Global Expertise
Trusted Standards
Improved Health

U.S. Pharmacopeial Convention

Cell and Tissue-based Therapies: Pharmacopeial Perspective

Dr. Fouad Atouf, Director
Biologics and Biotechnology

Cell and Tissue-based Therapies at USP

- Regulatory Pathways in the US
- Tissue-based products
  - Human or animal origin
  - Decellularized vs. living cells containing products
  - How do we define scaffolds?
    - Level of residual DNA
    - Histological analysis
    - Collagen identification
- Cell-based therapies
  - Complex and multicomponent manufacturing= complex products
  - Unique raw materials that may or may not end up in final products

Regulatory Pathways – Complex Landscape

- "361" Human cells, tissues or cellular or tissue based products (HCT/Ps)
  - No pre-market review; follow 21CFR Part 1271
  - Compliance with regulations relies on inspection
- "351" Biological Products
  - 21CFR Part 1271 and other regulations
  - Pre-market review and approval (BLA)—must show safety, purity, potency
- Medical Devices
  - 21CFR Part 1271 and other regulations
  - Pre-market review and clearance/approval (510(k), PMA)—must show safety and efficacy

Regulatory Pathways (Cont'd)

- 21 CFR Part 1271
  - Establishment Registration and Product Listing
  - Donor Eligibility
  - Current Good Tissue Practice
- Section 361 of Public Health Service (PHS) Act—prevent the introduction, transmission, or spread of communicable disease
- Section 351 of t PHS Act— Pre-market review and approval (BLA)—must show safety, purity, potency

Tissue-based products and USP

- Increasing number of submissions of monographs for tissues and tissue-based products
  - Growth of this segment of the industry
  - A USP-NF monograph is effectively required for reimbursement by Center of Medicaid and Medicare Services (CMS)
- Challenges with monograph submissions
  - Naming and definition of products
  - Lack of testing information on some products (e.g. 361 products)
- Broad scope of products
  - processed vs. minimally processed tissues
  - Human or animal origin
  - Regulated under different pathways

Tissue Monograph vs. Drug/Biologic Monograph

Drug/Biologic Monograph	Draft Tissue Monograph

Monograph Title/Name
▸ Definition
▸ Identification
▸ Potency
▸ Purity Tests
▸ Specific Tests
▸ Sterility, Endotoxins
▸ Additional Requirements:
  ▸ Labeling,
  ▸ Packaging and Storage
	Monograph Title/Name
	▸ Definition
	▸ Test 1
	▸ Test 2
	▸ Test 3
	▸ Sterility, Endotoxins
	▸ Additional Requirements:
	  ▸ Labeling,
	  ▸ Packaging and Storage

NDA, ANDA, BLA	**BLA, GTPs, 510K, PMA**

Naming for Scaffold, Construct and Tissue Products

- **Naming Rationale:**
  - Captures essential product's information for monograph title
- **Naming Approach:**
  - [Primary Characteristic] [Species] [Origin] [Other Characteristics] [Other Modifiers]
- **Application of naming approach:**
  - Monographs published in PF since 2010
  - Official monographs are revised
  - Monographs under development

Naming Scheme- Revision

- [Primary Characteristic]: The primary characteristic is the term that will be used to index a specific animal/human tissue product in the USP-NF. The intent is to group similar products together. To date, three (3) primary characteristics have been identified:
  - **Scaffold** - indicates acellular/decellularized natural or synthetic substrate used alone or as part of a construct
  - **Construct** - indicates a product that comprises cells seeded or cultured on a scaffold.
  - **Tissue** – human source material which may be fresh, frozen, dehydrated, chemically preserved, but not intentionally decellularized.

- [Other Characteristics]: "…For human tissue products, if the donor and recipient are the same, the parameter "autograft" may be included. For human tissue products in which the donor and recipient are different, the parameter "allograft" may be included."

Tissue Products Naming- Examples

Original Title/Name (Official or proposed)	Proprietary Name (Manufacturer)	Proposed Name (New Scheme)
Cryopreserved Human Fibroblast-Derived Dermal Substitute	Dermagraft (Advanced BioHealing, Inc.)	Construct Human Fibroblasts in polyglactin scaffold
Human Fibroblast-Derived Temporary Skin Substitute	TransCyte (Advanced BioHealing, Inc.)	Construct Human Fibroblasts in bilayer synthetic scaffold
Graftskin	Apligraf (Organogenesis, Inc.)	Construct Human Keratinocytes and Fibroblasts in bovine collagen scaffold
Small Intestinal Submucosa Wound Matrix	Oasis® Wound Matrix; Surgisis® Soft Tissue Graft (Cook)	Scaffold Porcine Small intestinal submucosa
Bovine Acellular Dermal Matrix	PriMatrix (TEI)	Scaffold Bovine Dermis
Human Acellular Dermal Matrix	Graftjacket (Lifecell, Wright)	Scaffold Human Dermis
Under Development		
Decellularized Human Peripheral Nerve	AVANCE™ (Axogen)	Scaffold Human Peripheral Nerve
Porcine Acellular Dermal Tissue Matrix	Strattice (LifeCell)	Scaffold Porcine Dermis
Collagen Nerve Conduit	NeuroMend™, NeuroMatrix™ (Collagen Matrix Inc)	Scaffold Bovine Tendon **Collagen***
Collagen Nerve Guide	NeuraGen, Neurarap (Integra)	
Crosslinked Collagen-GAG Scaffold	TenoGlide™, Integra™ (Integra)	Scaffold Bovine Tendon Collagen and Shark Chondroitin Sulfate
Scaffold Human Amniotic Membrane Dehydrated Wound Covering	*AmnioFix*™, EpiFix™ (MiMedx Group)	Tissue Human Amniotic Membrane
Cellular Repair Matrix	Grafix®(Osiris)	Tissue Human Amniotic Membrane Cryopreserved

Monograph Definition- Example 1

- **Scaffold Human Dermis** is derived from donated allograft human dermis that is processed to remove cells and freeze-dried to remove moisture while preserving biologic components and structure of the dermal matrix. It is biocompatible and supports remodeling by the recipient's own tissue. The matrix is composed of native human dermal architecture, consisting of collagen (mainly collagen Type I, with additional components of collagen Types III and IV), chondroitin sulfate and hyaluronic acid glycosaminoglycans, and elastin. The product can be provided in a sheet or powder form.
- The donated human skin is processed in a manner that removes all cellular components, including the epidermal layer and dermal cells. The resulting product is then rendered into a particulate form (microsized), with a mean particle size of less than 100 µm, by processing with a freezer mill. Scaffold Human Dermis does not contain intact cells, cell nuclei, or chemically induced crosslinks. Human skin used to produce Scaffold Human Dermis is obtained from sources that have passed applicable donor eligibility requirements for relevant communicable diseases. Scaffold Human Dermis is manufactured using sterile solutions and equipment under aseptic conditions. The final product is inspected and tested to ensure that the product meets specifications.

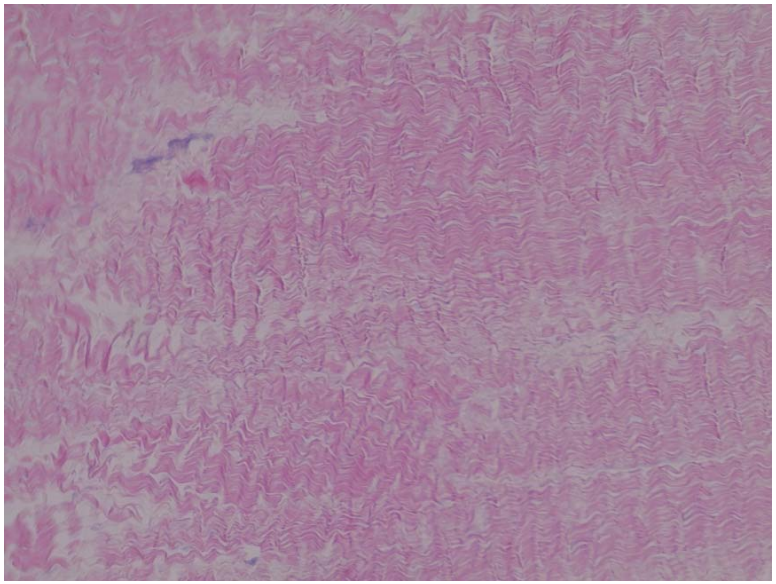
Monograph Definition- Example 2

- **Scaffold Human Peripheral Nerve** is Scaffold flexible non-permanent tubular implant designed to provide a conduit for axonal growth across a nerve gap. It is derived from donated allograft human peripheral nerve tissue. The final product is frozen to preserve the biological components and structure. It is biocompatible and supports remodeling by the recipient's own tissue. The Scaffold Human Peripheral Nerve is comprised of the native human peripheral nerve architecture consisting of the intact three dimensional extracellular matrix-ECM (endoneurium, perineurium and epineurium) that is composed primarily of laminin, Type IV collagen, proteoglycans and other components.
- The donated human peripheral nerve is processed in a series of proprietary phosphate buffer solutions (pH 7.4), detergents and enzyme washes to remove cells and cellular debris such as fat, blood, axons and myelin from the tissue while leaving the inherent and relevant structural characteristics (components, handling, tubular form) of the extracellular matrix (ECM) intact.

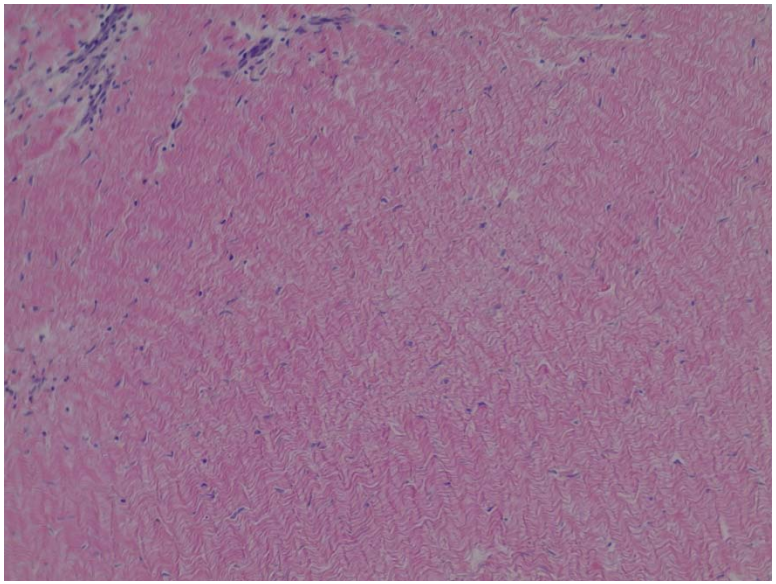
Evaluation of the Decellularization Process

- H&E staining, look for the absence of cells or cellular debris
- Absence of intact cells and/or cell debris

Product Sample



Control, Untreated Tissue

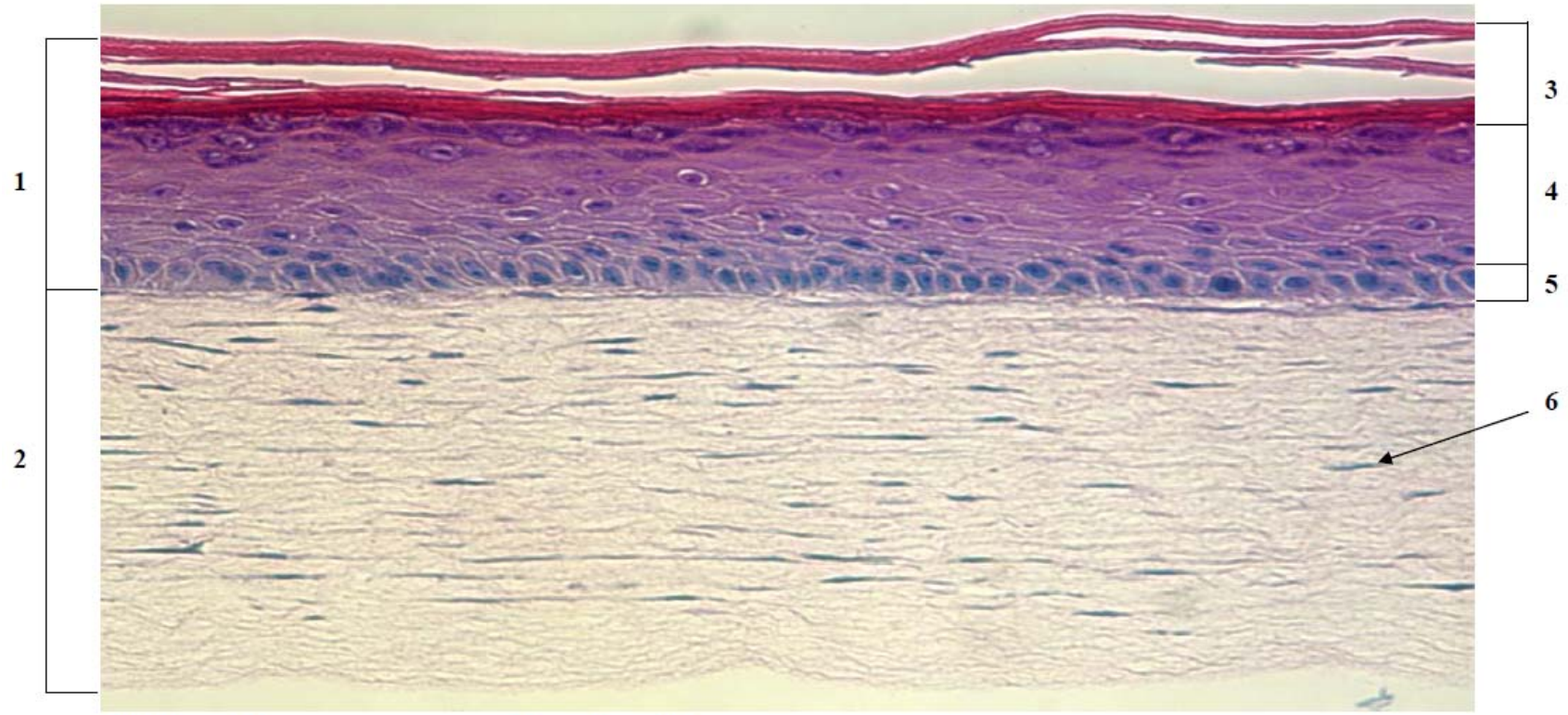


Graftskin

- Living, bi-layered skin substitute derived from neonatal foreskins
- Upper epidermal layer-human keratinocytes
- Inner dermal layer-human fibroblasts in bovine collagen lattice
- Cell banks generated and screened for microbial and viral contaminants
- Characterization tests
  - Histology
  - Gene expression profile
  - Barrier integrity
  - Metabolic activity
- Scope of histology testing
  - Epidermal coverage
  - Epidermal development
  - Keratinocyte aspect
  - Fibroblast density
  - Matrix aspect

Graftskin: Authentic Visual References (AVR): Example of Passing Unit

PASSING MORPHOLOGY SAMPLE

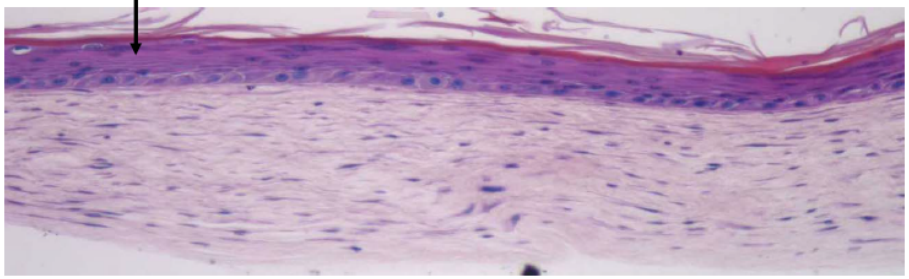


1.Epidermal Layer
2.Dermal Layer
3.Cornified Layer
4.Suprabasal Layer
5.Basal Layer
6.Fibroblast

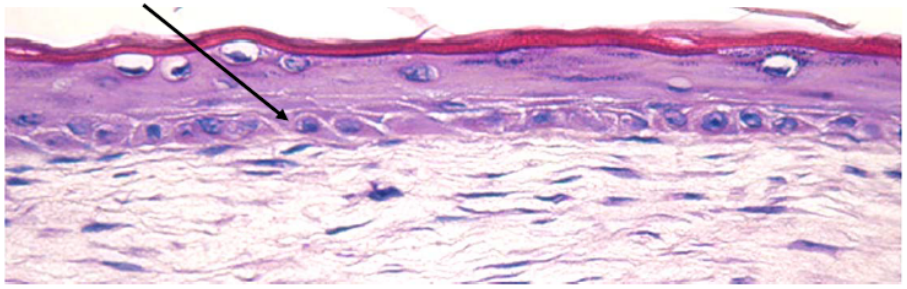
USP AVR Standards are used to visually aid the analyst in determining whether the product under analysis passes the *Histological evaluation* test.

Graftskin: AVRs for Failing Samples

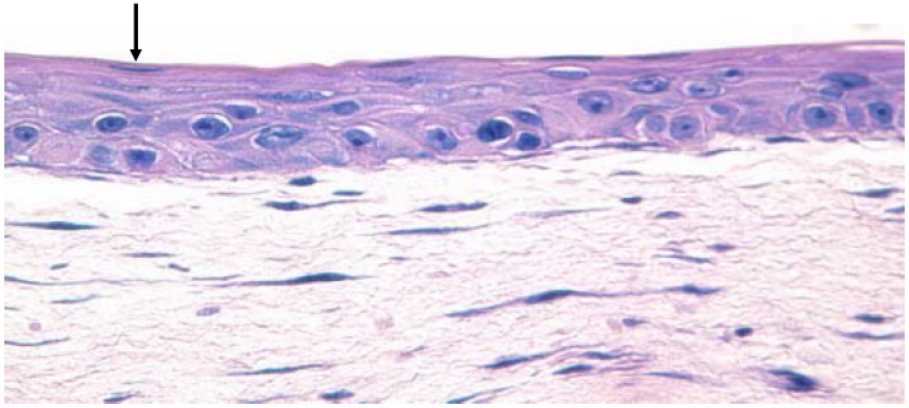
2. Inadequate epidermal development



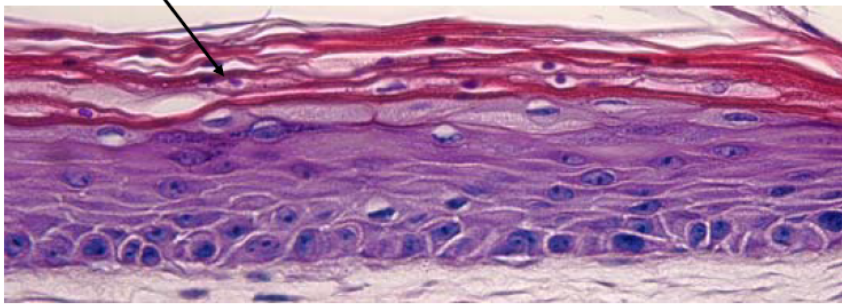
3. Squamous basals



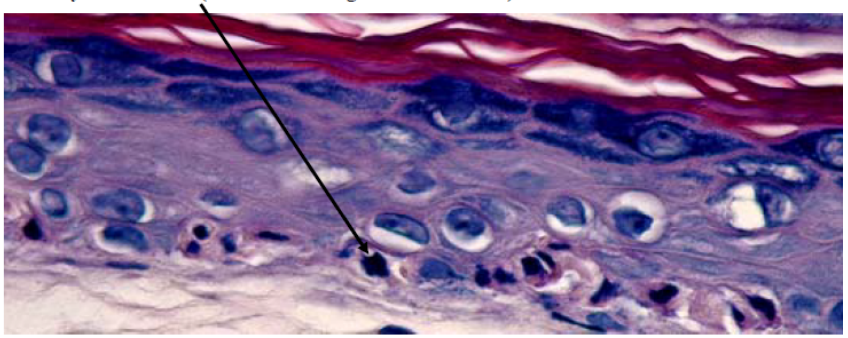
4. No cornified layer



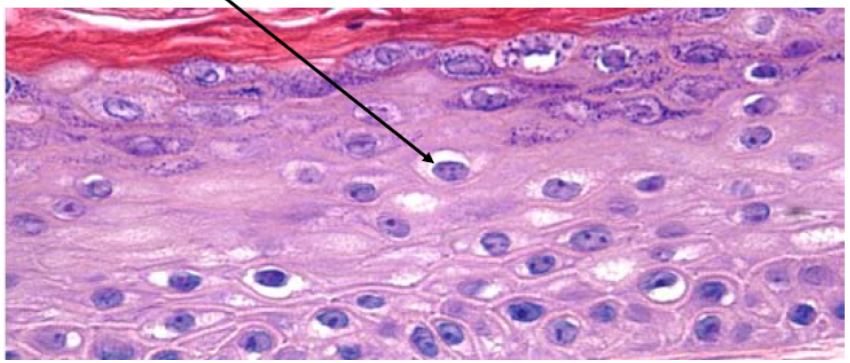
5. Parakeratosis (persistent nuclei in the cornified layer)



6. Pyknotic nuclei (nuclear shrinkage of necrotic cells)



7. Suprabasal "halos"



Amniotic Membrane-based Products – Example 3 for Candidates for Monograph development

Marketed Product	A	B1	B2	C	D
Tissue Origin	Human placental tissue	Amniotic membrane and amniotic fluid components	Human amnion allograft	Amniotic membranes of human placental tissue	Human amnion and umbilical cord
Amnion or Chorion	Amnion and chorion placental tissue	Amnion	Amnion	Amnion/ Chorion/ epithelial cells	Amnion and umbilical cord
Cryopreserved	Yes	Yes DMSO	No	No	Yes
Tissue Stabilization	Not described	No	Yes Glutaralde-hyde	Yes, Mechanical Dehydration	Yes, Cryopreservation
Process to remove cells	No	Partly to Completely decellularized, non-viable cells	Partly to Completely decellularize d non-viable cells	Yes, washes with saline and proprietary reagents (Chemical)	Yes, washed with PBS

Amniotic Membrane-based Products – Candidates for Monograph development, Cont'd.

Product	A	B1	B2	C	D
Test for Cell Viability	Yes, Trypan Blue	No	No	No	No
Processing Steps	Not described in draft submitted.	Separated from placenta, morselized using cryogenic milling Fluid-processed to remove urine	Separated from placenta using NaCl rinses, glutaraldehyde treatment and ethanol treatment	dehydration Yes, washes to remove cells, cellular debris	Thawed, aseptically processed, blunt dissection for amniotic tissue layer, rinsed and cut into sheets for cryopreserva-tion.
Product Release Tests	Test for presence EGF (ELISA), Cell Viability, FACS to identify MSC, Immunogenicity testing (MLR)	Donor idenification/testing Serological testing (bioburden) Endotoxin testing	Donor idenification/testing Serological testing (bioburden) Endotoxin testing	H&E Stain Stain positive for: Collagen Types IV, V and VII, EGF, FGF, laminin	H&E Stain, look for presence of ECM collagen and have positive staining throughout epithelium.

Points to consider when defining Tissue Products- lessons from monograph development

- Elements of definitions:
  - Naming approach?
  - What are the relevant elements for defining these products?
  - Role of quality tests for identification of major components (e.g. collagen content) in defining substances

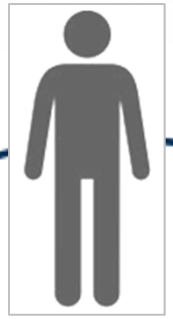
- Scaffold derived from animal sources
  - Species identification
  - And what is the substance in these products? Tissue source?

- Surgical mesh/scaffold vs. regenerative medicine product
  - Do we take into account how the product function?

Cell and Tissue-based Therapies at USP

- Regulatory Pathways in the US and implications on pharmacopeial standards
- Tissue-based products
  - Human or animal origin
  - Decellularized vs. living cells containing products
  - How do we define scaffolds?
    - Level of residual DNA
    - Histological analysis
    - Collagen identification
- Cell-based therapies
  - Complex and multicomponent manufacturing= complex products
  - Unique raw materials that may or may not end up in final products

Cell Therapy Products: Multi-Components Manufacturing Process



Release
Tests/QC
Identity , Purity, Potency, Safety

Formulation Media
Excipients
Container closure
Testing for defined media, suitable excipients
Leachables/ extractables

Procurement , storage
Collection & storage in media/buffer
Viral Testing
Donor screening
Stability

Starting Material: Tissue/Cells

Cell Therapy Product

Initial tissue/cell processing
Process qualification, Validation of enzyme removal, Residual testing stability

Storage or Cryopreservation
Qualification of storage media

Culture with media, growth factors, Serum
Qualification of all components, Functionality, Suitability for use

Cell Culture and Expansion

Cell Selection

Purification/Assembly
Antibodies, Devices
Quality attributes
Suitability for use

Cell passaging and/or differentiation (proteolytic enzyme treatment)
Component Qualification, Validation of removal, Residual testing

Cell Therapies: Examples of approved or in development (advanced stage) products

Approved	Company
Dermagraft	Shire
Osteocel	NuVasive
PureGen	Alphatec Spine
Cupistem	Anterogen
ReCell	Avita Medical
BioDfactor	BioDlogics
BioDfence	BioDlogics
Provenge	Dendron
LaViv	Fibrocell
Carticel	Genzyme
Epicel	Genzyme
Maci	Genzyme
Cartistem	Medipost
Apligraf	Organogenesis
Grafix	Osiris Therapeutics
Heartcelligram	Pharmicell
ChondroCelect	TiGenix
Gintuit	Organogenesis

In Development Products	Company
Ixmyelocel-T	Aastrom
Prochymal	Osiris
ABH001	Shire
Cx601	Tigenix
Mesenchymal Precursor Cells	Mesoblast
StemEx	GamidaCell
Placental Expanded Cells	Pluristem
Lenti-D	Bluebirdbio
Chondrogen	Osiris
Multistem	Athersys
Endometrial Regenerative Cell	Medistem
AMR-001	Amorcyte

Cell populations, Key Element of Definitions

Company	Product	Cell type
Genzyme, a Sanofi company	Epicel	Keratinocytes
Genzyme, a Sanofi company	MACI	Chondrocytes
Medipost	Cartistem	Chondrocytes
NuVasive	Osteocel	Osteogenic cells and osteoprogenitor cells
Organogenesis	Apligraf	Keratinocytes and Fibroblasts
Organogenesis	GINTUIT	Keratinocytes and Fibroblasts
Orthofix	Trinity / Trinity Evolution	Osteogenic cells and osteoprogenitor cells
Osiris Therapeutics	Grafix	Keratinocytes and Fibroblasts?
Pharmicell	Heartcelligram-AMI	MSC
TiGenix	ChondroCelect	Chondrocytes
Zimmer and ISTO Technologies	DeNovo NT	Chondrocytes

Gintuit: cellular and non-cellular components

"GINTUIT is indicated for topical application to a surgically created vascular wound bed in the treatment of mucogingival conditions in adults.

Cellular components:

- (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen) - neonatal foreskin derived cells

.

Other components (may or may not be in finished product)

Process enzymes: trypsin, and collagenase.
Keratinocyte cultured on bovine type I collagen.
Fibroblasts cultured in DMEM, 10%FCS.
Keratinocytes: MSBM and calcium–free DMEM (3:1) and Ham's F12.
Dermal collagen lattices: DMEM, insulin (5µg/ml), triiodothyronine (20pM), transferrin(5µg/ml) and sodium ascorbate (50µg/ml).
Epidermal development : modified MSBM supplemented with serine, choline cloride, ethanolamine and o-phosphorylethanolamine.
Other cell culture supllment: L-glutamine, gentamicin, EGF, hydrochortisone, insulin, adenine, BSA, linoleic acid-BSA complex.

Dermagraft: cellular and non-cellular components

Cellular components:

- This is a construct that contain living cells seeded into the scaffold. The living monolayer of skin substitute is derived from neonatal foreskins. Human fibroblasts are seeded onto a mesh scaffold composed of polyglactin. Fibroblasts proliferate to fill the interstices of the scaffold

Other components (may or may not be in finished product)

Polyglactin
Media, FBSHS
Hydrocortisone
Antibiotics
Trypsin
DMSO
Glycerol

Insulin, Growth Hormone, CSF, EPO, EGF

Raw/Ancillary Materials
Where these can be captured?

- Raw materials may or may not remain in the final therapeutic product as active substances or as excipients
- Ancillary products may exert an effect on a therapeutic substance (e.g. a cytokine may activate a population of cells), but they are not intended to be in final formulation
- Some components are more critical than others!
  - A critical material will come in contact with cells with a potential to alter either the growth characteristics of the cells or the ability of the cell culture to meet lot release specifications.

U.S. Pharmacopeial Convention

Global Expertise
Trusted Standards
Improved Health

Questions

Global Expertise
Trusted Standards
Improved Health

www.usp.org

Thank You