like laminin and BM-40. Six different alpha chains have been identified for Collagen Type IV. The most prevalent collagen Type IV is formed by a heterotrimer of two alpha1 chains and one alpha2 chain. Specific basement membranes (kidney glomerular basement membrane, neuromuscular junction, and so forth) contain heterotrimers containing the alpha3-6 chains. The exact composition of these heterotrimers is not clear. Goodpasture and Alport syndromes have been associated with Collagen Type IV (**26**, **27**). Collagen Type VII (see below), although not classified as a basement membrane collagen, forms tight associations (anchoring fibrils) with basement membranes in skin, the oral mucosa, and the cervix.

X1.3.6 *Multiplexins*—Collagen Types XV and XVIII have been grouped as Multiplexins (collagens containing multiple-triple-helix domains with interruptions). Both collagens are expressed widely. A fragment of Collagen XVIII, called endostatin, has been shown to inhibit angiogenesis.

X1.3.7 *MACITs*—Two members form the group of membrane-associated collagens with interrupted triple helices. Types XIII and XVII both contain triple helices in the extracellular domain and globular domains with transmembrane domains, attaching the molecules to the cell surface. Type XIII is widespread, and Type XVII is found in hemidesmosomes. It also presents as an autoantigen in the blistering disease bullous pemphigoid.

X1.3.8 *Other Collagens*—Collagen Types VI and VII do not belong to the other classifications and have been grouped separately. Collagen Type VI is the major component of beaded microfibrils. The heterotrimeric molecule contains a central triple helix flanked by globular domains. Dimers and tetramers are formed by disulfide exchange. Collagen Type VII is the major component of anchoring fibrils connecting the basement membrane in stratified squamous epithelia with adhesion plaques in the papillary dermis. Some of the subtypes of acquired and dystrophic forms of epidermolysis bullosa are associated with defects in Collagen Type VII (**28**, **29**).

X1.4 Occurrences

X1.4.1 Table X1.1 summarizes the location of major collagen types (**1**). Collagen Types XIII–XX are less well characterized and are excluded.

TABLE X1.1 Occurrences of Types I to XII Collagen

Collagen Type	Alpha Chains	Distribution
I	a1-2(I)	widespread; skin, bone, tendon, cornea, etc.
II	a1(II)	cartilage, vitreous body of the eye
III	a1(III)	skin, tendon, aorta, cornea
IV	a1-6(IV)	all basement membranes
V	a1-3(V)	widespread; skin, bone, tendon, ligament, etc.
VI	a1-3(VI)	widespread; skin, bone, cornea, etc.
VII	a1(VII)	skin, oral mucosa, cervix
VIII	a1-2(VIII)	Descemet's membrane
IX	a1-3(IX)	cartilage, vitreous body
X	a1(X)	hypertrophic and mineralizing cartilage
XI	a1-3(XI)	Cartilage
XII	a1(XII)	all Collagen Type I-containing tissues

X1.5 Type I Collagen Biosynthesis and Assembly

X1.5.1 Only a small subset of the different collagen types demonstrate the capacity to self-assemble, the most prominent of these are classified as fibrillar collagens. Of the fibrillar collagens, Type I is the well-studied. It is the most abundant protein in the body and the predominant collagen of most tissues including bone, tendon, skin, ligament, cornea, and interstitial connective tissues. For this reason, Type I collagen is ideally suited for medical materials, tissue-engineered medical products, delivery of therapeutic cells/molecules, and *in-vitro* cell/tissue culture applications. The production of type I collagen by cells and its subsequent self-assembly into the fibril network of the extracellular matrix is a complex, multi-step process. While the Type I collagen molecule primary sequence is identical across tissues, posttranscriptional modifications and formation of intermolecular cross-links contribute to diversification of collagen Type I molecules, collagen-fibril networks, and therefore tissue form and function. In fact, cross-link chemistries appear to be more tissue-specific than species-specific. Type I collagen is a heterotrimer comprising two α1(I) chains and one α2(I) chain derived from the COL1A1 and COL1A2 genes, which express the component alpha chains of the collagen molecule. Component Type I collagen polypeptide (alpha) chains, termed protocollagens, are synthesized within the rough endoplasmic reticulum. These chains undergo a myriad of post-translational modifications including hydroxylation and glycosylation. It is these modifications that make production of functional collagen by standard recombinant technology so challenging. The protocollagens move to the golgi where they trimerize into a triple helical procollagen molecule consisting of two α1(I) chains and a single α2(I) chain. Procollagen is then secreted into the extracellular space by the process of exocytosis, where the amino- and carboxy-terminal propeptide ends are cleaved to form tropocollagen. Tropocollagen is the full length, functional monomer which participates in fibrillogenesis and hierarchical self-assembly. As part of the collagen self-assembly process, intermolecular cross-links are formed between collagen molecules by an enzyme-mediated process, imparting stability and strength to the fibril matrix. *In vivo*, normal physiologic cross-link formation and specification involves *(1)* intracellular hydroxylation of select lysine residues by lysyl hydroxylase; *(2)* extracellular oxidative deamination of ε-amino groups on select lysine and hydroxylysine residues within the amino- or carboxy-telopeptide regions by lysyl oxidase; *(3)* reaction of resultant lysine aldehydes or hydroxylysine aldehydes with lysine or hydroxylysine within the central helical domain of adjacent molecules to form intermediate alsol, hydroxyaldol, or ketoimine divalent cross-links; and *(4)* conversion of a subset of divalent cross-links into more stable, mature trivalent cross-links. During subsequent collagen fibril assembly, lysyl oxidase binds to and catalyzes cross-link formation between prefibrillar aggregates of staggered collagen molecules (monomers) to create covalently cross-linked oligomers (for example, at least to collagen molecules joined by a covalent cross-link). In turn, these different oligomer precursors (dimers or trimers) direct the progressive molecular packing and assembly that eventually gives rise to tissue-specific fibril architecture and

matrix function. In fact, cross-link chemistries derived from the lysine aldehyde (allysine) route predominate in soft tissues including skin, cornea and sclera. Alternatively, those derived via the hydroxylysine aldehyde (hydroxyallysine) route yield pyridinoline- and pyrrole-based cross-links, which are prominent in stiff tissues including bone, ligaments, and tendons. The number and type of collagen cross-links not only are critical determinants of tissue microstructure-mechanical properties, but also define tissue-specific collagen metabolism.

X1.6 Sources

X1.6.1 Type I collagen routinely used for basic research and TEMPs is solubilized from different source tissues (for example, skin, tendon) and animals (for example, cow, pig, human, rat). A variety of extraction and purification methods are applied including neutral salt, dilute acid solutions, or enzymes (pepsin and pronase). Human cell cultures and recombinant collagen from yeast and insect cell cultures have also been identified as potential additional sources. Most recently, synthetic chemistry procedures have been used to develop collagen-mimetic peptides which replicate the amino acid sequence repeats (Gly-X-Y or modifications thereof) found within the triple helical region of the collagen molecule. It is important to note that the purity and integrity of the collagen molecules (for example, presence of telopeptide regions, specific functional domains (integrin binding, collagenase), and intermolecular cross-links) is a critical determinant of the polymerization capacity as well as the microstructure, physical, and biological properties of the self-assembled products.

X2. SOURCING ISSUES

X2.1 Tissue for Collagen or Collagen-Containing Medical Devices

X2.1.1 Tissues that have been obtained to produce collagen or collagen-containing medical products must be carefully selected, tested, and controlled. The age of the tissue may affect the degree of crosslinking of the collagen as well as the quantity of collagen. Using tissues from the same species and age will provide better process controls for collagen production. The safety of the animal tissues is also of utmost concern. Reference to 9 CFR 113 (FDA) for animal-sourced material should be considered, as well as reference to ISO 22442. The United States Food and Drug Administration implemented new guidelines in 2007 to minimize the danger of contamination by transmissible spongioform encephalopathies (TSEs), Proposed Rule: Use of Materials Derived from Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants (Federal Register, Vol. 72, Number 8, Jan 12, 2007, pp. 1581-1619). These include:

X2.1.1.1 Elimination of risk materials including skull, brain, trigeminal ganglia, eyes, vertebral column, spinal cord, dorsal root ganglia of animals over 30 months of age, and the small intestine and tonsils of cattle of all ages.

X2.1.1.2 Any material from “downer” cattle—those that cannot walk.

X2.1.1.3 Use of advanced meat recovery methods to prevent spinal cord contamination.

X2.1.1.4 Prevention of air-injection stunning.

X2.1.2 Animals shall be subjected to ante- and post-mortem inspection and be fit for human consumption.

X2.2 Requirements for a Closed Herd

X2.2.1 Tissues obtained from animals should be obtained from well documented herds. It is highly desirable to use closed herds to maximize biosecurity. A closed herd preferably includes the following:

X2.2.1.1 Enclosed property which is chosen to minimize exposure to environmental hazards around the land including the soil and water supply.

X2.2.1.2 Animals that are born, raised, and live their entire lives in the closed herd in isolation from other cattle, sheep, pigs, deer, and elk.

X2.2.1.3 The source and lineage of each animal is documented.

X2.2.1.4 The female parent of each new animal is a member of the herd.

X2.2.1.5 Artificial insemination from registered stock is the primary breeding method.

X2.2.1.6 Animals forage on closed herd pastures. Only purchased grain or hay from selected sources may be used to supplement closed herd pasture forage.

X2.2.1.7 The animals have never been fed ruminant (animal-derived) protein and therefore have not been exposed to the primary suspected source of TSE infection.

X2.2.2 Animal parts are harvested and controlled under documented procedures to minimize contact with brain and spinal cord tissues.

X2.3 Documentation

X2.3.1 Animal tissues serving as the starting material for downstream processing or fabrication into medical products should be well documented. The animal species, the specific tissue used, and the geographical history of the animal need to be described in detail. Maintenance of the herd is important to the consistency and quality of the material. As such, information on the long term health of the herd, frequency and type of veterinarian inspections, breeding history, animal traceability, animal feed history records, absence of TSE disease, and standard vaccinations such as live modified viruses which could co-purify in the desired tissue should be documented. Animal feed has the potential for introducing adventitious agents and the animal feed composition, diet, and labeling of feed composition at distribution locations should all be documented.

X2.3.2 When the animal is sacrificed the age of the animal should be documented, as well as the USDA status of the slaughter house, measures taken to reduce the risk of contaminating non-TSE tissues with material from tissues that could

contain TSE, and the results of the pre- and/or post-mortem inspection. The test used to release the tissue for further processing or incorporation into other tissues or medical products should be disclosed as well as the Certificate of Analysis. Records of the test results for each lot of material should be maintained at the manufacturing facility and submitted in regulatory documents when appropriate. Methods for maintaining records of the source material and testing should be disclosed in regulatory submissions.

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