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**IN VIVO EVALUATION OF ACELLULAR HUMAN DERMIS FOR
ABDOMINAL WALL REPAIR**

**For submission to the
Journal of Biomedical Materials Research Part A**

Abstract:

Limitations of synthetic biomaterials for abdominal wall repair have led investigators to seek naturally derived matrices, such as human acellular dermis, because of their excellent biocompatibility and their ability to naturally interface with host tissues with minimal tissue response. In this study we investigated two different biomaterials derived from human dermis (FlexHD Acellular Dermis and FlexHD Acellular Dermis – THICK) in a rabbit abdominal hernia repair model. One quarter of the abdominal wall was replaced with each biomaterial, and the animals were followed for up to 24 weeks. Rabbit hernias repaired with AlloDerm®, a commercially available acellular dermal matrix, and sham operated animals served as controls. Retrieved samples of these implants were assessed grossly and histologically. Collagen production measurements and tension studies were performed. FlexHD Acellular Dermis, FlexHD Acellular Dermis – THICK, and AlloDerm® maintained their strength in the rabbit hernia repair model with no incidence of hernia formation or bowel adhesion. The exact size measurements at 24 weeks were $217.0 \pm 20.9\%$ for FlexHD Acellular Dermis, $200.8 \pm 23.5\%$ for FlexHD Acellular Dermis – THICK, and $209.7 \pm 32.9\%$ for AlloDerm®. Macroscopic and microscopic evaluation showed excellent integration and tissue formation. All biomaterials studied harbored cells that produced new collagen fibers, and a 6-fold increase in these fibers was observed at 24 weeks. This study shows that acellular biomaterials derived from human dermis are suitable for abdominal hernia repair.

Keywords: Acellular matrices, biomaterials, human dermis, hernia repair

Introduction

Both synthetic implants and naturally derived tissue matrices have been used as biomaterials for abdominal wall repair¹⁻⁵. Although synthetic materials, such as polypropylene mesh, are able to provide the required tensile strength to support abdominal viscera, this material is associated with untoward effects, including fibrotic encapsulation, infection, erosion and mesh extrusion, and these issues remain a problem^{4,6-13}. Increasingly, naturally derived processed tissue matrices, such as human acellular dermis, human dura and bovine pericardium, have been used because of their biocompatible characteristics¹⁴⁻¹⁶. Biocompatibility is one of the most critical factors when considering a material for clinical use¹⁷. For this reason, investigators have sought a tissue matrix that could naturally interface with host tissues with minimal host response¹⁸⁻²¹.

An ideal biomaterial for abdominal wall repair should be biocompatible and non-immunogenic. It should also be able to promote the formation of new tissue through cellular ingrowth and be able to withstand the tensile forces created by the abdominal viscera and maintain structural integrity^{2,3,15}. Because acellular human dermis appears to possess these characteristics, it has been evaluated as a material for abdominal wall repair. FlexHD Acellular Dermis and FlexHD Acellular Dermis-THICK are matrices that are naturally derived, biodegradable, and primarily composed of collagen from human skin. Both materials were developed as biomaterials for surgical fascial replacement and have good tissue handling characteristics²². While there is a commercially available

product (AlloDerm®) that originates from the same tissue source, the processing techniques distinguish these materials from each other.

AlloDerm® is an acellular dermal matrix prepared from human skin. Skin is procured from cadaveric donors and aseptically processed to remove the epidermis. A hyperosmolar sodium chloride solution is used to separate the dermal and epidermal layers. Sodium deoxycholate is then utilized for cell removal. The acellular dermis is cryoprotected prior to freeze drying and packaging, leaving an intact matrix with basement membrane components²³. AlloDerm has been reported to be a suitable biomaterial in several clinical applications, including abdominal wall repair²³⁻²⁷ and breast reconstruction²⁸.

The present study was designed to assess the biocompatibility and feasibility of using this type of acellular dermis matrix as a fascial tissue substitute for abdominal wall repair. The objectives of this study were to test the ability of acellular dermal matrix to support tissue formation in the abdomen and to determine the utility of acellular dermis as a biocompatible fascial tissue substitute for abdominal wall repair. We examined whether acellular dermis provides adequate support and strength for *in vivo* abdominal applications.

MATERIAL AND METHODS

Human Dermis Preparation

Flex HD Acellular Dermis (group I) and FlexHD Acellular Dermis-THICK (group II) are derived from human allograft skin that is processed using proprietary procedures developed by MTF (Musculoskeletal Transplant Foundation, Edison, NJ). Prior to tissue harvest, the donor's medical and social histories are screened for any abnormal medical pathology, disease processes and other pertinent information that would cause the donor to be unsuitable for human transplant. The screening process is completed by the MTF's highly trained National On-Call Coordinators using the strict standards developed by the MTF Medical Board of Trustees. Donors are screened for a series of diseases including, but not limited to, HIV, HTLV I and II, hepatitis B and C and syphilis. MTF donor screening procedures and serologic and microbiologic testing policies meet current established industry standards. At MTF, skin is processed aseptically in a controlled clean room environment that meets current AATB standards²⁹.

Donor skin grafts are decellularized to reduce the occurrence of adverse immune responses in graft recipients^{30,31}. This decellularization procedure involves a series of soaks and water rinses performed under gentle agitation. The tissue is first soaked overnight at ambient temperature in a hypertonic, 1M sodium chloride solution, followed by water rinses. The skin is then soaked in 0.1% Triton X-100 non-ionic detergent to remove cellular fragments. A thorough water rinse is then performed to remove detergent residuals from the tissue. A residual detergent test using Gas Chromatography - Mass Spectrometry (GC-MS) and High Pressure Liquid

Chromatography (HPLC) is performed to ensure detergent levels are sufficiently low. The measured concentration of sodium chloride and Triton X-100 must be lower than 10 PPM. The acellular dermis is then subjected to a disinfection process in a solution containing alcohol, peracetic acid [less than 1% (v/v), prepared from 35-40% stock solution], and propylene glycol. The matrix is gently agitated under vacuum and then rinsed with sterile water. Residual analysis of reagents used in the preparation of acellular dermis are then performed using GC-MS and HPLC to confirm that residuals are below established safety limits (0.001%). Matrix integrity is assessed throughout processing using light and electron microscopy to verify normal collagen bundle patterns and macroscopic collagen matrix structure, as well as to confirm the removal of cellular material. Following disinfection, the dermal matrices are immersed in 70% ethanol solution and packaged in a sterile impermeable pouch. The biomaterials are tested for sterility in accordance with procedures in the current United States Pharmacopeia (USP) ³².

The MTF dermal disinfection process has been validated to demonstrate a six log reduction in a panel of microorganisms most commonly found in the body or on the skin. These include *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Clostridium sporogenes* and *Streptococcus pyogenes*. In addition to its effectiveness for bacterial inactivation, this process has been demonstrated to be effective for inactivation of certain viruses. The decontamination process was evaluated in accordance with the International Conference on Harmonization for Viral Clearance Studies of Biotechnology

Products. Studies established that this decontamination process was effective in inactivation of human immunodeficiency virus type I (HIV-I), human hepatitis A virus (HAV), porcine parvovirus (PPV) as a model for human parvovirus B19 (HPV), human polio virus, bovine viral diarrhea virus (BVDV) as a model for human hepatitis C virus (HCV), and pseudorabies virus (PrV) as a model for human cytomegalo (CMV) herpes virus.

Furthermore, the safety and biocompatibility of acellular dermis prepared following these procedures is evaluated using a test strategy developed in accordance with ISO 10993, Biological Evaluation of Medical Devices – Part 1: Evaluation and Testing and the FDA G95-1 Memorandum that guides the use of this international standard. Acellular dermis is evaluated in consideration of the biomaterial as an implant with contact to tissue. Tests include evaluation of cytotoxicity, sensitization, irritation, acute systemic toxicity, material-mediated pyrogenicity, genotoxicity, implantation, chronic toxicity, and carcinogenicity. Test results indicate that acellular dermis is non-cytotoxic, not a sensitizer, not an acute toxin, and non-pyrogenic. Genotoxicity studies indicated that extracts of acellular dermis were non-clastogenic in the Chinese hamster ovary in vitro test system and in the in vivo Mouse Micronucleus test and were non-mutagenic in the Ames bacterial test system.

The main difference between the FlexHD Dermis and Dermis-THICK grafts is, as the name implies, the thickness. Human Dermis (group I) consists of the dermal layer of human skin and has a median thickness of 0.45 ± 0.15 mm. The FlexHD Dermis-THICK

(group II) consists of human dermis and parts of the hypodermis, and has a median thickness of 1.0 ± 0.5 mm. The AlloDerm® (LifeCell Corporation, Branchburg, NJ) used in this study is commercially available. The material thickness ranges from 1.8 mm to 3.3 mm according to the package labeling.

Biomaterial characterization and ultra structure

Prior to *in vivo* implantation all 3 biomaterials were analyzed using scanning electron microscopy (SEM). For ultrastructural analysis, the biomaterials were placed in PBS and frozen at -80°C . After lyophilisation under vacuum, the samples were sputtered using a gold platinum target and analyzed using a scanning electron microscope at various magnifications. Morphometric analysis was performed to evaluate the pore size by measuring 15 random pores. In oval shaped pores the smaller diameter was recorded. Further, pepsin-soluble collagen was measured by collagen assay and biomechanical testing was performed (see below).

Animal Model

The animal study was performed in accordance with the Animal Care and Use Committee (ACUC) at Wake Forest University. A total of 48 New Zealand White rabbits weighing approximately 3 kg were randomized into 4 groups (Table 1): Group I animals received FlexHD Acellular Dermis grafts and group II animals received FlexHD Acellular Dermis-THICK grafts. Two control groups were used. In Group III, the commercial product AlloDerm® was used, and in group IV a sham operation was performed. Four animals from each group were sacrificed at 6, 12 and 24 weeks after implantation.

Surgery Preparation and Anesthesia

The animals were sedated with an intramuscular injection of 35 mg/kg ketamine and 5mg/kg xylazine. The animals were intubated and anesthesia was maintained with 2% to 3% isoflurane. One milliliter of blood was drawn for analysis (hematogram) prior to graft implantation and at the time of sacrifice. All animals received a single dose of antibiotics (Enrofloxacin 2.5 mg/kg). The surgical site was shaved and prepped in a sterile manner. The animals were placed in the supine position.

Abdominal Surgery

A midline abdominal incision was made and the subcutaneous tissue layer on the left side of the abdomen was bluntly dissected. A 4 x 6 cm defect was created by excising a full thickness portion of the abdominal wall. Care was taken not to harm internal organs. All biomaterials were soaked for 10 minutes in normal saline followed by a soak in 0.1% gentamicin solution for 10 minutes, after which the material was trimmed to fit the defect. The biomaterial was sutured to the abdominal musculature with 3-0 Prolene (non-absorbable) running sutures and the exact size was measured and recorded.

The sham operation included a midline abdominal incision with dissection of the same subcutaneous layers, but without removal of tissue. The abdomen was then closed with 3-0 Prolene sutures in a running fashion. The wound closure was performed in a running fashion with subcutaneous 3-0 Vicryl (absorbable) and 3-0 PDS (absorbable) sutures for the skin.

Postoperative Care

At the end of the procedure, the animals were extubated and transferred to the postoperative care unit. All animals were closely observed for at least 4 hours until fully awake. An intramuscular dose of buprenorphine (0.02 mg/kg) was given every 12 hours for the first 72 hours post-operatively, and as needed thereafter. Fecal and urinary output were monitored and recorded.

Implant retrieval and gross examination

Immediately after euthanasia, the implant site was inspected. Parameters assessed via gross examination included hernia formation, size and integration of the biomaterial into surrounding connective tissue. The sites were also inspected for evidence of fibrosis, infection and inflammatory responses. The dimensions of each biomaterial segment were measured and the average change was expressed as a percent of the original size. The size of the biomaterials prior to surgery was defined as 100%.

Measurements of abdominal bulging and hernia formation were made after euthanasia, with the abdomen still closed, by laying the animal in supine position and gently pressing on the abdominal wall surrounding the implant. Any extrusion of the abdomen with significant ballooning of greater than 3cm was recorded as bulging. Hernia was defined as macroscopic rupture of the biomaterial at the time of retrieval.

The implanted biomaterials were harvested through a midline incision. To reduce the risk of perforation of the implanted grafts, the abdomen was opened with an incision through the rectus muscle on the opposite side. Adhesion to the biomaterial was scored for extent (%) using the Modified Diamond Scale (0 = 0%, 1 \leq 25%, 2 = 25-50%, 3 > 50%)³³. After assessing the levels of adherence, the biomaterial was freed from the

surrounding tissues. The entire implant was removed en bloc and measured. The harvested biomaterial was cut into separate segments for histology, biomechanical studies, collagen assay and storage at -80 °C. A 5 x 6 cm piece of the abdominal muscle was harvested from the sham operated animals as a normal control.

Abdominogram

Abdominograms were performed by injecting 20 ml of contrast media (Optiray 240, Malinckrodt Inc, St. Louis, MO) into the abdominal cavity at the time of sacrifice. After waiting for several minutes for the contrast media to collect at the site of the hernia repair, lateral radiography was performed (Siemens Siremobil compact L, Germany).

Histology

The retrieved tissue specimens were fixed in 10% neutral formalin for 12 hours. All specimens were rinsed 3 times in PBS and placed in the tissue processor (Citadel 1000, Thermo-Shandon). Five micron thick sections were obtained from the paraffin embedded blocks (RM2145, Leica). Some sections were stained using hematoxylin and eosin (H&E), a staining method widely used for the assessment of local inflammatory response. Furthermore, Masson's Trichrome staining was used to assess collagen deposition. For each group, evidence of inflammatory response and quality of the collagen layer were noted. No quantitative analyses were performed.

Biomechanical Studies

Rectangular tissue strips (50 mm x 10 mm) were obtained from the abdominal hernia repairs. Strips of native abdominis externus fascia of equal size were used in the sham

group (normal control). Tensile tests (Instron model 5544 with 2716-016-1kn Grips, Norwood, MA, USA) were performed by elongating the tissue strips longitudinally at a speed of 0.05 mm/second with a preload of 0.2 N until failure. The grip-to-grip spacing was 2 cm. All specimens were tested at room temperature and kept moist. The maximum tensile strain and stress forces were determined. For the determination of strain the grip to grip distance was taken into account. The results of the 6, 12 and 24 weeks time points were analyzed.

Collagen Assay

Two rectangular pieces of the biomaterial (5mm x 5mm) from each animal were used to quantify the pepsin-soluble collagen fraction. This is a well established assay to define the amount of newly produced collagen present in a sample 34,35. Both samples were taken 1 cm from the suture line. Fat and peritoneal layers were carefully removed and the samples weighed. All samples were placed in 0.5 N acetic acid containing 1 mg pepsin per 10 mg tissue. Samples were kept on an orbital shaker for 12 hours. After centrifugation at 2000g for 10 minutes, 100 µl supernatant was assayed using a kit according to the manufacturer's protocol (Sircol, Biocolor Ltd., Newtownaffey, Ireland). Briefly, 1ml of Sircol dye reagent was added and the samples were mixed for 30 minutes. After centrifugation, the pellet was suspended in 1 ml of alkali reagent included in the kit and read at 540 nm with a spectrophotometer. Standards were produced using the collagen supplied in the kit. The results were expressed as µg collagen per mg of biomaterial (wet weight).

Statistical Analysis

All numerical data are expressed as averages and the corresponding standard error of the mean (SEM). For statistical analysis we used SPSS v11 (SPSS Inc). Differences between the groups and different time points were analyzed by one-way analysis of variance (ANOVA) followed by a Bonferroni test for multiple comparisons. A p value of less than 0.05 was considered significant.

Results

Characterization and ultra structure of the biomaterials

The ultrastructural analysis with SEM demonstrated a distinct difference between the biomaterials (Figure 1). FlexHD Acellular Dermis showed a layered structure with dense protein layers. The FlexHD Acellular Dermis-THICK demonstrated large pores throughout the biomaterial and the layering was partially interrupted. AlloDerm® showed distinct layering of protein dense structures, with few pores connecting the layers. The morphometric measurement of the pore size found similar widths for all materials ($9.7 \pm 1.0 \mu\text{m}$ for the FlexHD Acellular Dermis, $24.8 \pm 2.9 \mu\text{m}$ for the FlexHD Acellular Dermis-THICK and $17.8 \pm 1.4 \mu\text{m}$ for the AlloDerm®).

In summary, the layering was most dominant in AlloDerm ® (AlloDerm ® > FlexHD Acellular Dermis > FlexHD Acellular Dermis-THICK). The thickness of the biomaterial and the pore diameter was the largest for FlexHD Acellular Dermis-THICK (FlexHD Acellular Dermis – Thick > AlloDerm ® > FlexHD Acellular Dermis). All 3 biomaterials consisted mainly of mature collagen fibers. The level of pepsin soluble pre-collagen remained consistently low with values of $0.5 \pm 0.1 \mu\text{g}/\text{mg}$ for FlexHD Acellular Dermis, $0.5 \pm 0.1 \mu\text{g}/\text{mg}$ for FlexHD Acellular Dermis–THICK and $0.6 \pm 0.2 \mu\text{g}/\text{mg}$ for AlloDerm® (see also collagen assay below).

In Vivo Animal Studies

There were no surgical complications or postoperative problems. One animal expired during intubation and was replaced immediately with a new rabbit, since this death was unrelated to the study.

Gross examination

The implants and the surrounding tissues were inspected grossly after euthanasia (Figure 2). There were no hernias observed at the time of retrieval in any of the groups. However, bulging of the biomaterial without tearing was observed in all groups but sham. The FlexHD Acellular Dermis group contained 6/16 animals with bulging (2 at 6 weeks, 2 at 12 weeks and 2 at 24 weeks), the FlexHD Acellular Dermis-THICK group had 5/16 (2 at 12 weeks and 3 at 24 weeks) and the AlloDerm® Group had 3/16 (3 at 24 weeks). The exact size of the biomaterial was measured at the time of implantation and at sacrifice. All biomaterials used in this study showed a distention of approximately 200% at 24 weeks (Figure 4). The exact measurements for FlexHD Acellular Dermis at 6, 12 and 24 weeks were $179.8 \pm 27.8\%$, $208.0 \pm 33.7\%$ and $217.0 \pm 20.9\%$, for FlexHD Acellular Dermis-THICK $160.1 \pm 10.8\%$, $200.3 \pm 20.2\%$ and $200.8 \pm 23.5\%$, and for AlloDerm® $140.7 \pm 2.5\%$, $151.5 \pm 16.4\%$ and $209.7 \pm 32.9\%$.

Further, none of the animals developed bowel adhesions to the biomaterial. Occasionally, a small adhesion to the suture material (PDS 4-0) was seen and bluntly dissected.

Gross examination of the retrieved tissues 6 weeks after implantation was similar for all 3 biomaterials. All acellular grafts showed evidence of early vascularization with visible blood vessels on the peritoneal side of the biomaterial. There were no signs of inflammation, infection or fibrosis and the biomaterials were stably embedded into the host subcutaneous tissues. Further, no seromas or shrinkage of the biomaterials were recorded. One animal from the FlexHD Acellular Dermis group showed the formation of

a small nodule. A small biopsy of this nodule was sent off for bacteriological examination, but was negative for both aerobic and anaerobic bacterial growth.

At 12 weeks after implantation, the biomaterials were well integrated into the abdominal wall and the subcutaneous tissue. At this time, some biomaterials showed signs of thinning, becoming more transparent compared to the starting material. These transparent areas were framed by fibrous connective tissue. In the FlexHD Acellular Dermis all implantations showed some thinning. In the FlexHD Acellular Dermis-THICK group, only one biomaterial showed this thinning. In the AlloDerm® group, the biomaterials from 3 animals showed the formation of thin, transparent areas between fibrous connective tissue.

This thinning was more prominent at 24 weeks (Figure 3) and some of the samples had the appearance of a thick spider web. In the FlexHD Acellular Dermis group, 3 out of 4 samples were very thin, and in the FlexHD Acellular Dermis-THICK group 2/4 implants demonstrated this thinning. All AlloDerm® implants had some thin areas with sparse collagen fibers, but the AlloDerm® implants appeared thicker than the implants from the other groups. One animal from the AlloDerm® group showed signs of calcification within the biomaterial. The sham operated group showed a tight wound closure with no sign of inflammation at the suture line after 6, 12 and 24 weeks of implantation.

Blood Analysis

Blood analysis was performed prior to implantation and at the time of sacrifice. The white blood counts (WBC) before implantation and at 6, 12 and 24 weeks were all within the range of healthy animals. None of the animals showed a significant rise of WBC

(>30%) commonly seen during stress or infection³⁶. This indicates that there is no systemic reaction to any of the biomaterials used.

Abdominogram

Abdominograms were performed in 2 random cases for each experimental group at each time point. No contrast media leakage into the abdominal tissue or herniation was observed in the studied animals. All groups showed a smooth contour of the peritoneal tissue layer without signs of bowel attachment.

Histology

Hematoxylin and Eosin and Trichrome staining were performed on tissue from each group. The matrix structure was intact in all groups at all time points. FlexHD Acellular Dermis, FlexHD Acellular Dermis-THICK and AlloDerm® implants showed longitudinal collagen bundles, parallel to the tension lines of the abdominal wall, at all time points (Figure 5). Histologically, the appearance of the collagen bundles did not change during the entire study period (Figure 6). No lymph follicle formation was present in any of the abdominal graft samples. The findings in the sham animals were unremarkable, and included a low grade inflammatory response and some fatty degeneration of the muscle along the suture line.

At the 6 week time point, the FlexHD Acellular Dermis group showed an inflammatory response characterized by an infiltration of host cells that appeared to be lymphocytic in nature. The inflammatory cells were concentrated at the interface of biomaterial and muscle. FlexHD Acellular Dermis, FlexHD Acellular Dermis-THICK and AlloDerm®

implants showed extensive neovascularization with small blood vessels on the abdominal side of the biomaterial. At 12 weeks, cellular infiltration into the middle biomaterial was complete and blood vessels found in the material were larger than those observed at 6 weeks. There were no cell-free patches within the biomaterial. At 12 weeks a shift from inflammatory cells towards the presence of spindle shaped fibroblasts was evident, when compared to 6 weeks. At 24 weeks, a well organized fibroblastic ingrowth was present and new tissue formation was seen.

The findings in the FlexHD Acellular Dermis-THICK group were very similar. An early inflammatory response in the interface regions could be seen. Complete cellular penetration and the appearance of aligned, spindle shaped cells were observed after 12 weeks. The histology at 24 weeks closely resembled the findings of native external abdominal aponeurosis.

Alloderm® demonstrated a rapid infiltration of host cells from the suture line at 6 weeks. However, the central regions of the implanted material remained free of cells for more than 12 weeks. At 24 weeks, the levels of cellular penetration were comparable to the FlexHD groups. Further, the spindle shaped cells present within longitudinal bundles of collagen were aligned similar to normal fascia.

In summary, all three biomaterials showed a mildly inflammatory, wound healing response at 6 weeks. The levels of neovascularization were comparable for all materials. Ingrowth of cells and infiltration of spindle shaped cells were greater for FlexHD Acellular Dermis and FlexHD Acellular Dermis-THICK group. AlloDerm® had cell free areas for over 12 weeks. However, the final outcome was very similar after 24 weeks.

Biomechanical Studies

The biomechanical characteristics of the investigated biomaterials were measured at 6, 12 and 24 weeks and compared to native abdominal fascia (Figure 7). FlexHD Acellular Dermis and FlexHD Acellular Dermis-THICK grafts demonstrated a decrease in maximal tensile stress (MPa) from 6 to 12 weeks followed by a significant increase in strength from 12 to 24 weeks. At that time, values similar to native fascia were observed. AlloDerm®, on the contrary, was stronger at the start of the experiment but gradually showed a sustained loss of maximal tensile strength over time.

The maximal strain at rupture showed values that are comparable to native connective tissue for all biomaterials. The strain of FlexHD Acellular Dermis decreased from 6 to 12 weeks and regained normal values at 24 weeks. Both the FlexHD Acellular Dermis-THICK graft and AlloDerm® began with a higher strain than normal tissue at 6 weeks, which decreased in a linear fashion over time. However, both materials remained within an acceptable range of strain values during the study period.

The Young's modulus (E), a measure of the stiffness of a given material, was used to evaluate the elastic modulus of the biomaterials at 6, 12 and 24 weeks. All sampled tissues were within the range of native soft tissue. Both FlexHD Acellular Dermis grafts showed a loss of modulus from 6 to 12 weeks, but the values increased to normal values from 12 to 24 weeks. AlloDerm® showed a loss in modulus over time, but still remained close to native controls.

Collagen Assay

Quantification of newly synthesized collagen was performed using a pepsin-soluble collagen assay. This assay is able to quantify the cumulative synthesis of new collagen within the biomaterial^{37,38}. All investigated biomaterials harbored cells that produced new collagen. A 6-fold increase was observed at 24 weeks (Figure 4). In the FlexHD Acellular Dermis group, production of collagen remained low until 12 weeks. However, at 24 weeks, production of collagen by ingrown cells was similar to the other groups. The FlexHD Acellular Dermis-THICK showed a 2-3 fold increase in the newly formed collagen at 6 and 12 weeks, while the AlloDerm® showed a 3 fold increase at 6 and 12 weeks. At 24 weeks, all biomaterials showed similar amounts of newly produced collagen. There were no statistical significant differences between the groups.

Discussion

The current standard for abdominal hernia repair is the use of a propylene mesh. However, this non-absorbable biomaterial is known to induce the formation of a fibrotic capsule through overproduction of collagen by infiltrating fibroblasts. Although this response can successfully repair a hernia, many patients report discomfort due to its stiffness³⁹. Acellular biomaterials derived from tissues have been suggested as an alternative to polypropylene mesh in abdominal fascial repair surgery. These materials have been shown to have excellent biocompatibility in other systems^{3,17,18,20,40}. They appear to allow for cellular ingrowth and new tissue formation while maintaining biomechanical properties similar to native connective tissue^{1,14,18,41}. The findings presented here show that acellular biomaterials derived from human dermis meet the requirements needed for successful hernia repair. Biocompatibility was assessed in several ways, including gross examination of the biomaterial at retrieval, microscopic evaluation and testing for systemic response.

Further, the tissue handling and surgical characteristics were excellent as the biomaterials remained soft and malleable throughout the study period. The aims of this study were to investigate the ability of acellular dermal matrix to support tissue formation in the abdomen and to determine the utility of acellular dermis as a biocompatible fascial tissue substitute for abdominal wall repair. We compared two new acellular human dermis preparations (FlexHD and FlexHD-THICK) with a commercially available product (AlloDerm®). Although the origin of these biomaterials is the same, there are some distinct differences in the processing techniques used to obtain them.

While FlexHD is decellularized by a commonly used non-ionic detergent (Triton-X), LifeCell Inc. uses deoxycholic acid and EDTA. Further, FlexHD is decontaminated using an oxidative treatment alone (peracetic acid <1%), while AlloDerm® might contain residual gentamicin, cefoxitin, lincomycin, polymyxin B and vancomycin from different decontaminating washes, and these may potentially trigger an allergic reaction after implantation⁴²⁻⁴⁵. Lastly, for packaging and storage, AlloDerm® is freeze-dried and requires refrigeration, but FlexHD is soaked in ethanol before packaging and has a shelf life of 3 years at room temperature (Alloderm product information sheet, USFDA). This difference is important, because recent research has shown that dried acellular biomaterials may not perform as well in terms of cellular attachment and growth, which may be due to a change in ultrastructural morphology⁴⁶.

Despite these differences, all dermis implants showed regional tissue formation with fibroblastic infiltration, which developed an alignment parallel to the tension lines of the abdominal wall. However, there was no layering according to normal anatomy. Further, none of the biomaterials induced bowel adhesions, which are commonly seen when synthetic biomaterials are used⁴⁷⁻⁵¹. Collagen assays indicated that synthesis of new collagen fibers occurred within the biomaterials. This could be one possible explanation for the increased strength of the biomaterials after 12 weeks.

Microscopically, all biomaterials showed penetration of inflammatory cells and spindle shaped fibroblasts. At 12 weeks, cellular infiltration into the middle of the biomaterial was complete in both FlexHD grafts, while AlloDerm® still contained some cell-free regions between dense collagen bundles. Accelerated cell penetration could be beneficial, as it could decrease the risk of postoperative infection of the biomaterial and

expedite remodeling. On the other hand, this might lead to an early loss of strength through increased degradation of the biomaterial. However, at 24 weeks, there was no difference in cellularity, alignment of fibroblasts or vascularity between the study groups.

One key to a successful abdominal hernia repair is the selection of an appropriate biomaterial that can withstand changing forces, such as those that may occur during sneezing or coughing. In this regard, all of the biomaterials studied possess the necessary strain characteristics for a fascial substitute^{7,52-54}. None of the animals included in this study developed hernias. However, all groups contained some animals in which bulging of the biomaterial was evident. This is probably due to degradation of the biomaterial after implantation and the high variability of the structure of naturally derived biomaterials. This could result in weak areas of the material which stretch over time. The excised area corresponds to the replacement of approximately 1/4 of the abdominal wall by a biomaterial. Further, the horizontal orientation of the body axis maximizes the pressure on the implanted biomaterial. Taken together, the extension of the biomaterials to approximately 200% at 24 weeks without rupture is promising. The use of multiple layers of an acellular biomaterial may reduce variability and improve the strength of the material.

In this study we have applied a large piece of biomaterial, which may raise concerns that such a large amount of matrix may lead to untoward systemic effects^{55,56}. We have assessed the systemic response by evaluating the complete blood counts (CBC) and differentials. None of the animals showed significant changes due to the implantation of the biomaterial. Therefore, a systemic effect of acellular biomaterials derived from human dermis can be ruled out.

Summary

FlexHD Acellular Dermis, FlexHD Acellular Dermis – Thick, and AlloDerm® maintained their strength in the rabbit hernia repair model with no incidence of hernia formation and no bowel adhesion. Macroscopic and microscopic evaluation showed excellent integration, tissue formation and production of new collagen in the biomaterials. We conclude that FlexHD Acellular Dermis, FlexHD Acellular Dermis – Thick, and AlloDerm®, which are derived from human dermis, are suitable biomaterials for abdominal hernia repair.

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Legends

Figure 1. Biomaterial Ultrastructure: Scanning Electron Microscopy of FlexHD Acellular Dermis (A, D, G), FlexHD Acellular Dermis-THICK (B, E, H) and AlloDerm® (C, F, I).

The surface of the biomaterials (A-C), scale bar represents 200µm. FlexHD Acellular Dermis and AlloDerm® show strong collagen layers without significant pores throughout the material. FlexHD Acellular Dermis-THICK graft shows many pores penetrating the biomaterials top layer. Side view of the biomaterials (D-F), scale bar represents 500 µm. The difference in thickness is clearly visible (arrows). Further, FlexHD Acellular Dermis and AlloDerm® show a strict orientation of the collagen layers, this layering is less strict in FlexHD Acellular Dermis-THICK grafts. Higher magnification of side view, scale bar represents 50 µm (G-I), showing the different pore size, orientation and thickness of collagen fibers.

Figure 2. Gross Examination. Macroscopic view of the implants at retrieval after removal of the dermal and fat layer at 24 weeks. A- FlexHD Acellular Dermis, B- FlexHD Acellular Dermis-THICK, C- AlloDerm®, D- Sham operation. White dots indicate the corners of the biomaterial.

Figure 3: Gross Examination, peritoneal side. Direct macroscopic view of the peritoneal side of the biomaterial at 24 weeks showing the thinning areas surrounded by

thicker collagen bundles. A- FlexHD Acellular Dermis, B- FlexHD Acellular Dermis-THICK, C- AlloDerm ®,

Figure 4: Size of Biomaterial and Collagen Assay

A) All biomaterials showed a relaxation to approx. 200% at 24 weeks. AlloDerm® showed less distention at 6 and 12 weeks. However, at 24 weeks the expansion was comparable to FlexHD Acellular Dermis Grafts. B) All biomaterials showed the synthesis of new pepsin-soluble collagen through ingrown cells. Compared to FlexHD Acellular Dermis-THICK and AlloDerm®, FlexHD Acellular Dermis was lagging in collagen production at 6 and 12 weeks ($p=0.031$). However, at 24 weeks the amount of newly formed collagen was similar in all groups. The asterisk indicates a statistically significant difference between groups.

Figure 5: Histology. Hematoxylin and Eosin (12 Weeks).

The histology shows a mixed cell infiltration and areas with beginning fibroblastic alignment (arrows) in all biomaterials. There were no signs of infection or severe inflammation. A- FlexHD Acellular Dermis, B- FlexHD Acellular Dermis-THICK, C- AlloDerm ®, D- Sham operation. All images reduced from 100x

Figure 6. Histology. Trichrome Stain (12 weeks).

The collagen bundles (blue) remained intact throughout the duration of the study in all biomaterials. A- FlexHD Acellular Dermis, B- FlexHD Acellular Dermis-THICK, C- AlloDerm ®, D- Sham operation. All images reduced from 100x

Figure 7. Biomechanical Studies

A) Maximal tensile stress (MPa) at 6, 12 and 24 weeks demonstrating the loss and regain of tensile stress for FlexHD Acellular Dermis Grafts. AlloDerm® lost maximal tensile stress over time. At 12 weeks the differences were significant when compared to normal, FlexHD Acellular Dermis $p > 0.001$, FlexHD Acellular Dermis-THICK $p = 0.001$ and AlloDerm® $p = 0.023$. There was no significant difference between the 3 biomaterials. (B) The analysis of the maximum strain at rupture showed values well within the range of native soft tissues at all time points. There was a trend towards loss of strain over time for FlexHD Acellular Dermis-THICK and AlloDerm®. FlexHD Acellular Dermis shows a loss and regain of strain over the study period. C) The modulus, a measure for the stiffness of a material, demonstrated a continuous loss for AlloDerm® and a reduction and regain of modulus for FlexHD Acellular Dermis Grafts. At 12 week only FlexHD Acellular Dermis-THICK was significantly different when compared to normal fascia, $p = 0.009$. All values are compared to native fascia indicated as normal (2x SEM, standard error of the mean). The asterisk indicates a statistically significant difference between experimental and normal fascia groups. Values for native fascia are shown in the graphs as gray bars labeled with “normal” (2x SEM).

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