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Standard Guide for Tissue Engineered Medical Products (TEMPs) for Reinforcement of Tendon and Ligament Surgical Repair[1]

This standard is issued under the fixed designation F2903; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide is intended as a resource for individuals and organizations involved in the development, production, and delivery of tissue engineered medical products (TEMPs) intended to provide a mechanical (functional) reinforcement of the surgical repair of tendons and ligaments.

1.2 Surgical repair can include procedures that repair tendon to tendon, tendon to bone, tendon to muscle, ligament to ligament, and ligament to bone. In the context of this guide, a tendon is a fibrous cord or band that connects a muscle to a bone or other structure and consists of both dense collagenous fibers and rows of elongated tendon cells. In contrast, a ligament is a band or sheet of fibrous tissue connecting two or more bones, or cartilagenous structures.

1.3 Examples of TEMPs for use in reinforcement of tendon or ligament repairs include extracellular matrices (including allograft tissue, xenograft tissue, and tissue engineered extracellular matrix), polymeric matrices, membranes, or combinations of two or more of these, with or without cells and/or molecular mediators, where the function is to reinforce the surgical repair of tendon to tendon, tendon to bone, tendon to muscle, ligament to ligament, or ligament to bone.

1.4 The products may be rapidly degrading, slowly degrading, or non-degrading.

1.5 The guide is not intended to apply to TEMPs that have a primary function to induce a biological repair through cell or molecular action, although biologic activity may be a feature of the TEMPs. Examples of products or product concepts that are not included are *(a)* growth factors or cytokines applied to a biologic or synthetic scaffold, and *(b)* platelet-enriched plasma applied to or within a biologic or polymeric scaffold, where the primary function of the product is biologic.

1.6 The guide is not intended to apply to TEMPs that have a primary function to induce a chemical repair. An example of a product or product concept that would not be included would be a polymeric matrix containing reagents that glue collagenous tissues together.

1.7 The guide is not intended to apply to TEMPs that are designed to be used to achieve primary surgical repair of injured tendons and ligaments.

1.8 The guide is not intended to apply to TEMPs that are designed to replace tendons or ligaments.

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

1.10 *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:*[2]

D1004 Test Method for Tear Resistance (Graves Tear) of Plastic Film and Sheeting

D2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics

D3786 Test Method for Bursting Strength of Textile Fabrics—Diaphragm Bursting Strength Tester Method

D3787 Test Method for Bursting Strength of Textiles—Constant-Rate-of-Traverse (CRT) Ball Burst Test

D5035 Test Method for Breaking Force and Elongation of Textile Fabrics (Strip Method)

E139 Test Methods for Conducting Creep, Creep-Rupture, and Stress-Rupture Tests of Metallic Materials

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

F1978 Test Method for Measuring Abrasion Resistance of Metallic Thermal Spray Coatings by Using the Taber Abraser

[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 F04.44 on Assessment for TEMPs.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

F2150 Guide for Characterization and Testing of Biomaterial Scaffolds Used in Tissue-Engineered Medical Products
F2210 Guide for Processing Cells, Tissues, and Organs for Use in Tissue Engineered Medical Products
F2211 Classification for Tissue Engineered Medical Products (TEMPs)
F2212 Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)
F2312 Terminology Relating to Tissue Engineered Medical Products
F2383 Guide for Assessment of Adventitious Agents in Tissue Engineered Medical Products (TEMPs)
F2739 Guide for Quantitating Cell Viability Within Biomaterial Scaffolds

2.2 *ISO Documents:*[3]

ISO 10993 Biological Evaluation of Medical Devices
ISO 11135–1 Sterilization of Health Care Products—Ethylene Oxide—Part 1: Requirements for Development, Validation and Routine Control of a Sterilization Process for Medical Devices
ISO 11137–1 Sterilization of Health Care Products—Radiation—Part 1: Requirements for Development, Validation and Routine Control of a Sterilization Process for Medical Devices
ISO 22442–1 Medical Devices Utilizing Animal Tissues and their Derivatives—Part 1: Application of Risk Management

2.3 *U.S. Regulations:*[4]

21 CFR 610.12 Sterility
21 CFR 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products

2.4 *ICH Document:*[5]

Q1A(R2) Stability Testing of New Drug Substances and Products

3. Terminology

3.1 Unless provided otherwise in 3.2, terminology shall be in conformance with Terminology F2312.

3.2 *Definitions:*

3.2.1 *function, v*—the mechanism of producing the therapeutic effect of a medical product.

3.2.2 *reinforcement, n*—the process of strengthening the surgical repair of tendon or ligament.

4. Summary of Guide

4.1 It is the intent of this guide to provide a compendium of information that may be related to the functional characteristics of the TEMPs used to reinforce surgical repair of injured tendons and ligaments. TEMPs may be composed of the following individual components: biological products (for example, cells, organs, tissues, derivatives, and processed biologics), biomaterials (for example, substrates and scaffolds composed of natural and/or synthetic polymers), and active biomolecules (for example, recombinant proteins) (see Terminology F2312 for the complete definition). Examples of TEMPs are listed in Classification F2211.

4.2 Throughout this guide, the reader is referred to other documents that may provide specific information that can be applied in the manufacture and testing of TEMPs. Although many of these documents were not written with TEMPs in mind, parts are often applicable. Most of the potentially applicable position papers and guidance documents from many regions of the world can be accessed via the internet. New documents are produced continually.

4.3 The application of this guide does not guarantee clinical success of a finished product but will help to ensure consistency in the properties and characterization of a given TEMP developed for the purpose of mechanically reinforcing surgical repair of tendons and ligaments.

4.4 This guide does not suggest that all the listed tests be conducted. The decision regarding applicability or suitability of any particular test method remains the responsibility of the supplier or user of the material based on applicable regulations, characterizations, and preclinical/clinical testing.

5. Significance and Use

5.1 Injuries to tendons or ligaments are frequently treated by surgery to repair the damaged tissues and facilitate the healing process. The potential of TEMPs to enhance the outcomes (including function, pain, anatomy) of the surgical repair has been recognized.

5.2 Examples of tissues that when injured may be appropriate for repair using TEMPs: rotator cuff with a partial or full tear; Achilles tendon; Achilles tendon after harvesting for anterior cruciate ligament repair; patella tendon; patella tendon after harvesting for anterior cruciate ligament repair; quadriceps tendon; posterior cruciate ligament; medial collateral ligaments; lateral collateral ligaments; flexor tendons.

5.3 TEMPs may be used with the intent to improve the surgical outcome of tendon or ligament repair by *(a)* assuming some of the mechanical load experienced at the repair site to stabilize the surgical repair, *(b)* improving the natural biological healing process, or *(c)* a combination of these mechanisms.

5.4 TEMPs should improve clinical outcome. This may be accomplished by reducing or eliminating pain, returning function, shortening the recovery time following surgery, facilitating early mobility, improving return of strength, improving mobility, or other clinically relevant parameters.

5.5 The mechanism used by TEMPs to improve surgical repair should be understood and this conclusion should be supported by experimental results and should be supportive of the primary function of the TEMP.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.
[4] 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.
[5] 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.

5.6 TEMPs with the primary function of mechanical reinforcement may also have a secondary, biological function.

5.7 When the product is used to improve the body's natural biological repair process of tendons or ligaments, the product should allow cell attachment, migration, infiltration, extracellular matrix deposition and organization, formation of tendon or ligament repair tissue, integration with adjacent tendon, ligament or bone, tendon-bone attachment, or more than one of these actions.

5.8 When the TEMP is used to provide a mechanical support of the surgical repair of a tendon or ligament, the product may provide enhanced mechanical properties of the repaired construct immediately after the surgery. Ideally, TEMPs would have mechanical properties similar to the uninjured native tissue being repaired. After surgery, the TEMP should limit the amount of tendon/ligament separation from the bone, or separation of the fractured ends of the tendon or ligament, or reduce the number of patients that have these as outcomes of the surgery. The TEMP may allow functionality to return to the repaired tendon or ligament in a shorter time than without the use of the product.

6. Components of TEMPs for Tendon or Ligament Surgical Repair

6.1 The following describes the components (polymers, matrices, cells, bioactive molecules) that may be used to manufacture TEMPs, and if human cells or tissues are to be included, then 21 CFR 1271 may be used.

6.2 *Synthetically-Derived Matrices:*

6.2.1 *Polymer Types*—The polymers used should allow manufacture of a product that is biocompatible. The polymer may already be used in clinically marketed products, or may represent an untested material.

6.2.2 *Structure*—The material can be manufactured in sizes that can be cut to size, or in a size appropriate for surgical implantation without alteration. The shape, size (including thickness), and flexibility of the device should allow for easy positioning within the surgical site without requiring permanent surgical anatomic modification, and preferentially allow delivery to the surgical site using minimally invasive or arthroscopic surgery. The structure should allow for fixation of the product at the site such that the product will function as intended.

6.2.3 *Degradation*—The material may be rapidly degrading (for example, polyglycolic acid), slowly degrading (for example, poly L-lactic acid), or non-degrading (for example, polytetrafluoroethylene). The degradation features of the material should be selected for the particular tissue site and function of the implant, and the patient.

6.2.4 *Analyses*—Polymeric matrices in the manufactured product may be tested for chemical composition, purity and contaminants, porosity and void volume, mechanical properties, degradation rate, stability, leachable compounds, as well as residual solvents and crosslinking agents.

6.3 *Biologically-Derived Matrices:*

6.3.1 *Native Matrices*—Tissues derived from animal or human tissues may be used. These tissues are usually processed to remove cells, cell debris, and viral components. Additionally, the tissue may be crosslinked. They may contain a variety of extracellular matrix proteins, glycoproteins, carbohydrates, lipids, as well as growth factors. It is likely that most products will be predominantly composed of collagen.

6.3.2 *Other Naturally-Derived Matrices*—Other naturally-derived matrices used as a scaffold material in these TEMPs may be derived from human, animal, or other biologic sources. They are usually used in purified form, but may be used in combinations with other naturally-derived matrix components, or synthetic polymers. They may also be treated by crosslinking to provide additional mechanical properties and decreased porosity, and may be combined with other extracellular proteins.

6.3.3 *Cell Culture-Derived TEMPs*—The extracellular matrix may be generated by culture of cells (most likely with a scaffold) *in vitro*. The culture conditions will often be optimized to allow rapid deposition of extracellular matrix. If the scaffold is degrading rapidly, then it acts as a support for matrix deposition; if it is degrading slowly or not at all, it can also add mechanical properties to the construct.

6.3.4 *Analyses*—Tests may include cell content, cell membrane content, biochemical composition, growth factors and other mediators, and other biological activities. Biologically occurring matrices may also be tested as is done for synthetic polymers, for chemical composition, purity and contaminants, porosity and void volume, mechanical properties, degradation rate, and stability. Guide F2150 may be used for testing scaffolds for TEMPs, and Guide F2212 may be used when type I collagen is used as a component of the TEMP.

6.4 *Cells*—Cells may be included in the TEMP as a component of the product, secondary to the primary mechanism of mechanical reinforcement. The considerations raised in this section are general in nature, and more detailed characterization and control over manufacture and testing of a cellular component, if used, will be needed. General requirements may be found in applicable regulatory guidance documents, including Guide F2210.

6.4.1 *Cell Performance Requirements*—The cells should deposit an extracellular matrix that contributes to the TEMP's mechanical strength, or enhances biologic performance, or both. The cells should be able to proliferate to generate the appropriate number of cells required to form a TEMP, achieve appropriate characteristics during proliferation and matrix deposition, and be qualified as safe through appropriate testing. They may be autologous, allogeneic, or xenogeneic.

6.4.2 *Cell Types*—The cell types are likely to be tendon and ligament fibroblasts, but may also be other cell types or be derived from other cell sources, for example dermal fibroblasts or cells derived from a stem cell population. A careful characterization of the cell population is recommended to support development of appropriate quality assessment methods. If more than one cell population is to be used, then both the *in vitro* and *in vivo* cell-cell interactions at different cell ratios and concentrations should be understood.

6.4.3 *Analyses*—Analytical assessment of cells can include cell number, metabolism, extracellular matrix synthesis, proteomic profiling, gene expression, or other methods appropriate

to the product in question. Cell number may be determined using the Hoechst dye 33258. General metabolic activity can be assessed using the tetrazolium salt colorimetric assays MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) or other suitable methods. Guide F2739 may be used. Extracellular matrix synthesis may performed by 3H-proline labeling for collagen, and 35S-sulfate labeling for proteoglycans. Extracellular matrix synthesis may be assessed by deposition of the synthesized proteins, and can be assessed for collagen by hydroxyproline assessment, collagen types by electrophoresis, proteoglycans deposition may be assessed using the dye 1,9 dimethyl methylene blue, and different proteoglycans assessed by electrophoresis. Gene expression may be assessed using polymerase chain reaction (PCR) or quantitative PCR for tendon or ligament-specific genes including collagen I, collagen III, scleraxis, or others found to be useful for a particular product.

6.5 *Bioactive Molecules*—Bioactive molecules may be included in the TEMP as a component of the product, secondary to the primary mechanism of mechanical reinforcement.

6.5.1 *Source*—Bioactive molecules may be incorporated into the TEMPs during manufacture to elicit a specific biologic response before or after implantation, or may remain in the biologically-derived matrices as a residual of the processing and generate one or more biologic responses after implantation. Note that this may be a feature of the TEMPs covered by this standard, but the biologic activity of the molecules may not be the primary function of the TEMP. The bioactive molecules used may induce a specific biologic response associated with tissue repair, growth, or regeneration, and may include transforming growth factor betas (TGFbs), bone morphogenic proteins (BMPs), insulin-like growth factors (IGFs), platelet-derived growth factor (PDGF), fibroblast-derived growth factors (FGFs), and several others. Other molecules incorporated may be anti-inflammatory, anti-bacterial, or otherwise pharmacologically active.

6.5.2 *Analyses*—Characterization of molecules may typically involve assessment of biological activity and purity, and may be further analyzed for characteristics such as chemical composition, molecular weight, amino acid analysis, concentration, and protein sequence.

7. Sterility and Biocompatibility

7.1 The product should be delivered in a form that presents minimal risk of disease transmission. Procedures to ensure microbiological safety may include procedures to address sterility of source materials, aseptic processing, and where applicable, sterilization methods. If sterilization procedures are used, they should be selected to ensure functionality of the product after sterilization. Guide F2383, ISO 10993, ISO 11135–1, ISO 11137–1, and 21 CFR 610.12 may be used.

7.2 The product should be in a form that is biocompatible, as demonstrated by appropriate biocompatibility testing.

7.3 If the product has animal tissue as a component, then risk should be assessed, and ISO 22442–1 may be used

8. Characterization of the TEMP

8.1 The following methods can be considered for characterization of manufactured TEMPs for reinforcement of tendon and ligament repair, and should be performed on the product prior to implantation. Multiple characterization methods addressing different aspects of the TEMP should be used. Further, the following is not necessarily a comprehensive list, and other assessments may be appropriate. The precise set of evaluations will depend on characteristics specific to each individual product. Because TEMPs properties may be affected by sterilization, it is recommended that final processed and sterilized materials be studied in the assessment of material properties and characteristics.

8.2 The characterization analyses should focus on the safety and therapeutic effectiveness of the TEMP.

8.3 A comprehensive analysis is expected on the product, and a subset will be used as part of the manufacturing release criteria, again with a focus on safety and therapeutic effectiveness.

8.4 *Characterization Analyses:*

8.4.1 If the TEMP includes a polymeric matrix, recommended characterizations include polymer analysis, polymeric molecular weight, mechanical properties, and degradation rate.

8.4.2 If the TEMP contains an extracellular matrix, recommended characterizations may include histology, identification and quantitation of matrix components, and mechanical properties.

8.4.3 If the TEMP contains cells, recommended characterizations include histology, DNA content, and metabolic activity.

8.5 *Characterization Analyses:*

8.5.1 *Mechanical Properties*—Consideration for selecting the set of mechanical tests performed should include an assessment of the product indication, its surgical site, and the expected failure modes. The following mechanical tests represent exemplar methods to determine the mechanical integrity of the product and evaluate its contribution to tendon/ligament reinforcement but the list is not considered all encompassing.

8.5.1.1 *Tensile Properties*—One test method for tensile testing can be modeled after the cut strip test method as defined in Test Method D5035. Although the general testing protocols in Test Method D5035 may be applicable, additional considerations specific to TEMPs for tendon and ligament reinforcement may apply (for example, testing at physiologic temperatures in a fully hydrated environment, and use of an extensometer to measure strain particularly for products with a small gauge length). Thickness (mm) and width may be measured for each sample for use in calculation of stress. Outcomes may include breaking force (N), breaking stress (MPa), breaking strain (% elongation), elongation (mm), and modulus of elasticity (MPa).

8.5.1.2 *Suture Pull-out Strength*—There is currently no ASTM standard method, or other recognized standard method, for determination of suture pull-out strength. It is recommended that potential applications be considered when selecting a suture retention test method. Variables between methods include but are not limited to load rate, suture method or

pattern (for example, single pass suture versus mattress suture), suture type, and suture depth. There are a number of published studies that detail methods used to determine suture pull-out strength of materials (**1-5**).[6] It is recommended that the manufacturer establish a priori criteria for the necessary suture pull out strength that are based on their specific application and the expected forces present at that anatomical site.

8.5.1.3 *Burst Strength*—The burst strength may be measured using Test Method D3786 or Test Method D3787 with modifications as required.

8.5.1.4 *Tear Strength*—While not commonly used in the assessment of tendon/ligament reinforcement materials, determination of tear strength may be desired for certain TEMPs. Test Method D1004 may be used or adapted for the assessment of TEMPs.

8.5.1.5 *Creep/Hysteresis*—Creep and hysteresis characteristics of tendon and ligament TEMPs are important to assess and understand where the product is repetitively loaded. They may be assessed using Test Methods E139 and D2990, or published methods (for example, Bettinger et al (**6**)). However, these test standards are not designed for tendon or ligament testing, and modifications to the methodologies may be appropriate or necessary. The product may be exposed to constant low loads *in vivo* due to pretensioning effects in addition to repetitive loads. Therefore static creep properties should also be assessed.

8.5.1.6 *Fatigue Durability*—TEMPs may be assessed *in vitro* for their ability to resist fatigue due to repeat loading, as they will all undergo repeat loading in *in vivo*. This includes rapidly degrading TEMPs, as well as slowly degrading and non-degrading. If conducted, fatigue testing should be done using loads anticipated to be physiologic and the number of cycles that the product is likely to be expected to endure. Fatigue may also be assessed in an appropriately selected *in vivo* model, with the duration of implantation and the number of loading cycles being important parameters to consider for performance in clinical use.

8.5.1.7 *Abrasion Resistance*—The ability of TEMPs to resist abrasion may also be assessed. TEMPs may be exposed to abrasion *in vivo* during insertion into the surgical space, in particular if the product is used in an arthroscopic procedure. The product may be exposed to abrasion during its function *in vivo* through micromotion at the fixation sites, either to bone, tendon or ligament. The product may be exposed to abrasion if it is positioned to move over a bone. Test Method F1978 may be used or adapted for assessment of the abrasion resistance of TEMPs.

8.5.2 *Histology*—Histological analysis can include assessments of cell distribution and viability, matrix deposition and distribution, presence of a scaffold, and scaffold structure.

8.5.3 *Matrix Components*—Total protein, collagen, glycosaminoglycans, and water content are all common matrix assessments. Presence of particular proteins can be assessed using immunohistochemical staining, immunoassays, gel electrophoresis or biochemical analysis. Collagen is commonly assessed using histology, hydroxyproline analysis or gel electrophoresis. Glycosaminoglycans are commonly assessed using histologic analysis, gel electrophoresis, or biochemical analysis. Percent water content may be determined by measuring the material weight before and after drying.

8.5.4 *DNA*—DNA is frequently used as a measure of cellular content. Methods of detecting DNA include using Hoechst dye 33258, picogreen, or flow cytometry. In tissues that have been processed to remove cells, DNA content can be a measure of the effectiveness of cell removal.

8.5.5 *Metabolic Activity*—Metabolic activity may be used to measure general functionality of cells in TEMPs or of cells exposed to TEMPs. Methods of assessing general metabolic activity include using dyes 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assays. Specific metabolic activity can be assessed by qualitative and quantitative assessment of the synthesis of molecules. An indication of metabolism can be obtained by assessing cellular expression of mRNA of specific genes commonly expressed in tendon and ligament (for example, collagen type I, elastin, scleraxis).

8.5.6 *Degradation Rate:*

8.5.6.1 Dependent on the substrate material and processing, many of the aforementioned chemical, physical, mechanical, or biological properties may change while the scaffold is degrading, either *in vivo* or in cell culture. A thorough characterization may be made of property changes expected to occur under actual service conditions or expected conditions of use.

8.5.6.2 Degradation profiling can be conducted under specific controlled *in vitro* or *in vivo* conditions that model the intended application. When a material's degradation is primarily hydrolytic in nature, physiological conditions may be modeled *in vitro* at 37ºC under controlled pH conditions as described in Test Method F1635.

8.5.6.3 The product is expected to perform a mechanical function, and mechanical loading may affect various degradation characteristics of the TEMP. Therefore the degradation rate should be assessed under conditions that include the effect of loading (Test Method F1635).

8.5.6.4 For TEMPs that degrade through enzymatic or other *in vivo*-specific mechanisms, *in vivo* models are the preferred method of evaluation of degradation.

8.5.6.5 Assessments of TEMPs during degradation may include mechanical properties relevant to function, structure, mass, and molecular weight of polymeric materials.

8.5.6.6 Rapidly degrading TEMPs may be effectively degraded within approximately 30 days. Slowly degrading TEMPs may degrade over months or several years.

8.6 *Stability and Shelf Life*—The product, after sterilization and packaging, should be assessed for its ability to retain design compliance under anticipated storage conditions. The minimum time the design release criteria are retained by the product should allow the product to be delivered and used. Ideally, a shelf life will allow for efficient manufacturing and storage, and will minimize the opportunity for product to exceed the shelf life before being used. For some products it

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

might be appropriate to follow the Q1A(R2) ICH Guidelines Applicable to Stability Testing.

9. *In Vivo* Preclinical Tests

9.1 *In vivo* preclinical tests are used to assess biocompatibility and toxicity, and to evaluate proof of concept or therapeutic rationale. All products should be safe and demonstrate some indication of desired function.

9.2 *Biocompatibility and Safety Tests for TEMPs*—The safety and biocompatibility of the TEMP should be assessed using appropriate and preferably standardized methods.

9.3 *Animal Studies for Therapeutic Effectiveness*—Proof of concept or therapeutic effectiveness pre-clinical evaluation requires animal models that reflect the intended clinical indication by the primary mechanism of mechanical reinforcement as closely as possible. There are frequently limitations to animal models, and it should be understood what information is provided by the use of a particular model. The differences may include: *(a)* the anatomy of the animal compared to human; *(b)* acute versus chronic injury, *(c)* inaccurate replication of pathology, *(d)* recovery or rehabilitation protocol, or *(e)* age of the animal compared to the human patient.

9.3.1 Animal models can assess safety of the product when placed at the implant site, and can provide a preliminary indication of effectiveness or therapeutic rationale. Investigators should ensure that the outcome measures appropriately and accurately assess repair and function, at time points that provide useful data relevant to clinical studies.

9.3.2 For degradable products, a time point that exceeds the functional failure of the device. Interim time points should also be used to characterize the healing period. Longer time points may be needed to assess product safety issues that occur after functional failure. These safety issues could include but not be limited to long term effects of the degradation products of the TEMP on the tissue/body, potential for accelerated particulate generation after the product is not structurally intact, and the possibility that the rate/nature of product dissolution leads to poor tissue quality or remodeling. This may be performed using the same animal model that is used for therapeutic rationale studies, or a different animal model. In the cases that the product scaffold does not degrade completely, functionality of the repair should be continuous and any residual scaffold material should not interfere with function or biological outcomes.

9.3.3 For non-degradable products, a time point should be used that exceeds the expected time required for repair. Interim time points should also be used to characterize the healing period.

9.3.4 It is preferable that a large animal model be used for testing before clinical studies are performed. Appropriate species may include sheep, goat, pig, horse, and dog. Rabbits are generally regarded as a small animal and may be used in studies prior to a large animal model.

9.3.5 An appropriate animal model may not have been developed, established, or validated for assessing the TEMP for a particular therapeutic application. In that case, an animal model may need to be developed and validated for assessment of the product.

9.3.6 If a clear indication of therapeutic effectiveness is required, the studies should be statistically designed to test performance.

9.3.7 A TEMP may be designed to reinforce tendon and/or ligament at different sites (for example, rotator cuff tendons and flexor tendons), and this may require therapeutic assessment in multiple animal models. These may be the same species or different species of animals, depending on the requirement of the model.

9.3.8 Therapeutic rationale outcome measures should include mechanical properties of the tendon or ligament repair that relate to the expected function of the TEMP. For example, if the TEMP is expected to improve the tensile properties of the repair site, then tensile modulus and tensile failure loads should be assessed.

9.3.9 Therapeutic rationale outcome measures of biologic response typically include histology of the repair site and surrounding tissues. This can provide an indication of safety, and an assessment of biological processes in and adjacent to the implant site.

9.3.10 The degradation rate of a TEMP may be assessed *in vivo*, particularly for TEMPs containing an extracellular matrix where *in vitro* studies would have limited value. The preferred site for implantation is the same as the site used for clinical application, however subcutaneous sites may also provide useful information.

9.3.11 *Human Cadaveric Studies:*

9.3.11.1 TEMPs may be implanted in human cadaveric specimens to confirm feasibility of implantation and to aid in development of surgical implantation protocols.

9.3.11.2 When the product is expected to perform a mechanical function immediately upon implantation, the effect on mechanical properties of the product-cadaveric tissue construct may be tested for effect on mechanical functionality of the construct. This *in vitro* testing is not expected to precisely mimic *in vivo* function and performance after time zero, and will not provide any indication of biologic function.

10. Manufacturing of TEMPs for Tendon and Ligament Repair

10.1 TEMPs for ligament and tendon repair should be manufactured in accordance with the appropriate regulations.

11. Clinical Evaluation

11.1 The objective of the TEMP is to have a positive impact on current treatments. The products should be assessed using prospective, randomized controlled, patient-blinded clinical trial(s). The outcome measures should include (*a*) validated patient-reported general health-related quality of life instruments (for example European Quality of Life [EuroQoL, EQ-5d]), (*b*) condition/joint-specific instruments for pain and (for example American Shoulder and Elbow Shoulder Score, University of Pennsylvania Shoulder Score, UCLA Shoulder Score, Constant Shoulder Score (**7-10**)), (*c*) structural assessment by imaging to determine failed repair and/or gap formation, and (*d*) independent assessments of joint mobility, strength level, and post-operative pain levels.

12. Issues to Consider

12.1 There are many factors that can influence the development and use of this type of TEMP, and factors described below identify some of these.

12.1.1 Preclinical issues may include *(a)* identifying appropriate design criteria, *(b)* establishing useful release criteria, *(c)* development and use of relevant animal models.

12.1.2 Manufacturing issues may include *(a)* identification of release specifications to ensure uniform product, *(b)* establishing useful shelf life, *(c)* convenient storage conditions.

12.1.3 Clinical issues may include *(a)* identification of optimal surgical application techniques, *(b)* identification of appropriate patient populations, *(c)* demonstrating effectiveness or efficacy in specific patient populations using appropriate clinical studies.

12.1.4 Regulatory issues may include identification of primary function.

13. Keywords

13.1 Achilles; bone anchors; ligament; quadriceps; reinforcement; repair; rotator cuff; sutures; tendon

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