

Veritas[®] Collagen Matrix Safety Studies

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Veritas[®] Collagen Matrix is a remodelable bovine pericardium collagen matrix manufactured by Synovis Surgical Innovations. Veritas Collagen Matrix is indicated for implant as a urological sling to provide support and stability to the urethra just below the bladder. Safety of the Veritas Collagen Matrix is based on the results of several studies: biocompatibility, viral inactivation and animal implants.

Veritas Collagen Matrix Material

Veritas Collagen Matrix is non-cross-linked bovine pericardium. This material is exposed to 1.0N Sodium Hydroxide for 1 hour at room temperature to reduce or eliminate the potential of BSE transmissions and enhance decellularization. Using a proprietary process, the primary amine groups on the material are capped by propylene oxide treatment to make the material less immunogenic. The product is supplied terminally sterilized by E-beam in a double barrier package.

BIOCOMPATIBILITY

The urological sling indication classifies the device as having bone/tissue contact. The International Standards Organization (ISO) provides guidance to regulators and manufacturers regarding the testing necessary to establish the safety of any device. The Veritas Collagen Matrix is intended for use as a permanent tissue implant, and ISO 10993 with FDA modifications defines the battery of biocompatibility testing required to evaluate the safety of the material. The testing included:

- Cytotoxicity
- Sensitization
- Intracutaneous reactivity (irritation)
- Acute systemic toxicity
- Genotoxicity
- Implantation
- Hemocompatibility

All biocompatibility testing was completed at Toxikon Laboratories in Bedford, MA, in accordance with Good Laboratory Practice (GLP) requirements. These tests were conducted to demonstrate the safety of the Veritas Collagen Matrix. The results are presented in Table 1: Biocompatibility Testing.

Table 1: Biocompatibility Testing

Test	Conditions	Results	Conclusion
Cytotoxicity-L929 MEM Elution	Extracted in MEM for 72 hrs at 37°C	No biological reactivity	Pass
Sensitization Assay	Extracted in NaCl & CSO for 72 hrs at 37°C	0% sensitization	Pass
Intracutaneous Injection	Extracted in NaCl & CSO for 72 hrs at 37°C	No toxicity/irritation	Pass
Genotoxicity-reverse mutation	Extracted in NaCl for 72 hrs at 37°C	Non-mutagenic	Pass
Systemic Injection	Extracted in NaCl & CSO for 72 hrs at 37°C	Negative	Pass
Intramuscular Implant	Paravertebral implants (3/side)	Slightly toxic at 14 and 28 days; Non-toxic at 56 and 84 days post-implant	Slight toxicity is resolved with remodeling
Rabbit Pyrogen	Extracted in NaCl for 72 hrs at 37°C	Non-pyrogenic	Pass
Hemolysis – Rabbit Blood	Extracted in NaCl for 1 hour at 37°C	4.78% lysis	Pass
<i>In Vitro</i> Hemocompatibility	Mixed with blood 1 hour at 37°C	Fail	See text
Prothrombin Time	Mixed with plasma for 15 min at 37°C	Normal	Pass
Unactivated Partial Thromboplastin Time	Mixed with plasma for 15 min at 37°C	Normal	Pass
Complement Activation	Mixed with plasma for 1.5 hrs at 37°C	Normal	Pass

CSO = Cotton Seed Oil

The comprehensive biocompatibility evaluation, including the genotoxicity screening battery, provides a baseline rationale to determine if additional testing (chronic toxicity, carcinogenicity and reproductive/developmental toxicity) is required. Additional testing may be required if the initial tests produce remarkable results. Because the results obtained from the initial genotoxicity-screening assay of the Veritas Collagen Matrix were unremarkable, there was no adequate rationale or justification to conduct carcinogenicity and reproductive/developmental toxicity tests. Similarly, favorable results obtained from the implant, systemic toxicity, cytotoxicity, sensitization and irritation assays did not provide rationale or justification for performing the chronic toxicity test.

All the tests recommended according to ISO 10993 were designed and developed to elicit a specific reaction or response, either *in vitro* or *in vivo*. In toxicological studies, it is not uncommon to provoke a negative response, or to fail a particular test, and still have successful product implants. Although hemocompatibility studies were not required for

the urethral sling application of this product, the test battery was applied for purposes of determining future potential applications.

The results obtained from the *in vitro* hemocompatibility test and the borderline passing results obtained from the hemolysis test tend to suggest a potential incompatibility with blood. However, it is important to note that the remaining hemocompatibility battery (prothrombin time, unactivated partial thromboplastin time and complement activation) met the acceptance criteria for each test. These tests and the favorable results from the porcine studies demonstrate that the Veritas Collagen Matrix is compatible with most of the major components of blood.

In addition to the *in vitro* tests, numerous *in vivo* tests demonstrated that the Veritas Collagen Matrix is biocompatible. The three- and six-month porcine explants from the animal studies (Animal Implant Studies, page 5) demonstrate the excellent response of a living animal to the Veritas Collagen Matrix. This series of implants showed in-growth of surrounding tissue, neo-collagen formation and angiogenesis; all are signs of a favorable response to the implanted material in the pig model. The response of the porcine model indicates that there is successful production of new blood vessels into the implant material as well as proliferation of fibroblasts throughout the tissue.

During the Intramuscular Implant Test, all rabbit explants of the bovine pericardial tissue at 12 weeks fared better than the United States Pharmacopeia (USP) control plastic used as a negative control with no observation of fibrotic encapsulation of the implant. Although the rabbit implants did not demonstrate the same level of response as seen in the porcine studies, their favorable results, when compared to the USP control plastic at 8 and 12 weeks, provide the essential safety information required by this test.

In terms of safety, the results of the Intramuscular Implant Test are supported by the negative, or passing, results of the systemic toxicity, irritation, sensitization and cytotoxicity tests conducted in animals. All animals used in these tests were found to be healthy at the end of testing with signs of weight gain, where monitored, and no evidence of adverse reaction.

In conclusion, the results of all required tests are acceptable and meet the validation acceptance criteria and design requirements for the remodelable bovine pericardial material, Veritas Collagen Matrix. The results are indicative of a biocompatible product that is safe for implant and one that does not place the patient at undue risk.

VIRAL SAFETY

To identify the viral safety designed in Veritas Collagen Matrix, a viral inactivation study, designed to mimic production scale processing, was prepared jointly with ViroMed Laboratories in Camden, NJ. Pericardium to solution ratios were determined for each step then scaled down linearly for each solution so that manufacturing and laboratory ratios would be equivalent. Three steps in the manufacturing process were identified as potentially virucidal. These three steps were solution soaks of the

pericardial tissue in sodium hydroxide (NaOH), propylene oxide (PO) and ethanol (ETOH).

Temperature, time and pH were controlled during the study in a manner consistent with production practices. Inoculation viruses were selected based on relevance to the raw material (bovine pericardium) and for known resistance to inactivation methods. The viruses used were bovine parvovirus (BPV), bovine viral diarrhea virus (BVDV), reovirus type 3 (REO-3) and infectious bovine rhinotracheitis virus (IBR). This selection of viruses includes the major categories of virus: enveloped DNA, non-enveloped DNA, enveloped RNA and non-enveloped RNA.

Samples were drawn at several points throughout the processing to provide a kinetic basis to the inactivation results. The NaOH solution was tested at 0, 30 and 60 minutes: 60 minutes is the minimum routine processing time for the material. The PO solution was tested at 0, 24 and 48 hours: 48 hours is the minimum routine processing time for the material. The ETOH solution was tested at 0, 8, and 24 hours: 24 hours is the minimum routine processing time. Stock virus, freeze/filter and processing controls were included for each solution and virus tested.

The testing conducted represents a worst-case scenario to demonstrate the effectiveness of each solution. Maximum tissue-to-solution ratios were used with high viral inoculations. A wide spectrum of resistant model virus was chosen, and virus was allowed to absorb to the pericardium to present a challenge for the solution to penetrate the collagen matrix and provide inactivation.

Log reduction for each processing step may be determined and a total log reduction calculated for the entire process (no single process step is repeated and included in sum.) The results listed in Table 2: Log Reduction Summary, show the individual log reductions per virus at each processing step and the total reduction after completion of the entire three-step process.

Table 2: Log Reduction Summary

	NaOH	PO	ETOH	TOTAL
Duration	1 hour	48 hours	24 hours	
Virus				
BPV	>5.05	>2.31	N/A*	>7.36
BVDV	>4.97	>4.40	>4.78	>14.15
IBR	>5.57	>6.07	>6.25	>17.89
REO	4.53	>5.57	>5.87	>15.97

*Bovine parvovirus was not tested against 70% ethanol due to previous experience that indicates ethanol solutions are ineffective against BPV (less than 1.0 log reduction regardless of concentration or temperature)

BPV: Bovine parvovirus, non-enveloped DNA

BVDV: Bovine viral diarrhea virus, enveloped RNA

IBR: Infectious bovine rhinotracheitis virus, enveloped DNA

REO-3: Reovirus type 3, non-enveloped RNA

In all cases, each virus was reduced to non-detectable levels. Recovery assays were validated at ViroMed Laboratory, with minimal inter-assay variation BVP at +/- 0.5 log, BVDV and IBR at +/-0.3 logs, and REO-3 at +/- 0.1 log.

Results of the viral inactivation study provide a high degree of assurance that any viral contaminants, even at high titers, will be inactivated by the combined processing steps of sodium hydroxide, propylene oxide and ethanol treatments in the manufacture of the Veritas Collagen Matrix. Each solution, individually, has strong efficacy against a wide spectrum of viral types. In addition, each solution has rapid inactivation characteristics that reach the reported log reductions in less than half of the minimum processing times required during manufacturing. The results of the viral inactivation study substantiate the ability of the manufacturing process to inactivate related organisms and potential organisms currently uncharacterized.

ANIMAL IMPLANT STUDIES

Several animal implant studies were conducted to demonstrate the safety of the Veritas Collagen Matrix:

1. Subcutaneous implants in rats
2. Canine abdominal wall implants
3. Rabbit abdominal wall implants
4. Lung resection in rabbits
5. Urethral sling implants in minipigs

Subcutaneous and Abdominal Wall Implants:

The Subcutaneous implants in rats (conducted at Dr. Nimni's laboratory in USC) and the Canine abdominal wall implants (conducted at New England Medical Center) were the initial implant studies undertaken during the development of the Veritas Collagen Matrix. Rat implants revealed that the material is very biocompatible and caused no observable inflammatory reaction at 45 and 90 days post implantation. Furthermore, there was evidence for neo-collagen formation. Canine abdominal implants revealed that the material was "remodeled" in 28 days post implantation. At implant, the material is totally decellularized. At 28 days post implantation, the explanted material, when analyzed histologically, was indistinguishable from the host tissue and was populated with cells to the same extent the host tissue was cellularized. The Rabbit abdominal wall implants study (conducted at New England Medical Center) was pivotal in proving the vascularization of the implant in 4 weeks or less.

Lung Resection:

The lung resection study in rabbits was conducted at North American Science Associates (NAmsA), in Toledo, OH, in accordance with Good Laboratory Practice (GLP) Regulations. This study revealed that the Veritas Collagen Matrix is as good a

buttressing material as Peri-Strips Dry[®] buttressing material (available from Synovis and used to enhance staple line integrity in lung resections and gastric bypass surgeries). As an additional benefit, the Veritas Collagen Matrix demonstrated markedly less adhesion formation as compared to Peri-Strips Dry. This low adhesion property of the Veritas Collagen Matrix could prove advantageous when the material is used for indications where lack of adhesions is important.

Urethral Sling Implants

The urethral sling implant study was conducted at NAmSA in compliance with the GLP Regulations. The objectives of the study were to evaluate the systemic toxicity potential of the Veritas Collagen Matrix in swine at three and six months post-implant and to assess the response of the pig's tissue to a bladder neck suspension with the Veritas Collagen Matrix. The results were compared to a control material, human fascia lata, which was implanted in an identical manner.

A midline incision was made from the umbilicus down to the brim of the pelvic bone. The incision penetrated the peritoneal cavity. The bladder was dissected free of supportive connective tissue (top and both sides). The urethra and bladder neck were dissected free of colon. The sling (test or control material) was implanted into a 2-3 cm space between the urethra and colon and secured in place with 2-0 Prolene suture. Four points of device suture attachment included two to the pubic bone bilaterally and two to each side of the pelvic fascia connective tissue. When necessary to stabilize the urethra, the sling was sutured directly to the urethra.

Twenty minipigs were included in this study. Ten were implanted with Veritas Collagen Matrix and ten with the control material. Body weights were recorded prior to surgery. A pre-treatment blood specimen was collected from each animal and forwarded to a veterinary reference lab for complete blood cell count (CBC) with differential and blood chemistry. Twelve of the 20 animals were sacrificed at three months post implant (six test and six control) and the remaining eight were sacrificed at six months (four test and four control). At each of the two dates of sacrifice, body weights were recorded and a blood specimen was collected from each animal and forwarded to a veterinary reference lab for complete blood cell count (CBC) with differential and blood chemistry. Each animal was observed for signs of general health, behavioral changes, or toxicity.

In addition to visual examination of each implant at each time point, necropsy specimens were processed for histologic evaluation. Sections of each graft were preserved for subsequent histologic evaluation. Integrity of attachment, inflammation, fibrosis or capsule formation and any other obvious tissue responses were evaluated. Along with specimens taken from the implant site, certain organs were removed at sacrifice, weighed and histologic specimens prepared.

Clinical Observation: No behavioral changes or signs of toxicity were observed in any animal throughout the full term of the study. One control animal was noted to have

developed an abscess of the abdominal incision line at day 41 of the study. Upon antibiotic therapy, the condition resolved.

Body Weight: Individual and group mean body weights were considered to be clinically acceptable throughout the study. Stable body weights indicate that the animals were maintained in a general state of good health throughout the study.

Organ Weights: Organ weights to body ratios at each point of the study were similar between all the animals. There was no trend considered to be a result of the surgical procedure or the presence of the xenographic sling implant.

Necropsy: There were no macroscopic changes observed in the abdominal organs and tissue and no significant irritation noted at the implant sites. Upon necropsy at three months, an identifiable amount of the control or test sling was visible. The explants exhibited substantially intact ends still sutured to the adjacent host connective tissue. At six months, individual visual necropsy observations described that in two out of four control animals, the fascia lata sling was not readily identifiable. The Veritas Collagen Matrix sling, however, was observed to be present, but decreased in mass in all four test animals. In these four Veritas Collagen Matrix cases, definite strands or cords were oriented where the implant had been placed. Also observed in the Veritas Collagen Matrix animals was a thick host membrane-like connective tissue surrounding the urethra.

Hematology and Clinical Chemistry: The CBC with differential and 23 blood chemistry parameters were recorded prior to implantation and at three- and six-month termination points. All values were within acceptable limits and no trend was noted from the comparison of all animals at the three time points. Thus, there is no significant effect on the hematology and blood chemistry parameters as a result of the surgical procedure or the presence of the Veritas Collagen Matrix implant.

Histopathology: At three months, minimal to moderate inflammation and mild hemorrhage and hemosiderosis were commonly observed in regional lymph nodes collected from all animals. These findings, combined with the minimal to mild inflammation and fibrosis along the serosal surface of the urinary bladder and urethra, are regarded as expected and transient changes associated with the surgery and xenographic implants. Microscopic changes in the other organs examined are regarded as common, spontaneous changes in minipigs, thus indicating no detectable toxic or pathologic effects as a result of the implants. There was no evidence of suppurative or reactive changes in any animal (changes which are likely to progress to implant rejection or secondary bacterial infection.)

Average severity grades for chronic inflammation and fibrosis in the Veritas Collagen Matrix sites were less than those recorded for the control article. The Veritas Collagen Matrix was often difficult to identify in the suburethral implant sites, suggesting that the Veritas Collagen Matrix material is of low irritation potential and/or is resorbed by physiologic processes. The precise interface between the Veritas Collagen Matrix and

juxtaposed native tissue was difficult to identify in routine histologic sections. The Veritas Collagen Matrix was characterized by relatively thin linear deposits of devitalized connective tissue with minimal to mild deposits of calcium salts and small accumulations of inflammatory cells.

Linear deposits of compacted collagen-like fibers, circumscribed by moderate to marked infiltrates of mixed inflammatory cells were observed in the control sling material. Often, inflammatory infiltrates are associated with populations of proliferating fibroblasts, and the combined mixture of reactive cells frequently obscures the precise interface between the implant and the connective tissue. The central regions of most control implants appeared to consist of devitalized, fibrillar connective tissue with focal deposits of calcium salts (mineralization).

The presence of minimal vacuolation of renal tubular epithelial cells in two pigs with the Veritas Collagen Matrix was noted. The cause of this development was not observed. Vacuolar changes are considered to impair renal function or to represent a progressive nephropathy, i.e. necrosis.

At six months, test and control materials were difficult to identify in most histologic sections. The Veritas material again displayed lower extents of fibrosis and inflammation than did the control material, which confirmed that the Veritas Collagen Matrix is of relatively low irritation potential. At six months, the Veritas Collagen Matrix was resorbed/remodeled by the surrounding pig tissue to a greater extent than at the three-month interval.

Regional lymph nodes again exhibited mild inflammation in the test animals. These findings, combined with minimal to mild inflammation and fibrosis along the serosal surface of the bladder and urethra, are regarded as an expected result of the implantation surgery. All signs of inflammation indicated a diminution of this host response. As in the case of the three-month sacrificed animals, microscopic changes in other organs were regarded as expected, spontaneous and common changes. There were no reactive changes in any implantation site that would be likely to progress to implant rejection or secondary infection.

Study Conclusions:

At three months:

- There was no evidence of systemic toxicity resulting from the presence of the Veritas Collagen Matrix following suburethral surgical implantation in pigs. Daily clinical observations, body weights, organ weights and necropsy findings were within acceptable limits.
- There were no changes in hematology or clinical chemistry that are considered significant or dependent on the presence of the Veritas Collagen Matrix.
- Microscopic evaluation of selected organs/tissues revealed no evidence of a treatment-related response.

- A substantial amount of the original Veritas Collagen Matrix was still visible, with the sutured ends quite intact and the sutures remaining in place. There were no visible signs of implant rejection or implant site infection.
- Veritas Collagen Matrix remodeling was visible, with the bulk of the implant reduced in extent, as well as observation of new host connective tissue in the region where the sling was in contact with the urethra.
- Histology revealed a minimal degree of inflammation and minimal mineralized fibrotic encapsulation of the Veritas Collagen Matrix.
- Histologic staining revealed the presence of an abundant dispersion of pig connective tissue cells (fibroblasts) and new blood vessels distributed throughout the Veritas material's collagen matrix, indicating the early stages of remodeling of the implant and its integration into the host tissue.
- The Veritas Collagen Matrix is considered to be a non-irritant to the suburethral tissue of the pig.

At six months:

- There was no evidence of systemic toxicity as a result of the Veritas material's presence. Daily observations, body weights, necropsy findings and organ weights were within acceptable limits.
- There were no significant changes in terminal hematology or clinical chemistry values, indicating no detectable toxic or systemic pathologic host response.
- Microscopic examination of the organs taken revealed no evidence of a treatment-related response. Thus, neither the surgery nor the presence of the Veritas Collagen Matrix produced obvious and deleterious effects on the peripheral organs.
- At necropsy, there was visually less of the original Veritas Collagen Matrix visible. The implant changed from an original strip-like shape to that of several substantial strands or cords, which remained in the initial orientation around the urethra. More new pig connective tissue was associated with the implant, usually in the form of a thick membrane.
- Histology revealed that, at the time of necropsy there was tissue at the implant site that was oriented around the bladder neck, making microscopic identification of the Veritas material difficult.
- The Veritas Collagen Matrix was produced in an acellular state. Histologic examination of the implant sites taken at six month demonstrated the presence of connective tissue cells (fibroblasts and their mature counterparts, fibrocytes) and blood vessel development throughout the entire microscopic field. There was macroscopic identification of the Veritas Collagen Matrix either partially remodeled or replaced at the implant site, oriented as a wrap, usually in cord or strand configuration, around the urethra at the bladder neck. Thus, the presence of connective tissue cells and blood vessels in the initial stages of healing and remodeling throughout the entire bladder neck/implant site demonstrates the integration of the test article into the surrounding host tissue.

Summary: The necropsy results of the pigs at three and six months demonstrated that surgery and the presence of the Veritas Collagen Matrix as a sling implant created no detectable systemic or site-specific toxicity. The Veritas Collagen Matrix sling was

observed to be virtually non-inflammatory and relatively inert regarding mineralization and fibrosis. Average severity grades for chronic inflammation and fibrosis in Veritas Collagen Matrix implant sites were less than scores recorded for the control article (processed cadaveric fascia lata) suggesting that the test implant was less irritating than the control article. At both necropsy time points, histologic examination revealed the sling material to be readily remodeled and integrated into the adjacent host connective tissue. The active remodeling of the sling was macroscopically observed to result in a gradual alteration of the initial strap-shaped configuration of the sling to a cord or strand-like appearance. The process also involved the development of new pig connective tissue and its association with the implant.

Conclusions

There was no systemic or site-specific toxicity observed from the implantation of Veritas Collagen Matrix in various animal models in different positions. This material was produced in an acellular state. Histologic examination of the implant sites demonstrated the presence of cells (fibroblasts and their mature counterparts, fibrocytes) and blood vessel development throughout the entire microscopic field. The implanted Veritas Collagen Matrix was integrated into the surrounding host tissue as demonstrated by the presence of connective tissue cells and blood vessels in the initial stages of healing and remodeling throughout the implant site. Due to these findings it is concluded that the Veritas Collagen Matrix is a safe material to use as a urethral sling.