

# Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant: Mechanical and Biological Properties Following *in vivo* Implantation

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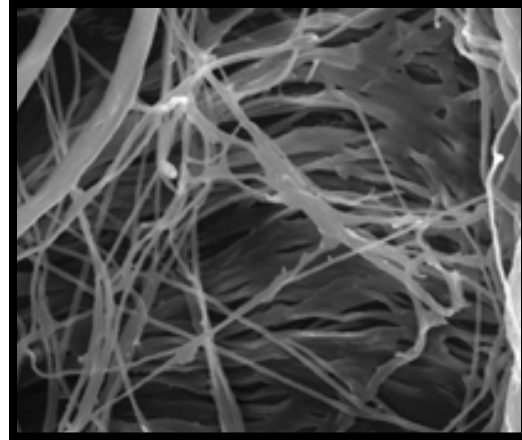
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## ABSTRACT

Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant is a sterile sheet of lyophilized, cross-linked, acellular, porcine dermal collagen and its constituent elastin fibers, indicated for use in soft tissue reinforcement, such as the repair of hernia and chest wall defects and/or damaged/ruptured soft tissue membranes. The primary objective of this study was to determine the *in vivo* mechanical and biological properties of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant over time. The study population consisted of 45 male Sprague-Dawley rats (350-400g). Following isoflurane anesthesia and aseptic surgical preparation, 40 rats were randomized to receive bilateral full-thickness muscular surgical defects to the left and right of the midline, which were repaired with one mechanical and one histological sample of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant. Animals were sacrificed at various time points (2, 4, 8, and 12 weeks; n=10/group) post-implantation, to assess the mechanical properties, host inflammatory response, and tissue ingrowth characteristics of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant over time. Five (5) additional rats were also utilized to determine the mechanical characteristics of rat abdominal wall (n=10; 2 samples/animal). Our data indicates a significant (rehydration-dependent) reduction in the mechanical tensile strength of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant at 2 weeks post-implantation, as compared to  $T_0$ . However, no significant difference was observed between 2, 4, 8, and 12 weeks *in vivo*. Interestingly, further mechanical analysis also demonstrated that the mechanical tensile strength of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant at 12 weeks post-implantation was not significantly different than non-implanted Bard<sup>\*</sup> Soft Mesh (a synthetic polypropylene mesh). A significant reduction in the mechanical stress of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant was observed between 2 and 12 weeks post-implantation, although it was significantly higher than the mechanical stress of rat abdominal wall at all observation time points. Further evaluation also revealed a significant reduction in the mechanical modulus (relative stiffness) of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant at 4, 8, and 12 weeks post-implantation, as compared to  $T_0$ . As modulus is inversely proportional to compliance, this data suggests an increase in the compliance of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant following *in vivo* implantation. Gross pathological analysis indicated that implantation of Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant led to a minimal foreign body reaction, promoted cellular growth, and the implant was surrounded with new host tissue by 2 weeks post-implantation. Angiogenesis was observed surrounding the device by 2 weeks, with further evidence of vascular penetration and remodeling of the device (through follicular channels) by 4, 8, and 12 weeks post-implantation. Further microscopic evaluation demonstrated a minimal host inflammatory/fibrotic response, dominated by macrophages and new collagen deposition at the perimeter (and through follicular channels) of the device. The primary collagen scaffold within Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant was observed to be intact and functional at 12 weeks post-implantation. Overall, data suggests that Bard<sup>\*</sup> CollaMend<sup>\*</sup> Implant represents a biocompatible biologically-derived prosthetic scaffold optimized to inhibit rapid enzymatic degradation, support cellular growth and maintain long-term mechanical properties *in vivo*.

## INTRODUCTION

Recent advances in synthetic materials and techniques for surgical hernia repair/soft tissue reconstruction have resulted in reduced patient recovery time, stronger repairs, and fewer complications<sup>1, 2</sup>. These repairs primarily require the permanent implantation of foreign

synthetic materials, such as polypropylene, polyester, and/or expanded polytetrafluoroethylene (ePTFE) mesh, to achieve adequate repair<sup>3</sup>. However, in some clinical settings the use of synthetic materials is contraindicated, thereby hindering the use of these traditional repair methods<sup>4</sup>. The development of biologically-derived prosthetics for hernia

repair/soft tissue reconstruction may provide a functional alternative in challenging cases, and potentially eliminate the need for long-term synthetic material retention in complex patient populations. Bard<sup>®</sup> CollaMend<sup>®</sup> Implant represents one such biologic innovation, which has been optimized for hernia repair/soft tissue reconstruction. Bard<sup>®</sup> CollaMend<sup>®</sup> Implant is a sterile sheet of lyophilized, cross-linked, acellular, porcine dermal collagen and its constituent elastin fibers. It is indicated for use in soft tissue reinforcement, such as the repair of hernia and chest wall defects and/or damaged/ruptured soft tissue membranes. Bard<sup>®</sup> CollaMend<sup>®</sup> Implant is chemically processed to achieve acellularity, reduce antigenicity, and also chemically cross-linked with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) to inhibit rapid enzymatic degradation. The primary objective of this study was to characterize the *in vivo* mechanical and biological properties of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant over time following repair of an abdominal surgical defect.

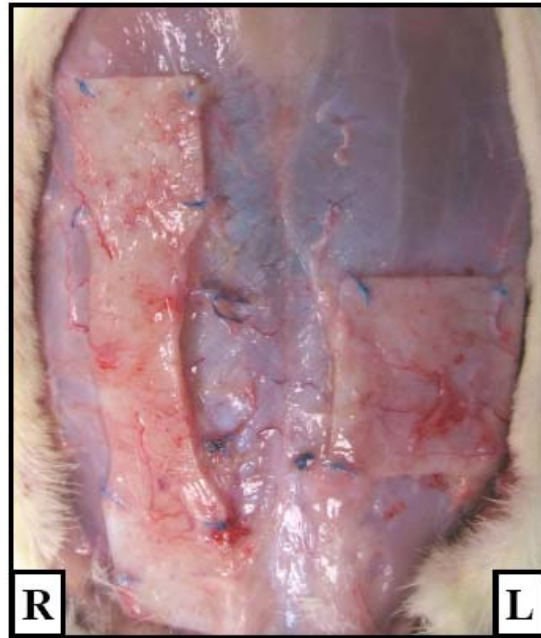
## METHODS

### Study Population:

The study population consisted of 45 male Sprague-Dawley rats (350-400 g). Forty (40) rats were randomized to be implanted with Bard<sup>®</sup> CollaMend<sup>®</sup> Implant for 2, 4, 8, and 12 weeks (n=10/group), and five (5) rats were also utilized to determine the mechanical characteristics of a rat abdominal wall (n=10; 2 samples/animal).

### Implantation:

Animals were initially anesthetized with 2.5-4% inhalational isoflurane, and maintained at 0.5-2.5% throughout the procedure. The ventral abdomen was prepared for aseptic survival surgery by clipping the fur over the entire abdominal region, cleaning the operative area with three alternating scrubs of povidine and 70% alcohol, and applying sterilized surgical drapes over the entire field. Following preparation of the ventral abdomen, an approximate 6-8 cm midline skin incision was



**Figure 1.**

Representative photograph of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant 2 weeks following subcutaneous abdominal implantation. The mechanical and histological test articles were secured over the right (R) and left (L) abdominal surgical defects respectively. Gross observations indicate biocompatibility, rapid host acceptance, angiogenesis, and initial tissue integration.

made. The skin was bluntly dissected to accommodate the placement of both test articles in the subcutaneous space (1 mechanical, 1 histological/animal). Two surgical defects were subsequently made in the abdominal muscle (0.5 cm length; 0.5 cm width; 1/side) to the left and right of the midline. Each surgical defect was repaired with the test articles following 5 minutes of rehydration in sterile saline (0.9% NaCl) prior to implantation. In each animal, the mechanical test article was secured over the right abdominal defect with eight interrupted 5-0 Prolene sutures (4 corners of the grip area, 4 corners of the middle area). The histological test article was secured over the left abdominal wall defect with four interrupted 5-0 Prolene sutures (1 at each corner). Following placement of the test articles, the skin was closed with 3 interrupted (inner) subcuticular 4-0 Vicryl sutures and a continuous (outer) 4-0 Vicryl suture using standard subcuticular technique (Figure 1; 2 weeks post-implantation).

Explantation:

At specified explantation intervals, euthanasia was achieved by CO<sub>2</sub> inhalation. Following euthanasia, the skin was dissected from the entire abdomen, and photographs were taken of each animal and each individual test article *in situ*. The entire body wall, including both the mechanical and histological specimens, was then explanted *en bloc*. After completion of the initial examination, each mechanical (dogbone-shaped) test article was excised with a 1 cm margin of surrounding tissue and immersed in saline (0.9% NaCl) for further mechanical analysis. Each histological (square-shaped) test article was removed with at least a 1 cm margin of surrounding tissue and immersion fixed in 10% Neutral Buffered Formalin for further histological analysis.

Mechanical Testing:

All mechanical testing of Bard\* CollaMend\* Implant was conducted by an independent biomaterial consulting firm (Altran Solutions, Inc., Boston, MA). Mechanical analysis was performed utilizing a calibrated ASTM fixed tensiometric servo-hydraulic testing system (Instron, MTS), and 50 pound (lbs) load cell (Omega LCCB-50). To assure adequate sample gripping characteristics, all loosely adhered tissue was removed such that pneumatic grips captured the mechanical test article and all tightly adhered tissue. Each test article (gauge length: 30 mm) was tested with a pneumatic grip pressure of 45 psi, strain rate of 5 mm/min, and test duration of approximately 5 minutes. The stress-strain data for each test article was digitally captured and stored for subsequent offline analysis. Tensile Strength was captured as the peak load (lbf) to achieve test article mechanical failure. Stress was reported as Tensile Strength (lbf)/inch<sup>2</sup> (psi). Modulus was calculated from the slope (tensile stress/strain) between 20% and 80% of the peak Stress (psi). Mechanical tensile strength testing of non-implanted Bard\* Soft Mesh (synthetic polypropylene mesh) was conducted at Davol Inc. utilizing the same testing parameters and test article configuration.

Pathological Analysis:

All histological analysis was conducted by an independent Pathologist (Paul Termin, D.V.M., Ph.D., Lincoln Associates, Inc., St. Paul, MN). Following gross and sub-gross pathological examination and photography, explanted histological (square-shaped) test articles were cut into specimens of 3-4 mm thickness, placed in processing cassettes, photographed, and routinely processed and embedded in paraffin. Sections 4-5 μm in thickness were mounted onto glass slides and stained with Hematoxylin and Eosin (H&E). All remaining slides were retained in archive with corresponding case numbers for future study.

Statistical Analysis:

Mechanical data was collected, analyzed, and graphically displayed with GraphPad Prism 4.03 statistical software. ANOVA with Tukey's post-hoc analysis was used for multiple comparisons, with statistical significance set at  $P < 0.05$ .

**RESULTS**Tensile Strength:

Data indicates a significant reduction in the mechanical tensile strength of Bard\* CollaMend\* Implant at 2, 4, 8, and 12 weeks post-implantation, as compared to T<sub>0</sub> (following 5 minutes of rehydration) (Figure 2A). (T<sub>0</sub> – 5 min = 31.28 ± 3.39 lbf; 2 weeks = 20.95 ± 2.01 lbf; 4 weeks = 15.18 ± 1.87 lbf; 8 weeks = 14.54 ± 2.64 lbf; 12 weeks = 11.68 ± 1.78 lbf). Interestingly, prolonged product rehydration (T<sub>0</sub>-24 hr) also led to a significant reduction in mechanical tensile strength, which was not significantly different than Bard\* CollaMend\* Implant at 2, 4, 8, and 12 weeks post-implantation. (T<sub>0</sub> – 24 hr = 19.39 ± 2.72 lbf). No significant difference in the mechanical tensile strength of Bard\* CollaMend\* Implant was observed between 2, 4, 8, and 12 weeks *in vivo*. However, a significantly higher mechanical tensile strength was observed for

**Figure 2.****A) Tensile Strength**

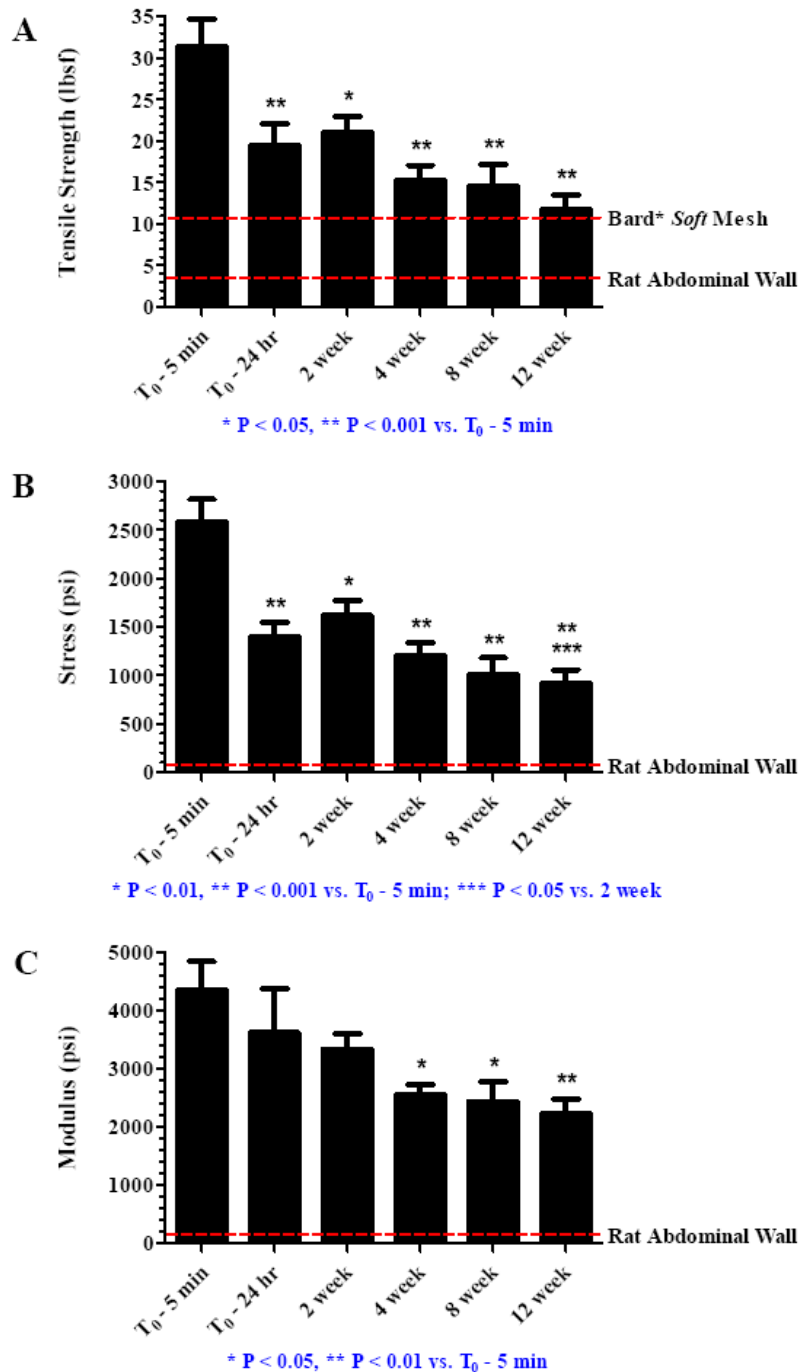
A significant (rehydration-dependent) reduction in mechanical tensile strength was observed at 2 weeks post-implantation, as compared to  $T_0$ . However, no significant difference was observed between 2, 4, 8, and 12 weeks *in vivo*. Further analysis also demonstrated that the mechanical tensile strength of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at 12 weeks was not significantly different than non-implanted Bard<sup>®</sup> Soft Mesh. The mechanical tensile strength of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant was significantly greater than that of rat abdominal wall at 2, 4, and 8 weeks post-implantation.

**B) Stress**

A significant (rehydration-dependent) reduction in mechanical stress was observed at 2 weeks post-implantation, as compared to  $T_0$ . A significant reduction in mechanical stress was also observed between 2 and 12 weeks *in vivo*. The mechanical stress of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant was significantly greater than that of rat abdominal wall at all observation time points.

**C) Modulus**

A significant reduction in mechanical modulus was observed at 4, 8, and 12 weeks post-implantation, as compared to  $T_0$ . The mechanical modulus of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant was significantly greater than that of rat abdominal wall at all observation time points.



Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at  $T_0$ , 2, 4, and 8 weeks post-implantation, as compared to rat abdominal wall. (Rat Abdominal Wall =  $3.41 \pm 0.24$  lbsf). Data also suggests that the mechanical tensile strength of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at 12 weeks post-implantation is not significantly different than

synthetic polypropylene mesh (Bard<sup>®</sup> Soft Mesh). (Cross Direction =  $10.78 \pm 0.23$  lbsf).

**Stress:**

Data indicates a significant reduction in the mechanical stress of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant

at 2, 4, 8, and 12 weeks post-implantation, as compared to  $T_0$  (following 5 minutes of rehydration) (Figure 2B). ( $T_0 - 5 \text{ min} = 2577 \pm 237 \text{ psi}$ ; 2 weeks =  $1614 \pm 158 \text{ psi}$ ; 4 weeks =  $1200 \pm 132 \text{ psi}$ ; 8 weeks =  $1003 \pm 176 \text{ psi}$ ; 12 weeks =  $917 \pm 140 \text{ psi}$ ). Interestingly, prolonged product rehydration ( $T_0 - 24 \text{ hr}$ ) also led to a significant reduction in mechanical stress, which was not significantly different than Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at 2, 4, 8, and 12 weeks post-implantation. ( $T_0 - 24 \text{ hr} = 1392 \pm 158 \text{ psi}$ ). No significant difference in the mechanical stress of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant was observed between 2, 4, and 8 weeks *in vivo*,

however a significant reduction was noted between 2 and 12 weeks. Data also demonstrates that the mechanical stress of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at 2, 4, 8, and 12 weeks post-implantation is significantly higher than rat abdominal wall. (Rat Abdominal Wall =  $86 \pm 6 \text{ psi}$ ).

#### Modulus:

Data indicates a significant reduction in the mechanical modulus of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at 4, 8, and 12 weeks post-implantation, as compared to  $T_0$  (following 5 min rehydration)



**Figure 3.**

Representative photographs of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant (histological test article) at 2, 4, 8, and 12 weeks following abdominal surgical defect repair and subcutaneous implantation. Angiogenesis and tissue incorporation were observed at both the muscular and subcuticular interface. Evidence of angiogenesis was also observed through follicular channels at 4, 8, and 12 weeks post-implantation.

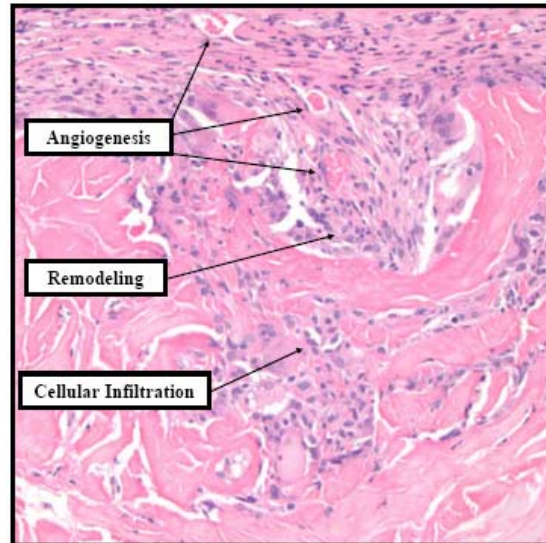
(Figure 2C). ( $T_0 - 5 \text{ min} = 4354 \pm 488 \text{ psi}$ ; 2 weeks =  $3337 \pm 261 \text{ psi}$ ; 4 weeks =  $2548 \pm 183 \text{ psi}$ ; 8 weeks =  $2436 \pm 331 \text{ psi}$ ; 12 weeks =  $2218 \pm 263 \text{ psi}$ ). Prolonged product rehydration ( $T_0 - 24 \text{ hr}$ ) did not lead to a significant reduction in mechanical modulus, as compared to  $T_0$  (following 5 min rehydration). ( $T_0 - 24 \text{ hr} = 1392 \pm 158 \text{ psi}$ ). A significantly higher mechanical modulus was also observed for Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at 2, 4, 8, and 12 weeks post-implantation, as compared to a standard rat abdominal wall. (Rat Abdominal Wall =  $163 \pm 15 \text{ psi}$ ).

#### Pathological Analysis:

Gross pathological analysis indicated that implantation of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant led to a minimal foreign body reaction, promoted cellular growth, and was rapidly surrounded with new host tissue by 2 weeks post-implantation. Angiogenesis was observed surrounding the device by 2 weeks, with evidence of vascular penetration and remodeling of the device through follicular channels by 4, 8, and 12 weeks post-implantation (Figure 3). Further microscopic pathological analysis demonstrated that implantation of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant resulted in a minimal inflammatory/fibrotic response, dominated by macrophages at the perimeter (and through follicular channels) of the device. Evidence of cellular infiltration, angiogenesis, and remodeling (localized to the perimeter and through follicular channels) of the device was observed at 4, 8, and 12 weeks post-implantation (Figure 4). The primary collagen scaffold within Bard<sup>®</sup> CollaMend<sup>®</sup> Implant was observed to be intact and functional at 12 weeks post-implantation.

#### **DISCUSSION**

The emergence of novel biologically-derived prosthetic materials for hernia repair / soft tissue reconstruction provides the surgeon with several alternatives to traditional synthetic mesh repair, particularly in the complex cases<sup>5</sup>. Both allograft (human) and xenograft (porcine and bovine) products are currently available which possess unique characteristics that may affect their overall mechanical and biological performance *in vivo*. An ideal biologically-



**Figure 4.**

Representative H&E photomicrograph of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant at 4 weeks following abdominal surgical defect repair and subcutaneous implantation (10X Objective). Angiogenesis, remodeling, and cellular infiltration was observed at the perimeter (and through follicular channels) of the device at 4, 8, and 12 weeks post-implantation.

derived material for hernia repair / soft tissue reconstruction should possess the following mechanical and biological characteristics following *in vivo* implantation:

1. Provide a primary scaffold for long-term mechanical reinforcement during the post-operative period
2. Resist rapid enzymatic degradation
3. Biocompatible, with low antigenicity
4. Support cellular growth
5. Promote angiogenesis and remodeling
6. Facilitate natural wound healing

In the present study, we describe the mechanical and biological characteristics of Bard<sup>®</sup> CollaMend<sup>®</sup> Implant. Bard<sup>®</sup> CollaMend<sup>®</sup> Implant is a sterile sheet of lyophilized, acellular, porcine dermal collagen and its constituent elastin fibers, which has been chemically cross-linked to resist rapid enzymatic degradation *in vivo*. Analysis of the pre- and post-implantation

mechanical characteristics of Bard\* CollaMend\* Implant indicate several properties representative of a biologically-derived material with long-term mechanical strength *in vivo*.

Data demonstrates an initial reduction in the mechanical tensile strength of Bard\* CollaMend\* Implant at 2 weeks post-implantation. However, no significant difference in tensile strength was observed between 2, 4, 8 and 12 weeks *in vivo*. Additional mechanical testing of Bard\* CollaMend\* Implant at T<sub>0</sub> (following 24 hrs. of rehydration) revealed a similar phenomenon. It is therefore interpreted that further product rehydration which occurs *in vivo*, may account for this early reduction in mechanical tensile strength. Hilger *et al.* and Dora *et al.* describe a similar proportional reduction in the mechanical strength for dermal products following abdominal implantation in rabbits<sup>6,7</sup>. However, it is important to note that in relation to synthetic polypropylene mesh, we demonstrate that the mechanical tensile strength of Bard\* CollaMend\* Implant at 12 weeks post-implantation was not significantly different than non-implanted Bard\* Soft Mesh (a light-weight large-pore synthetic polypropylene mesh). Data also suggests an initial significant (rehydration-dependent) reduction in the mechanical stress of Bard\* CollaMend\* Implant at 2 weeks post-implantation. Interestingly, a significant reduction in mechanical stress was also observed between 2 and 12 weeks *in vivo*. However, even at 12 weeks the mechanical stress of Bard\* CollaMend\* Implant was significantly greater than that of rat abdominal wall.

A recent shift in the field of hernia repair / soft tissue reconstruction has led to the development of light-weight large-pore synthetic materials in an effort to achieve a less stiff, more compliant surgical mesh<sup>8</sup>. In the present study, we demonstrate a significant reduction in the mechanical modulus (relative stiffness) of Bard\* CollaMend\* Implant at 4, 8, and 12 weeks post-implantation. As modulus is inversely proportional to compliance, this data suggests an increase in the compliance of Bard\* CollaMend\* Implant as *in vivo* remodeling of the device occurs.

Macroscopic and microscopic pathological analysis demonstrated that implantation of Bard\* CollaMend\* Implant led to a minimal foreign body reaction, with biocompatibility at 2, 4, 8, and 12 weeks post-implantation. These results are consistent with those reported by Macleod *et al.* and Zheng *et al.* following subcutaneous abdominal implantation of acellular porcine dermis in rats<sup>9,10</sup>. Interestingly, Zheng *et al.* also report a significantly reduced host inflammatory response to acellular porcine dermis (which also resolves more quickly) than that provoked by polypropylene mesh. Taken together, these data suggest that acellular porcine dermis is well accepted by the host following implantation.

Angiogenesis represents a critical event in the wound healing cascade, and provides a direct conduit for device cellular infiltration and collagen remodeling *in vivo*<sup>11,12</sup>. We observed evidence of angiogenesis surrounding Bard\* CollaMend\* Implant by 2 weeks, with vascular penetration and remodeling at the perimeter (and through follicular channels) of the device by 4, 8, and 12 weeks post-implantation. However, the primary collagen scaffold within Bard\* CollaMend\* Implant was observed to be intact and functional at 12 weeks post-implantation. These biological characteristics highlight the ability of Bard\* CollaMend\* Implant to promote angiogenesis and support cellular growth, while resisting rapid enzymatic degradation that may affect mechanical integrity *in vivo*.

## CONCLUSION

Overall, data from this study suggests that Bard\* CollaMend\* Implant represents a biocompatible biologically-derived prosthetic scaffold, optimized to inhibit rapid enzymatic degradation, support cellular growth, and maintain long-term mechanical properties *in vivo*.

## ACKNOWLEDGEMENTS

This study was conducted at DaVinci Biomedical Research Products, Inc., S. Lancaster, MA, an AAALAC accredited pre-clinical laboratory testing facility. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of DaVinci Biomedical Research Products, Inc., and was performed in compliance with all regulations regarding the humane treatment of laboratory animals. Funding for this study was provided by Davol Inc.

## COVER PHOTO

Scanning electron microscopy (SEM) photomicrograph of Bard® CollaMend® Implant prior to *in vivo* implantation (10,000X).

## DISCLAIMER

This study represents a pre-clinical evaluation of Bard® CollaMend® Implant following *in vivo* implantation in an animal model. The data presented herein is not intended to imply clinical product performance and/or circumvent sound clinical judgment.

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