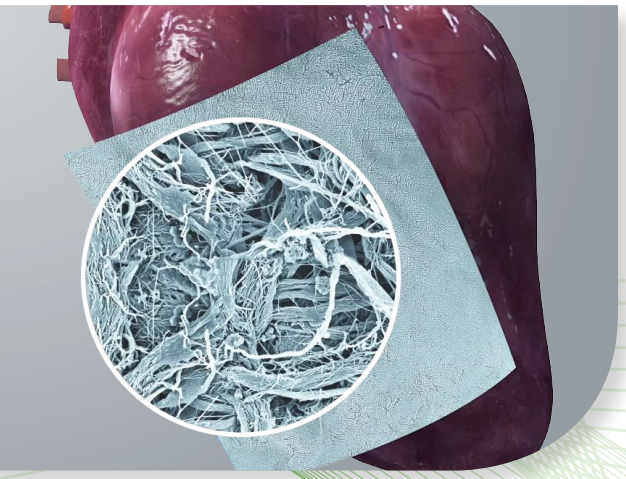
TECHNICAL BROCHURE

rti surgical™



Tutopatch™ ECM Extracellular Matrix

A **HIGHER** STANDARD™

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Tutopatch™ ECM Extracellular Matrix

The Strong New Choice
A Safe and Natural Biologic Option

Tutopatch™ ECM extracellular matrix is an acellular collagenous matrix derived from bovine pericardium and has been shown to be non-crosslinked.[1] Tutopatch ECM is processed through the validated Tutoplast® Tissue Sterilization Process which inactivates or removes potential pathogens. The process leaves behind an acellular structure that is intended to be a scaffold for host cells and blood vessels to repopulate providing a safe and natural biologic option for soft tissue repair.

The Tutopatch ECM implant offers three important components of a biologic implant: safety, strength and support for revascularization & remodeling. The handling characteristics of Tutopatch ECM allow the implant to conform to anatomic structures, making it well suited for cardiothoracic surgeries.

Tutopatch ECM is intended for use to reinforce soft tissue where weakness exists in general and plastic surgery applications, and is indicated for repair of pericardial structures.

WHAT IS AN EXTRACELLULAR MATRIX?

Extracellular Matrices (ECMs) are naturally occurring bioscaffolds found in all animals to act as the highways of communication and transportation of cells. Through the use of integrins ECMs can regulate a cell's behavior through:

- Cellular adhesion
- Cellular differentiation
- Cellular division
- Cellular migration

These functions occur on a cell's surface through various interactions with the components of the ECM (Figure 1). The complex matrix plays an integral role in coordinating all functions of tissue repair and remodeling.

This complicated network of various proteins and proteoglycans act as a scaffold for cells and tissues to provide support, structure and a means to trigger the body's own natural healing mechanisms.

Once implanted, a desired ECM will signal surrounding native cells to migrate into the matrix and populate the ECM scaffold. During this angiogenic stage, a naturally occurring resistance to infection takes place via the body's own defense mechanism. As an ECM is remodeled and revascularized, the site-specific tissue will fully replace the bioscaffold leaving behind minimal artifact and scarring.

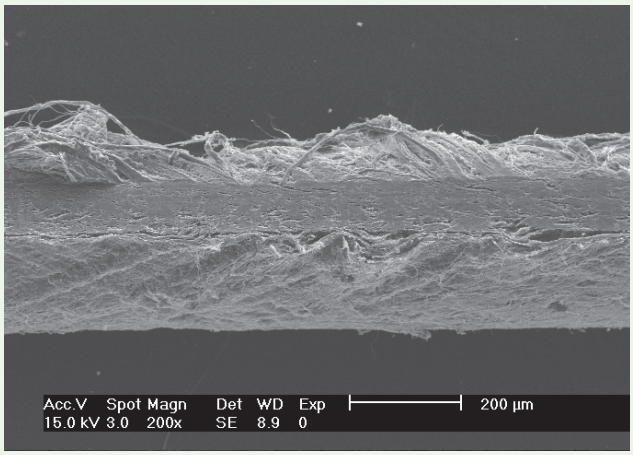


Figure 1. SEM micrograph of cross-section of bovine pericardium after the tutoplast process

Pericardial Repair and Soft Tissue Reconstruction

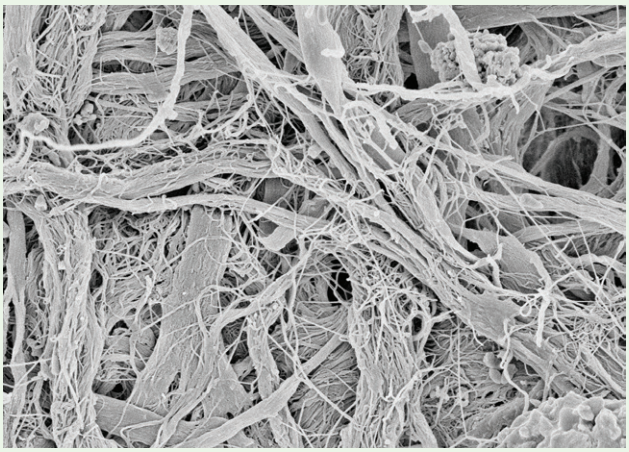
REPAIRING THE PERICARDIUM AFTER RESECTION

During an open heart procedure, the pericardium is resected, giving the cardiothoracic surgeon access to the heart along with the corresponding structures. Upon resection, the body signals cells to migrate to the incision area initiating an inflammatory response. The pericardium acts as a physiological barrier for cardiac protection and maintains proper cardiac function through monitoring septal motion and regulating cardiac pressures. Once the surgery is complete, closing the pericardium becomes a challenge due to its retracted nature after incision. Trying to re-approximate the native pericardium may cause cardiac tamponade or risk excess pressure on the grafts. A desired ECM allows for angiogenesis without the long term risk of scar tissue or residual artifact as the patch is remodeled into the body.

Tutoplast® Tissue Sterilization Process

Tutopatch ECM is preserved using the multi-step Tutoplast Tissue Sterilization Process which gently cleans and dehydrates the tissue. The membrane is terminally sterilized by gamma irradiation to a sterility assurance level (SAL) of 10^{-6}.

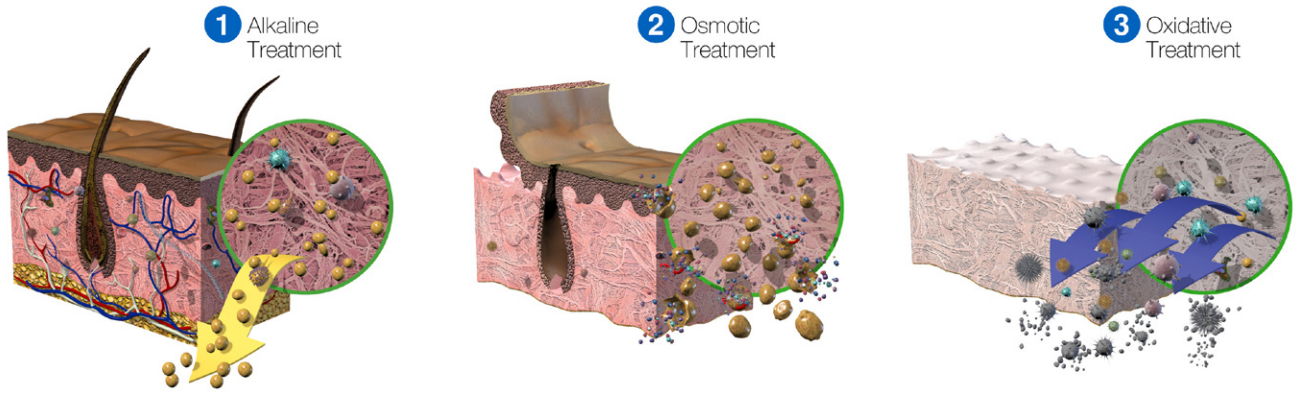
With more than three million sterilized implants distributed with zero confirmed incidence of implant-associated infection, the Tutoplast Process has a 40 year history of reliability and safety.



Bovine pericardium after the Tutoplast Process, Smooth surface, 5000x magnification.

HOW DOES THE TUTOPLAST® PROCESS WORK?

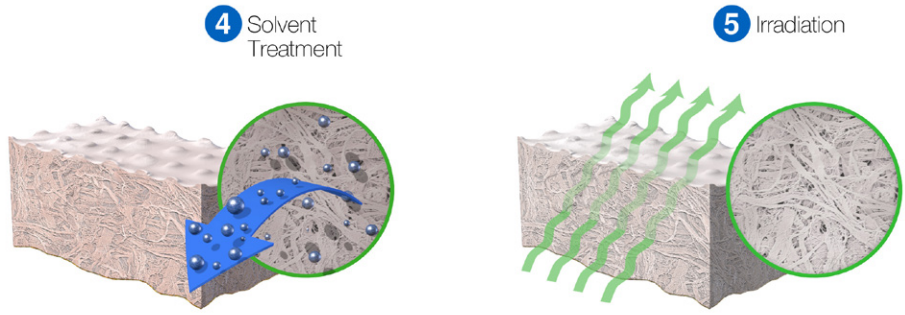
Osmotic, oxidative and alkaline (if indicated) treatments break down cell walls, inactivate pathogens, and remove bacteria. Solvent dehydration allows for room-temperature storage of tissue without damaging the native tissue structure. Low-dose gamma irradiation ensures a minimum sterility level (SAL) of 10^{-6} of the final packaged graft.



Alkaline Treatment
Removes cells and lipids which interfere with healing.

Osmotic Treatment
Disrupts cell membranes to allow easier removal of cellular components.

Oxidative Treatment
Removes immunogenic structures, enveloped and non-enveloped viruses.



Solvent Treatment
Removes water from tissue, preserves the natural tissue matrix and allows for a five-year shelf life.

Irradiation
Low-dose irradiation produces a terminally sterile graft, while preserving structural integrity.

**Images depict dermal processing.*

TUTOPATCH™ BOVINE PERICARDIUM COMPARED TO VERITAS[2]

The objective of this study was to characterize and compare the in vitro properties of the two bovine pericardium products and measure their in vivo performances (rat model). The in vitro morphology, collagen integrity, mechanical strength and enzyme susceptibility of the two products prior to implantation were analyzed using scanning electron microscopy (SEM), histological staining, biomechanical testing and collagenase digestion assay. The biological responses including cell repopulation, neovascularization and inflammation of the two implants were evaluated using an athymic rat implantation model.

PRESERVED COLLAGEN STRUCTURE AFTER RIGOROUS PROCESSING

Histological View (Picro Sirius red stain and polarized light)

Under polarized light examination, Tutopatch bovine pericardium showed normal collagen structure and birefractive properties comparable to non-processed bovine pericardium, but Veritas did not show similar birefractive properties (Figure 2). The loss of birefractive properties is an indication of a considerable alteration or damage of the collagen structure in Veritas.

Both SEM and histological analysis revealed that Veritas exhibited a dramatically altered collagen structure, and Tutopatch bovine pericardium maintained collagen structure similar to that of non-processed bovine pericardium.

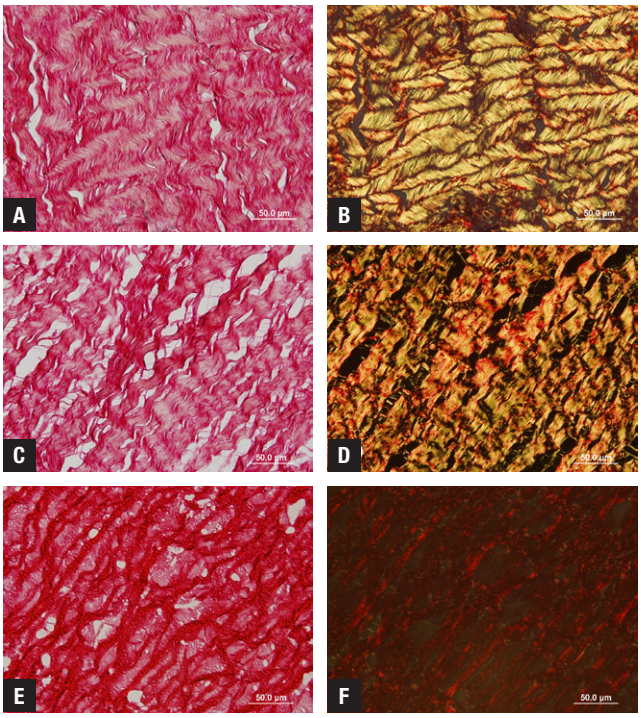


Figure 2. Micrographs of PSR stain of non-processed bovine pericardium (A, B), Tutopatch bovine pericardium (C, D) and Veritas (E, F) pre implants (t=0) under normal light (A, C, E) and polarized light (B, D, F).

Scanning Electron Microscope (SEM) Cross Section

The morphology of cross-sections and surfaces of Tutopatch bovine pericardium and Veritas were examined using SEM. Non-processed bovine pericardium was also examined for comparison purposes. The cross-section examination revealed that Tutopatch bovine pericardium had similar thickness and morphology compared to the non-processed bovine pericardium, whereas Veritas appeared thicker than non-processed pericardium (Figure 3). In addition, collagen bundle separations were observed in the fibrosa layer of Veritas (Figure 3).

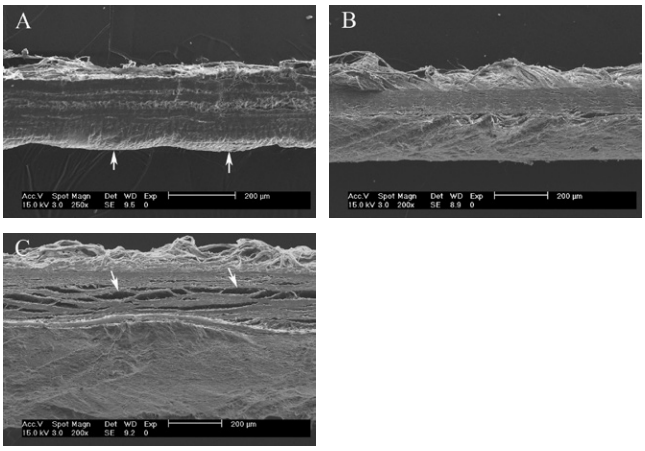


Figure 3. SEM micrographs of cross-sections of non-processed bovine pericardium (A), Tutopatch bovine pericardium (B) and Veritas (C). A smooth cell layer was present on serosa layer in nonprocessed bovine pericardium (indicated by arrows in A). Collagen bundle separations were evident in fibrosa layer of Veritas (indicated by arrows in C).

Histological View (H&E stain)

Histologic analysis revealed that Tutopatch bovine pericardium had more favorable remodeling characteristics compared to Veritas during the three-month implantation period. At four weeks, the Tutopatch bovine pericardium implant showed intact collagen structure whereas Veritas had an amorphous appearance (Figure 4). The drastic differences in collagen structures of Tutopatch bovine pericardium and Veritas were observed at eight and 12 weeks as well. The Tutopatch bovine pericardium implant exhibited significantly greater cell infiltration and neovascularization than Veritas at four weeks (Figure 4). By 12 weeks, the Tutopatch bovine pericardium implant showed increased cell infiltration across the graft whereas Veritas had limited cell infiltration localized at the periphery of the implant and host interface. The dramatic alteration of collagen structure in Veritas could be a key factor that contributed to the slow cell infiltration and neovascularization during the postoperative period.

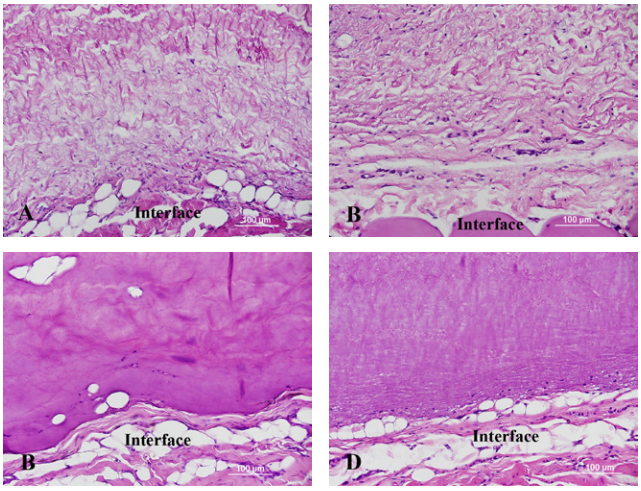


Figure 4. H&E representative micrographs of Tutopatch bovine pericardium explants at 4 (A) and 12 weeks (B) and Veritas explants at 4 (C) and 12 (D) weeks post implantation in a subcutaneous implantation animal model.

TUTOPATCH ECM COMPARED TO CORMATRIX ECM[3]

The objective of this study was to characterize and compare the in vitro properties of the two implants and evaluate their in vivo performances in an animal model. The in vitro morphology, collagen integrity, DNA content, mechanical strength, and enzyme susceptibility of the two implants prior to implantation were analyzed using scanning electron microscopy (SEM), histological staining, PicoGreen assay, ELISA, biomechanical testing, and collagenase digestion assay. The biological responses including cell repopulation, neovascularization, inflammation and graft resorption of the two implants were evaluated using a Sprague Dawley rat subcutaneous implantation model.

MORPHOLOGICAL ANALYSIS

Scanning Electron Microscope (SEM) Cross Section

The cross-section examination revealed that Tutopatch ECM had an integrated structure, whereas CorMatrix ECM exhibited a laminate structure (Figure 5). The layers in CorMatrix ECM appeared knitted to each other with small separations between the individual layers. The uniform thickness of Tutopatch ECM compared to the multilayered CorMatrix ECM product may eliminate concerns of delamination.

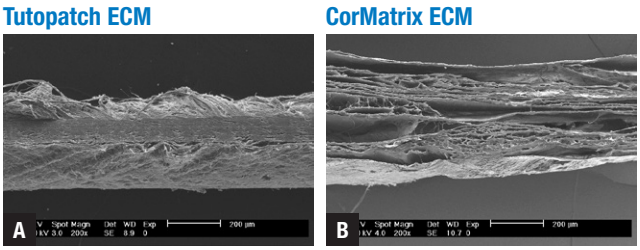


Figure 5. SEM micrographs of cross-sections of Tutopatch ECM (A) and CorMatrix ECM (B) pre-implants (t=0).

Histological View (H&E stain)

H&E staining revealed that Tutopatch ECM appeared mostly acellular, whereas CorMatrix ECM exhibited extensive nuclei staining indicating incomplete cell and DNA removal (Figure 6). Further analysis showed the amount of residual DNA content of CorMatrix ECM to be significantly higher than Tutopatch ECM (Figure 6). Incomplete DNA removal has been shown to solicit an unwanted inflammatory reaction from the body that may lead to compromised tissue remodeling.

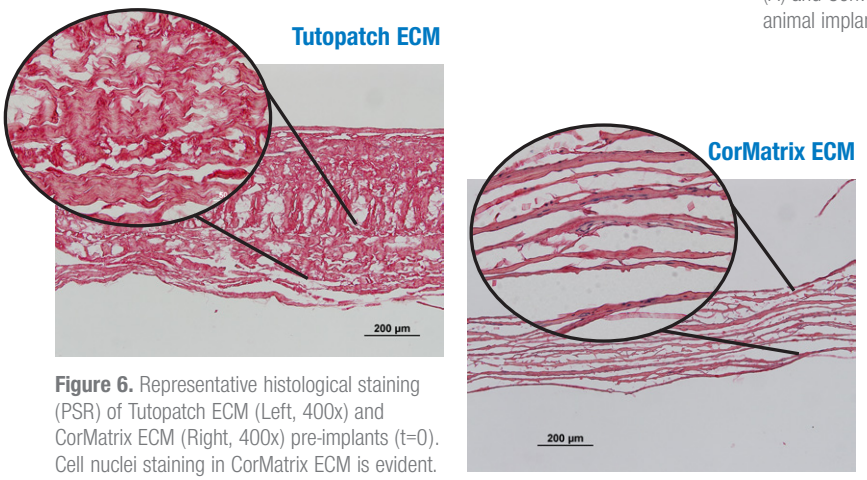


Figure 6. Representative histological staining (PSR) of Tutopatch ECM (Left, 400x) and CorMatrix ECM (Right, 400x) pre-implants (t=0). Cell nuclei staining in CorMatrix ECM is evident.

Histological Evaluation

At 4 weeks, intense inflammation was associated with CorMatrix ECM, whereas Tutopatch ECM showed a mild response (Figure 7). The CorMatrix ECM inflammatory response consisted of numerous macrophages, lymphocytes, plasma cells, foreign body giant cells and an increase in eosinophils as compared to Tutopatch ECM. Delamination of the CorMatrix ECM material was also apparent in the subcutaneous animal implantation model (Figure 7).

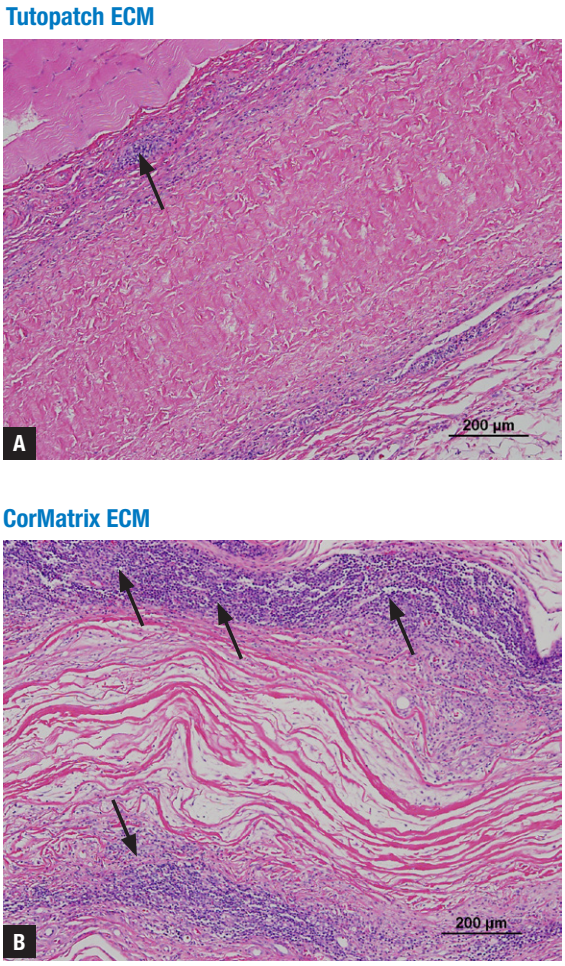


Figure 7. Representative histology staining (H&E, 100x) of Tutopatch ECM (A) and CorMatrix ECM (B) following 4-week) of implantation in subcutaneous animal implantation model. Key: Arrows = Inflammation.

DNA ANALYSIS

DNA analysis revealed that CorMatrix ECM had significantly higher DNA residual content than Tutopatch ECM (Figure 8). The finding was consistent with the histological observations.

	DNA CONTENT (µg/g wet weight)
Tutopatch ECM	60.43±20.03
CorMatrix ECM	155.93±4.89

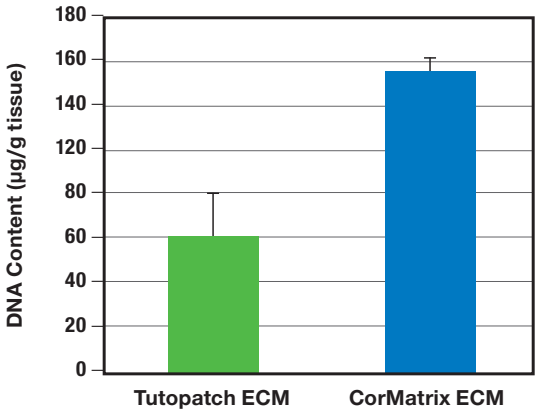
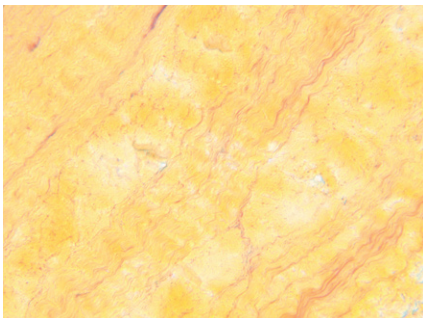


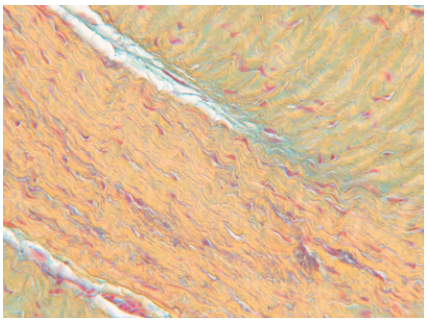
Figure 8. DNA content in Tutopatch ECM and CorMatrix ECM.

RAPID REVASCULARIZATION, REPOPULATION & GRADUAL REMODELING

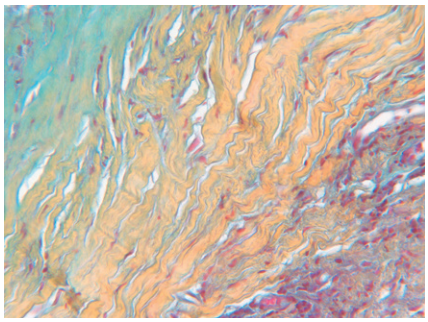
TUTOPATCH BOVINE PERICARDIUM IMPLANT HISTOLOGY[4] (CLINICAL BIOPSY)



Pre-Implantation
Collagen is stained in yellow and elastin fibers appear in red.



9 Months After Implantation
Collagenous tissue with blood vessels indicates revascularization.



36 Months After Implantation
Tissue shows revascularization with blood vessels and cells, completely remodeled.

Histology depicts a Tutopatch bovine pericardium implant used for hernia repair.

**Performance data from animal studies may not be representative of performance in humans.*

To order, call RTI directly: **888.778.8771**

ORDERING INFORMATION

For more information, contact your RTI Surgical representative.

CODE	DESCRIPTION	QUANTITY
BPE410	Tutopatch ECM, 4 x 10cm	5
BPE710	Tutopatch ECM, 7.5 x 10cm	5
BPE814	Tutopatch ECM, 8 x 14cm	5

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4. Urbach V., Linderman M., Shaheen I., Paolucci V. Data on file. Department of General Surgery and Visceral Surgery, Kettler Hospital, Offebach, Germany.

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