



# PURION® Processed Dehydrated Human Amnion/Chorion Membrane Allografts

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## INTRODUCTION

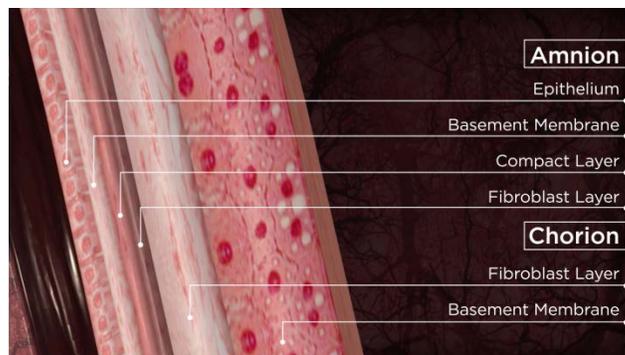
Human amniotic membrane allografts have been used for a variety of reconstructive surgical procedures since the early 1900s. Interest in utilization of amniotic membrane waned in the early 1980's as a result of communicable diseases such as HIV/AIDS, Hepatitis, etc. In the late 1990's and early 2000's amnion re-appeared in cryopreserved form for the treatment of ophthalmic wounds. In 2007, the use of the amniotic membrane as an allograft accelerated because Surgical Biologics, a MiMedx Group Company, developed the PURION® Process, which among other things allowed the tissue to be dehydrated and sterilized. This produced an easy to use graft with room temperature storage and a five-year shelf life. To date, 70,000 allografts have been distributed for human implantation in various surgical applications. The PURION Process allografts were first used in ophthalmic surgery where there have been over 20,000 implants to date without any adverse events associated with the tissue. Most recently, amniotic membrane has been utilized as a potent facilitator of wound healing in various fields within medicine including lower extremity ulcers but also eye surgery, burns, gynecologic surgery, orthopedics, and a variety of other applications.<sup>1,2,3,4,5,6,7</sup>

## AMNIOTIC MEMBRANE ALLOGRAFT DESCRIPTION

Human amniotic membrane is comprised of the innermost layer of the placenta and lines the amniotic cavity. The membrane is composed of multiple layers which include a single layer of epithelial cells, a basement membrane and an avascular connective tissue matrix. The tissues of the placenta present a very complex interrelationship of materials that possess numerous physiologic characteristics, which can in turn change in importance with the appropriate stage of gestation. During pregnancy, the placenta permits the passage of nutrients, metabolites and metabolic gases, and provides physical and immunological protection to the developing fetus. In addition, it produces a variety of steroids and important metabolic hormones.<sup>8</sup>

Amniotic membrane is a unique material and its composition contains collagen types IV, V, and VII. Amniotic membrane is composed of structural extracellular matrix (ECM) which also contains specialized proteins fibronectin, laminins, proteoglycans and glycosaminoglycans. In addition, amniotic membrane contains essential, active, healing growth factors such as epidermal growth factor (EGF), transforming growth factor beta (TGF- $\beta$ ), fibroblast growth factor (FGF), and platelet derived growth factor (PDGF).<sup>8</sup> Amniotic tissues have shown little to no HLA-A, B, C antigens and  $\beta$ 2 microglobulin.<sup>3</sup>

**Figure 1: Layers within the Amniotic Membrane**



Surgical Biologics was the first to perfect the process of safely separating placental tissue, cleaning, and reassembling the amnion and chorion layers, and embossing the graft. Patents have been filed on a number of these embodiments and processes. This intellectual property began development in 2006, and has been improved over the last several years.

In addition, the dehydrated amnion/chorion membrane allograft can also be micronized which allows it to be administered as a topical powder or mixed with saline to create an injectable solution or a topical gel.

Use of amniotic membrane has recently increased clinically as an allograft material for chronic and acute wound care management, for scar tissue reduction, as a barrier membrane, and as a soft tissue regeneration graft.

### THE PURION PROCESS

The PURION Process is a patent pending process that safely and gently separates placental tissues, cleans and reassembles various layers, and then dehydrates this tissue in a way that preserves the key elements associated with healing. The sterilized tissue is packaged and stored at room temperature and has a 5-year shelf life. The tissue may be delivered in a dried sheet configuration using an on-lay surgical or clinical technique. Dehydrated amnion/chorion membrane allograft can also be micronized to create a powder configuration which can be administered as a topical powder or can be mixed with a saline to create an injectable solution.

PURION processed dehydrated amniotic tissue is regulated under Section 361 of the Public Health Service Act by the United States Food and Drug Administration (FDA). PURION processed dehydrated allografts are minimally manipulated and intended for homologous use.

**Table 1: FDA Regulatory Classifications for Tissue and Cell Based Products**

Regulatory Pathway	Material	Requirements
361 HCT/Ps (Human Cell Tissue/ Products)	Human Tissue (Allograft)	Minimally manipulated, intended for homologous use. No clearance or pre-market approval needed. Requires FDA Good Tissue Practices (GTP)
510(k) Clearance	Medical Device (Example: decellularized human dermis, xenografts, collagen dressings, bone void filler)	Requires FDA Substantial Equivalence, shorter submission and less required verses PMA. Based on predicate device. Requires FDA Current Good Manufacturing Practice (cGMP)
Premarket Approval (PMA)	Medical Device (Example: human living skin substitutes, bone substitute)	Requires extensive FDA Pre-Market approval process, including comprehensive clinical trials. Requires FDA Current Good Manufacturing Practice (cGMP)
New Drug Application (NDA)	Drug (Example: living stem cells non-autologous, or second degree relative)	Requires extensive FDA Pre-Market approval process, including comprehensive clinical trials. Requires FDA Current Good Manufacturing Practice (cGMP)

## TISSUE CONFIGURATIONS

**Membrane allograft:** This configuration is delivered as a dry sheet embossed with the monogram “SB”, which enables the user to identify the correct orientation prior to the application of the graft. The allograft may be used as presented on opening the package, or alternatively by moistening prior to use with normal saline solution. The tissue is for single use and single patient application.



**Micronized dehydrated amnion/chorion membrane allograft:** This configuration presents as a powder that can be mixed with sterile saline to create a liquid (micro-grafts are suspended in the saline) for injection into or adjacent to the targeted damaged soft tissue. The tissue is for single use (multiple injections sites) and single patient application.



All PURION processed amniotic membrane allografts are dehydrated and packaged aseptically into an inner peel pouch and sealed with an outer peel pouch system within a clean room environment. The outer peel pouch is NOT considered sterile. The inner pouch, which contains the graft, is considered sterile unless damaged or compromised.

## SOURCE OF AMNIOTIC TISSUE



Eligible amnion donors are living mothers that have delivered a live birth through Cesarean section. All tissues are recovered under full informed consent of the donor. Each donor must then answer a series of questions to ensure the donor has not engaged in behaviors to place her at an increased risk for the transmission of infectious diseases and to ensure the donor has not shown signs or symptoms of illnesses. Donor procurement and screening processes were developed to prevent the transmission of infectious diseases from donors to recipients of the material. These processes follow the FDA regulations and American Association of Tissue Banks (AATB) standards.

The donor screening process also includes the completion of a physical examination of the donor mother by the attending physician. This exam is performed to ensure the donor mother does not exhibit any physical evidence of high risk factors, such as infectious disease. A physical exam is performed and documented according to established procedures. Donor testing is performed on a blood specimen from each donor which meets the requirements of the FDA and the AATB. In Table 2 see a list of communicable disease tests performed on amniotic tissue allografts before undergoing the PURION Process.

In addition to donor testing and screening, an additional element of testing includes performance of microbial cultures from the tissue at the time of recovery to detect the presence of bacterial contaminants. All test results for serology, bacteriology and infectious diseases are reviewed prior to the release of the donor tissue. Only tissue from donors with acceptable test results according to the standards of the company as well as the standards of all state and federal regulatory bodies are released.

## TISSUE SAFETY

All serological testing for bacterial, viral and infectious diseases are performed on blood specimens for each donor and meets the requirements of the FDA regulations and AATB standards, specifically section D4.354, *Required Infectious Disease Tests*, of the Standards for Tissue Banking. The full testing panel includes:

**Table 2: Infectious Diseases Screened for in Tissue Transplantation**

HIV-1 & HIV-2 Antibody	Hepatitis B Core Antibody	Hepatitis C Virus (Nucleic Acid Test)
HIV Type 1 (Nucleic Acid Test)	Hepatitis B Surface Antigen	CMV Total Antibody
HTLV-1 & HTLV-2 Antibody	Hepatitis C Antibody	Syphilis (Serological Test)

To verify the ability of the PURION Process to provide microbial reduction, a disinfection validation was originally performed by an ISO certified laboratory. Amnion tissue samples were contaminated with 5% bovine serum containing *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Candida albicans*, and *Bacillus subtilis*. Bioburden extractions were performed to determine the number of viable organisms present on one positive control for each organism. Amnion tissue samples contaminated with the organisms were disinfected through the PURION Process, and another bioburden assay was performed to determine the bioload reduction of each amnion sample. Results obtained during the decontamination process validation testing were all below the established acceptance criteria. Therefore, test results demonstrate that the decontamination process can be considered an effective supplement to the standard terminal sterilization process.

## TISSUE PROCESSING

Surgical Biologics has created the proprietary and patent pending PURION Process for amniotic membrane preparation. The proprietary process has been specifically designed to deliver a safe, effective and minimally manipulated allograft tissue. All placental tissues are recovered under sterile conditions from patients who have been screened for underlying infectious disease. At no time are the recovered tissues ever subjected to ultra-low or ultra-high temperatures during quarantine, processing, or storage. No chemicals are used in the PURION Process which might result in chemical cross-linking or decellularization.

Tissue stabilization is accomplished by a dehydration step. Using this methodology, the delicate collagen matrix remains structurally intact. During the dehydration process, a patent pending orientation embossment (“SB”) is incorporated onto each graft in the PURION Process. This also helps to ensure proper graft placement during surgical implantation. After processing of the tissue is complete the tissue graft provides no living cells to the patient. Finally, an inner and outer peel pouch system is used for packaging which is then sterilized with Electron-beam irradiation which addresses any potential remaining bioburden. The final implantable PURION processed dehydrated human amnion/chorion allografts can be stored at room temperature (0° to 38°C/32°F to 100°F) for up to five-years. The final product is inspected prior to release to confirm the manufacturing process has been completed according to procedure.



The PURION Process also employs some unique steps that are unlike some traditional allograft tissues processes:

**Step 1:** The placenta is recovered under sterile conditions within an operating room environment immediately following child delivery. The placenta is then placed into a sterile transfer container with a proprietary hypertonic solution. At no time is the recovered tissue subjected to ultra-low temperatures during quarantine storage.

**Step 2:** The placental tissues are transferred from quarantine storage to a Class 100 biological safety cabinet. The membrane layers go through a series of proprietary gentle washes and sterile water rinses to remove unwanted biological material.

**Step 3:** Tissue stabilization is accomplished by dehydration. Using this methodology, the delicate extracellular matrix (ECM) (collagen, elastin, etc) and cells remain structurally intact. In comparison, lyophilization or freeze drying would introduce ice crystal formation that could cause damage to cellular architecture. During the dehydration process, an orientation embossment is incorporated onto each graft. This ensures proper graft placement during surgical implantation. The embossment process does not change the biologic or structural integrity of the graft.



**Step 4:** An inner and outer peel pouch system is used for packaging both membrane and micronized versions of the dehydrated human amnion/chorion allografts. The outer pouch is considered non-sterile with the inner being sterile. All packaged tissues are then dosed with Electron-Beam irradiation which addresses any potential remaining bio-burden.

## TISSUE STERILITY

Sterilization validation was conducted according to the standards established by the International Organization for Standardization (ISO) and the Association for the Advancement of Medical Instrumentation (AAMI) for the substantiation of a chosen sterilization dose to attain a sterility assurance level of  $10^{-6}$  substantiating the ability of the selected radiation dose to produce a sterile product.

Specific insight into donor suitability determination is provided by the Surgical Biologics medical director, a pathologist certified by the American Board of Pathology in the areas of clinical pathology and blood banking/transfusion medicine.

The infectious disease test results, together with the consent document, donor medical history and behavioral risk assessment, physical examination, available records, and along with tissue procurement test results are evaluated by a medical director and must be determined to be sufficient to indicate that the donor suitability criteria have been met before the release of donor tissue for transplant. Strict guidelines are maintained for each step of the tissue processing. All recovery and processing operations, including donor eligibility determination, are performed in strict adherence to FDA Regulations and the standards that have been established by the AATB as well as applicable state and local regulatory requirements.

## SCIENTIFIC EVIDENCE

Laboratory tests have been conducted to provide proof that PURION processed human amniotic tissue allografts do contain active growth factors to enhance soft tissue wound healing.

### Hypothesis: Do PURION processed dehydrated amniotic tissue allograft membranes deliver essential growth factors and cytokines?

In vitro tests were conducted on amniotic membrane to confirm the presence of essential soft tissue healing growth factors such as PDGF AA & BB, bFGF, TGF-b1, and EGF.<sup>9</sup> Of 13 cytokines present in natural Amnion/Chorion, all were found in the PURION processed tissue.

Cytokines Present in Amnion/ Chorion Tissue													
Cytokines	PDGF-AA	PDGF-BB	TGF-a	TGF-b	bFGF	EGF	VEGF	IL-10	IL-4	PIGF	TIMP-1	TIMP-2	TIMP-4
<b>Natural</b>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
<b>Purion processed</b>	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Cytokine test completed by a 3<sup>rd</sup> party laboratory: Data on file

### Hypothesis: Do micronized PURION processed dehydrated amniotic tissue allografts deliver essential growth factors over time?

PURION processed dehydrated human amnion/chorion was micronized into a powder like material. Neutral saline was used to rehydrate the micronized amniotic tissue and then centrifuged for ten minutes to create a supernatant liquid and solid material pellet. The supernatant liquid was tested for PDGF-AA, PDGF-BB, bFGF, TGF-b1 and FGF using ELISA protocols. The results showed that less than 50% of the TGF-b, bFGF, PDGF-AA, and PDGF-BB were found to be released into solution, except for EGF. EGF released 63% in to solution.

As the remaining solid is absorbed over time, it has been proposed that the growth factors still bound into the extracellular matrix are released into the surrounding tissue, providing a continual release of growth factors during the tissue regeneration process.<sup>9</sup>

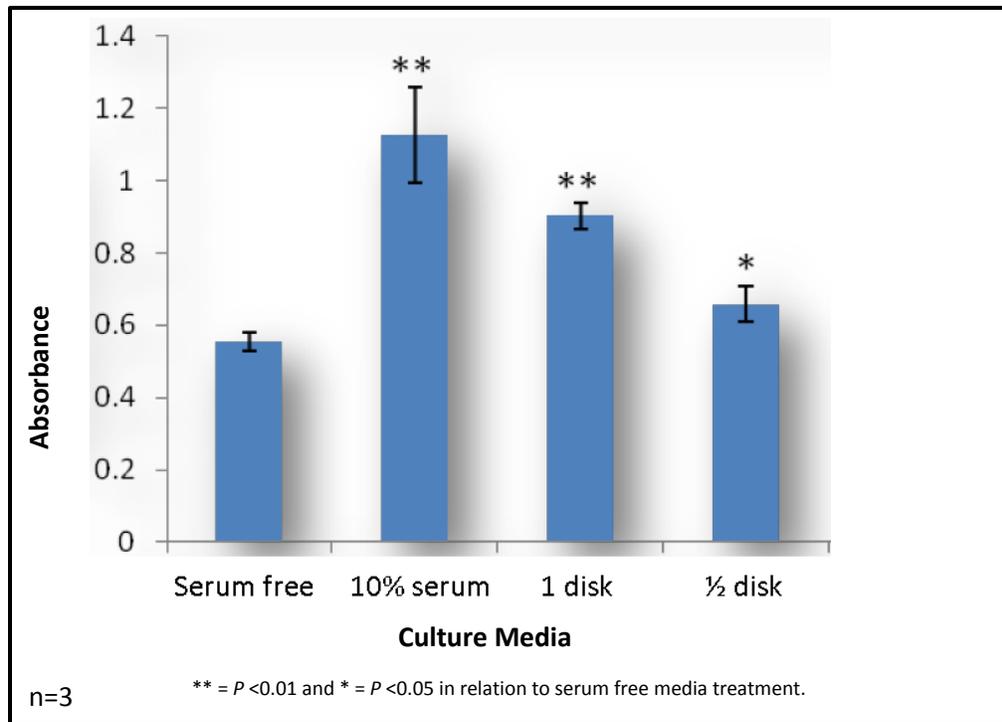
Native Growth Factors Present	Relative Amount Determined by ELISA Assay (n=5)	Release Profile (n=5)	
		24 hr incubation period at 4°C	Remaining in Tissue
PDGF-AA	++++	13%	87%
PDGF-BB	+	4%	96%
bFGF	++	44%	56%
TGF-b1	++	31%	69%
EGF	+	63%	37%

Determined by ELISA Assay (N=5)

## Hypothesis: Do PURION processed dehydrated human amniotic tissue allografts promote cell proliferation?

The effects of dehydrated human amniotic membrane on cell proliferation were assessed in vitro using human dermal fibroblasts (HDF).<sup>10</sup> Test media was generated through the incubation (37°C for 16 hours) of 2 ml serum free culture media with varying amounts of dehydrated human amniotic membrane (1 and ½ of a 16 mm diameter membrane disk). In addition serum free and 10% serum culture media were also incubated to provide negative and positive controls respectively. The HDF cells were incubated (37°C, 5% CO<sub>2</sub>) with test media, negative and positive controls for 72 hours. Cell proliferation was then measured using XTT cell proliferation assay. In addition, test culture media was analyzed for PDGF-AA using an ELISA in order to relate cell proliferation levels to growth factor release.

**Chart 1: Cell proliferation amounts based on membrane size**

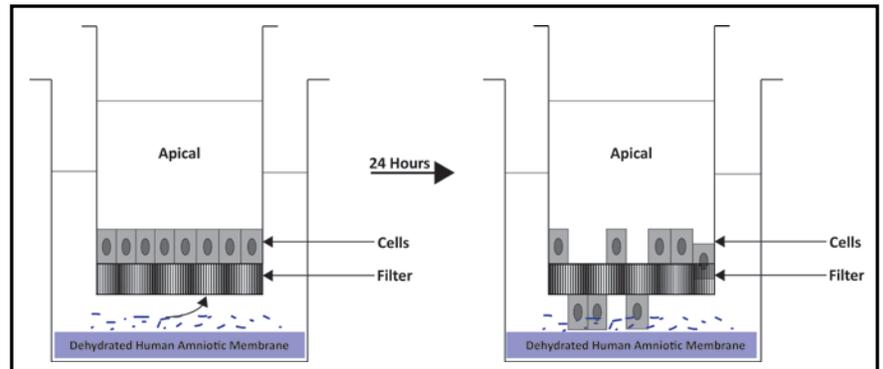


In all cases, the PURION processed dehydrated human amniotic membrane material showed statistically significant increase of cellular proliferation when compared to serum free control.

## Hypothesis: Do PURION processed dehydrated human amniotic tissue allografts promote cell migration?

The effects of dehydrated human amniotic membrane on human cell migration were evaluated in vitro in a trans-well culture apparatus, Diagram 1, according to established methods. Human cells were cultured in the upper chamber and PURION processed dehydrated human amniotic membrane was placed in the lower chamber. <sup>11</sup>

**Diagram 1: Trans-well culture apparatus**

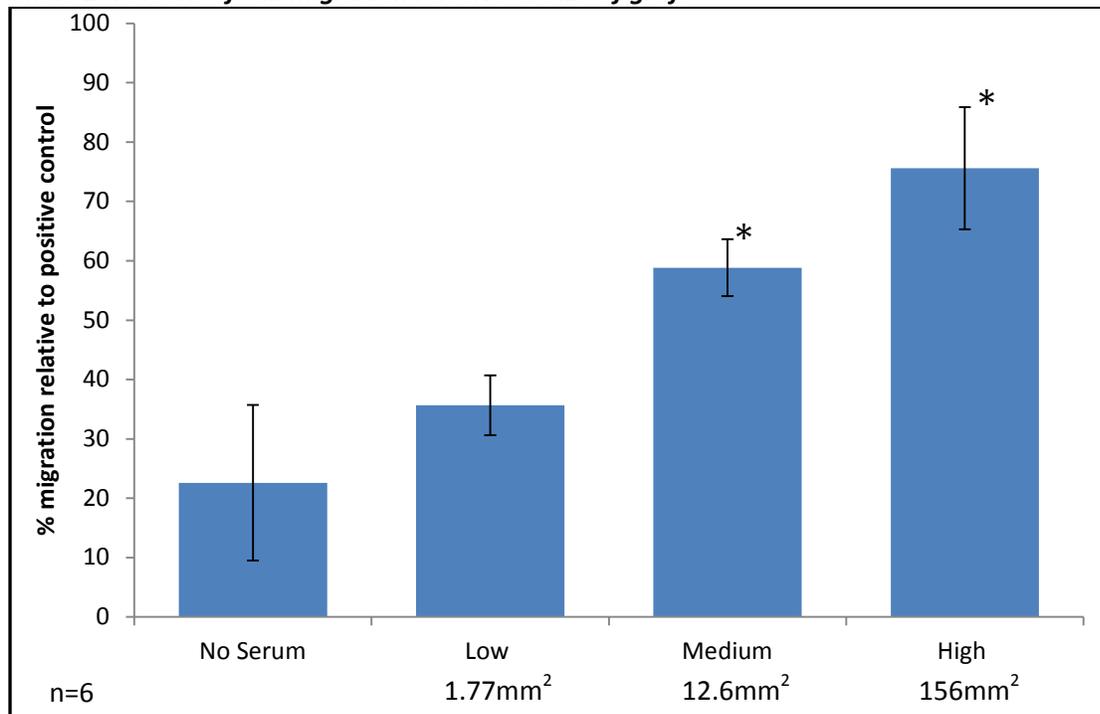


The number of human cells migrating through the porous membrane was counted after 24 hours of culture. Cell counts of migrating cells per micrograph relative to the PBS positive control are depicted in Chart 2.

Statistically higher cell counts were observed in High (12x13mm or 156mm<sup>2</sup>) and Medium-sized (4mm diameter disk or 12.6mm<sup>2</sup>) samples relative to the Low (1.5mm diameter disk or 1.77mm<sup>2</sup>) counterpart and the No Serum negative control.

Chart 2 demonstrates that increased cell migration correlates with higher amounts of PURION processed dehydrated human amniotic membrane.

**Chart 2: Percent of cell migration relative to size of graft**



\* indicates significantly higher ( $p \leq 0.05$ ) migration rate than both No Serum and Low group

## CLINICAL USES

Amniotic tissue has unique properties that reduce scar tissue formation, reduce inflammation and support soft tissue regeneration. The potential medical uses of the tissue inside and outside of the body are broad. This tissue can be utilized in procedures where reduction in scar tissue or regeneration of a soft tissue is beneficial. Amniotic membranes have been employed in the treatment of full and partial-thickness acute internal and external and chronic wounds. Both membrane and micronized dehydrated amnion/chorion membrane allografts have been used in the treatment of internal soft tissue healing including, but not limited to tendons, ligaments and skin. The dehydrated human amnion/chorion allografts can be used in a powder form for acute suture lines and hard to cover areas.

Some major clinical uses or proposed uses for membrane and micronized dehydrated human amnion/chorion allografts include:

### **Wound Healing of acute and chronic, full and partial thickness wounds**

- Delayed healing ulcers of various etiologies including diabetic foot ulcers, venous leg ulcers, arterial ulcers, pressure ulcers, post-surgical or post traumatic wound dehiscence, burns, acute suture line repairs, etc.
- Subcutaneous wound tunnel repair

### **General/ OBGYN/ Vascular Surgery (to reduce scar tissue formation and enhance soft tissue healing after primary repair)**

- General use in dermal scar revision by plastic/reconstructive surgery
- Incision with primary closure using graft
- Vascular surgery vessel repair grafts
- Reconstruction/revision, flap graft with tissue
- OBGYN laparoscopic procedures where material is used as a tissue barrier to reduce scar formation
- Inguinal hernia repair with mesh
- Ventral umbilical hernia repair
- Prostatectomy surgery for protection of nerve plexus
- Craniotomy surgery, to prevent scarring in flap scar reduction

### **Spine/ Orthopedic/Sports Medicine (to reduce scar tissue formation and enhance soft tissue healing after primary repair)**

- Spine surgery – as a cover for dura to reduce scar formation
- Total knee arthroplasty / Total knee replacement
- Shoulder arthroscopy
- Rotator cuff/ labial repair
- Tendonitis, bursitis, plantar fasciitis (golfer's and tennis elbow, jumper's knee)

### **Ear, Nose, and Throat/ Dental**

- Tympanoplasty (ear drum repair)
- Nasal septum repair
- Periodontal surgery and periodontal repair

## PATIENT SAFETY

MiMedx's focus on patient safety is evident in the development and validation of the PURION Process as well as continuous monitoring of the use of the tissue. MiMedx has attained voluntary AATB accreditation and complies with rigorous AATB standards. In addition, the innate properties of the human amniotic tissue provide an immunoprivileged material which reduces the likelihood of rejection or allergic reaction. Thousands of applications of PURION processed amnion/chorion composite grafts in the eye, a highly sensitive indicator of irritation, further support the continuous use of the tissue and the lack of reaction by the patient. Ocular irritation tests including GLP ISO tests<sup>12</sup> carried out by an independent lab further demonstrated that the material does not cause an inflammatory response in the standard test.

## CONCLUSION

The use of human amniotic membrane over the past 100 years has produced a significant amount of data in multiple areas of medicine. Based upon relevant immuno-histochemistry, ELISA, cell migration and proliferation studies conducted by MiMedx and independent laboratories on PURION processed dehydrated human amniotic membrane it is clear the patent pending PURION Process provides a minimally manipulated and carefully preserved amniotic tissue which contains essential growth factors and extra-cellular matrix within the membrane. The sterilized dehydrated human amniotic membrane is processed to provide an easy to use, safe surgical option for multiple surgical applications while providing a 5-year shelf-life at room temperature. Initial scientific evidence supports the hypothesis that many of the key components present in natural amniotic membrane are preserved during the gentle preservation of the PURION Process, which accounts for the advantageous clinical properties observed when the allografts are used in the clinical applications described.

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