Development of a Novel Acellular Dermal Matrix (ADM) Designed Specifically for Plastic and Reconstructive Surgery

MTF Biologics Research and Development Department
125 May Street, Edison, NJ 08837

READ THE STUDY

FIGURES AND TABLES

INTRODUCTION

Human allograft dermal tissue has been widely accepted for clinical use in general surgery for decades. More recently, the use of dermis has expanded to other plastic and reconstructive surgery applications. The requirements for the ideal tissue matrix may vary depending on the procedural application. As such, MTF Biologics has developed acellular dermal matrices (ADMs) tailored to different applications.

Through detailed analysis, differences in the microstructure of human dermis have been observed. It is recognized that these differences can be utilized to provide matrices with different structural properties to meet procedural application needs. It is these structural properties that affect the behavior of the material under certain loading and biologic conditions.

The microstructure of skin is shown in Figure 1. With the originally available dermal tissue form, FlexHD® Structural, the epidermal layer is removed via processing and the upper layer of the dermis structure is decellularized and aseptically processed to meet sterility testing requirements[1]. FlexHD Structural has been proven to be a clinically successful implant for abdominal hernia repair[2,3,4,5,6,7], with tensile strength and stiffness exceeding other commercially available biologic materials[8].

While the strength and stiffness of FlexHD Structural are necessary characteristics of a graft in an application requiring substantial strength, there are other applications where pliability and ability to conform to natural body contours are more important. Accordingly, MTF sought a route to provide a more flexible, pliable dermal tissue while maintaining the natural structure and sufficient tensile strength.

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The key requirement, based on surgeon feedback, was to establish a predictably supple and flexible material with a uniform pliability and thickness sufficient to stretch and expand without tearing during tissue expansion. Finally, the ideal material would also allow rapid and efficient cellular ingrowth.

Based on the microstructure of human dermis and MTF's proprietary process for achieving sub-cuts to isolate specific layers of the dermis, a new tissue form was developed known as FlexHD® Pliable. This human ADM is derived from the reticular layer, a deeper cut of dermal tissue compared to traditional ADMs, taking advantage of the difference in matrix structure of this region compared to the region directly adjacent to the epidermis. The collagen structure is more loosely packed and open in this sub-cut. This leads to increased pliability and improved handling characteristics, which make the tissue more drapeable and conformable. It also provides a more open collagen architecture, which may allow for quicker cellular infiltration and integration.

In order to evaluate the biomechanical properties of this novel ADM, FlexHD Pliable was compared to FlexHD Structural, and two commercial human ADMs commonly used in Plastic and Reconstructive surgery applications: AlloDerm® and AlloDerm® RTM Ready to Use (LifeCell, Branchburg, NJ). This paper reports the results of these material characterizations.

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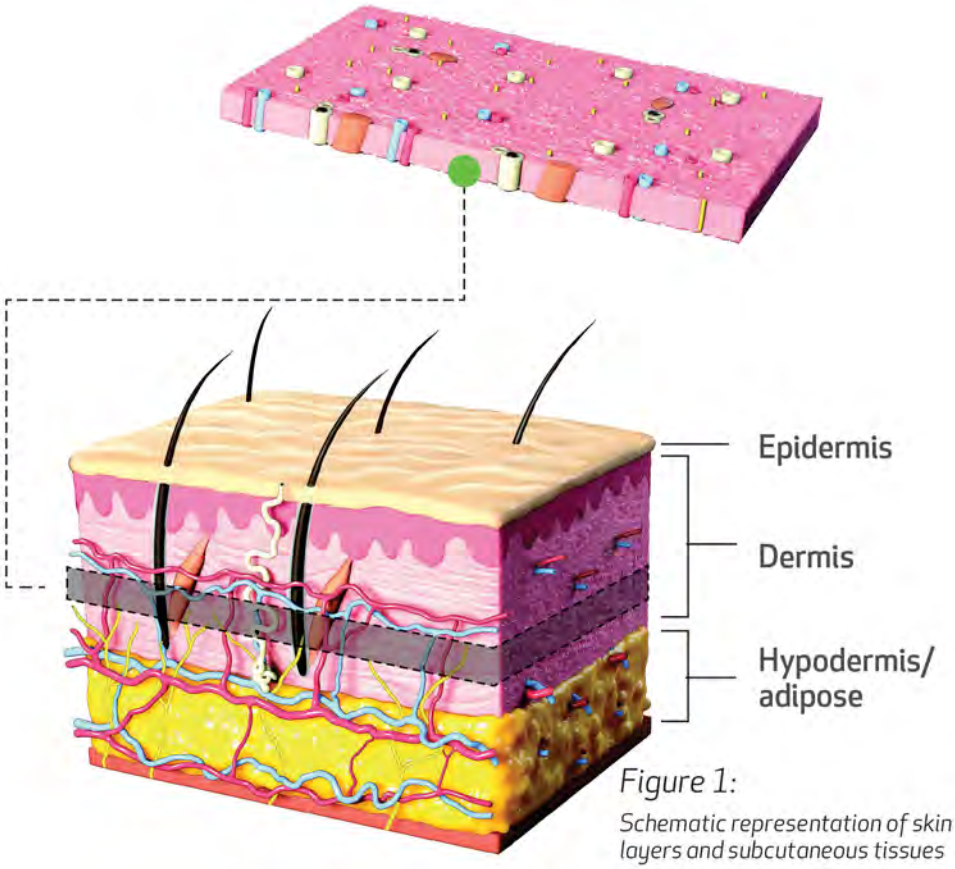

Figure 1:
Schematic representation of skin layers and subcutaneous tissues

MATERIALS AND METHODS

Tissue Preparation

For this study, human allograft skin was recovered from donors after receiving consent from the donor family.

To recover the deeper dermal tissue with uniform thickness and precision, a proprietary separating process was used. A cut was made into the reticular lower layer to remove the hypodermis containing the adipose and muscle tissue. A second cut was then made close to the papillary-reticular dermis interface. This effectively removed the dense collagen present in the papillary region. MTF designates this tissue FlexHD Pliable.

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The tissue was then decellularized and processed by treating with saline, detergent, peracetic acid, ethanol and propylene glycol. The tissue was then washed with sterile water to remove residual processing chemicals. The disinfected and acellular tissue was cut to the desired size. The final tissue was treated with aqueous ethanol and then packaged to provide a hydrated collagen matrix. All of these processes are in compliance with FDA Good Tissue Practices regulations under FDA 21 CFR 1271. Sterility was ensured via USP<71> guidelines and bacterial endotoxin testing was performed using the Kinetic Chromogenic Limulus Amebocyte Lysate (LAL) method.

Histology (H & E staining). Tissue sections were fixed in 10% neutral buffered formalin prior to paraffin embedding, sectioned and stained via hematoxillin and eosin (H & E). All histological processing was done at Premier Laboratory (Longmont, CO). Imaging was also done at Premier using AperioScope software (Vista, CA). Representative images were taken at 40x magnifications.

Scanning electron microscopy (SEM). Tissue samples were lyophilized and coated with a 10nm layer of gold. Images were taken using a Field Emission Zeiss Scanning Microscope (Carl Zeiss, Inc., Thornwood, NY) with a working distance of 5–10mm and voltage range of 30-200kV. All images were taken at the Department of Ceramics and Material Science at Rutgers University, New Brunswick, NJ.

In vitro fibroblast attachment

7mm punches of each tissue sample were prepared and seeded with 1 x 105 BJ neonatal human foreskin fibroblasts (ATCC Manassa, VA) on both sides in Eagles Minimum Essential Medium plus 10% fetal bovine serum. After 30 minutes, the tissue sections were washed to remove any non-adherent cells and incubated at 37°C for 1 hour in complete growth medium. Attached cells were quantified using CyQuant Cell Proliferation Assay (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Non-cell seeded controls were measured for all samples. The test was replicated with each sample set. Values for cell fluorescence were reported. Tissue from multiple donor lots was collected, processed as described and tested.

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Tensile properties

Tissue samples were tested on an MTS 858 Mini Bionix System. Sample thickness was first measured with a laser micrometer (Z Mike, Benchmike 4050S). Samples in dogbone configuration (1cmx7cm; ASTM 638) were positioned in pneumatic action grips set at 29psi pressure at a gauge length of 26mm. Tissue was pulled to break at a strain rate of 50.6mm/min. Ultimate tensile strength, modulus of elasticity and elongation at break were recorded.

Suture Retention Strength

A size 0 PDS® II suture with a 40mm, 1/2 circle tapered needle (Ethicon, Inc., Somerville, NJ) was placed 5mm from the edge of a 6cm x 1cm test sample. With one end fixed, the suture was pulled through the material until failure, and the load to failure recorded on an MTS 858 Mini Bionix System. AlloDerm RTM Ready to Use has not yet been tested for this parameter.

Endotoxin testing

Endotoxin testing of representative samples from every donor lot of FlexHD has been implemented by MTF Biologics since January 2018. The test is performed using the Kinetic Chromogenic Limulus Amebocyte Lysate (LAL) method (EndoScan-V, Charles River Laboratories, Wilmington, MA) that relies on the extreme reactivity of horseshoe crab (Limulus polyphemus) amebocytes to endotoxin.

Statistical Methods

Data of tensile properties were analyzed by ANOVA using a General Linear Model, Bonferroni Method at a 95% confidence interval.

RESULTS

Histology

For each sample, the image is a low magnification (10x) representative scan of the entire thickness of the tissue sample. In all images, the epidermal side is on the upper part of the scan.

As expected, FlexHD Structural shows a dense structure with an even topography on the epidermal side. Towards the dermal side, the structure becomes less dense, with the tissue directly adjacent to the cut edge showing a more open architecture. FlexHD Pliable exhibits a more uniform and open collagen matrix throughout the thickness of the graft with no distinguishable differences between the epidermal and dermal sides. (See Figure 2)

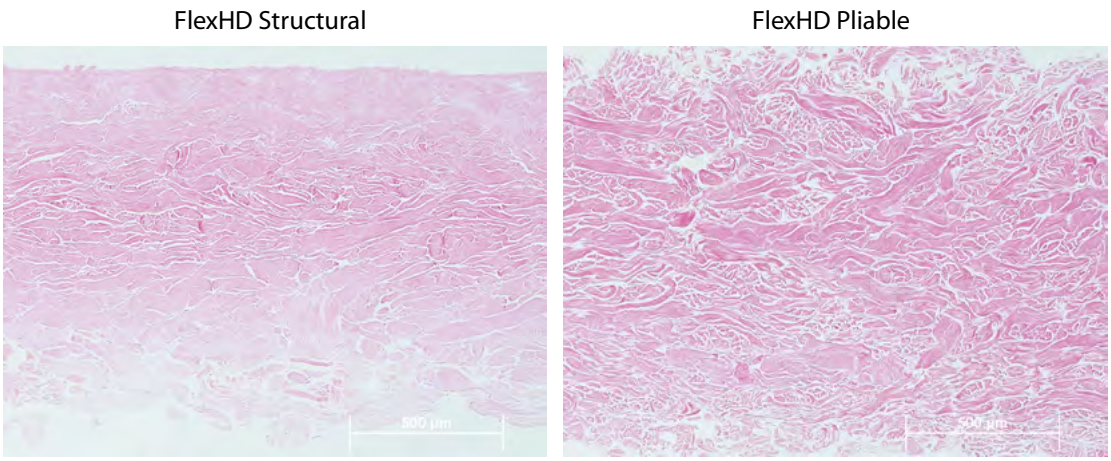

Figure 2: Histological (H&E) depiction of FlexHD structural (superficial papillary/reticular layer) and FlexHD Pliable (deeper/reticular layer)

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SEM

Representative images were taken at 250x for all samples (See Figure 3). In all cases, the epidermal side is on the left and the dermal side is on the right. The epidermal side of FlexHD Structural, AlloDerm and AlloDerm RTM Ready to Use shows a more solid, closed architecture, indicative of the tighter collagen matrix in this region. The dermal side is more open, similar to that of both the epidermal and dermal sides of the FlexHD Pliable. Both sides of the FlexHD Pliable graft demonstrate a more open architecture, suggesting greater porosity.

In vitro fibroblast attachment

Epidermis side of tissue:
FlexHD Pliable demonstrates a statistically higher fibroblast attachment as compared to FlexHD Structural: 7246 vs. 2620 fluoresence units. AlloDerm and AlloDerm RTM Ready to Use show much lower cell attachment than either FlexHD Structural or FlexHD Pliable tissues. The difference between the cell attachment level for FlexHD Pliable is statistically higher than AlloDerm or AlloDerm RTM Ready to Use. See Table 1, Figure 4.

Dermis side of tissue:
FlexHD Pliable demonstrates a statistically higher fibroblast attachment as compared to FlexHD Structural: 8379 vs. 6047 fluorescence units. AlloDerm has a statistically greater fibroblast attachment as compared to AlloDerm RTM Ready to Use: 4568 vs. 2028 fluorescence units. Finally, the number of attached fibroblasts for FlexHD Pliable is much greater than for either AlloDerm or AlloDerm RTM Ready to Use. These differences reached statistical significance. See Table 1, Figure 4.

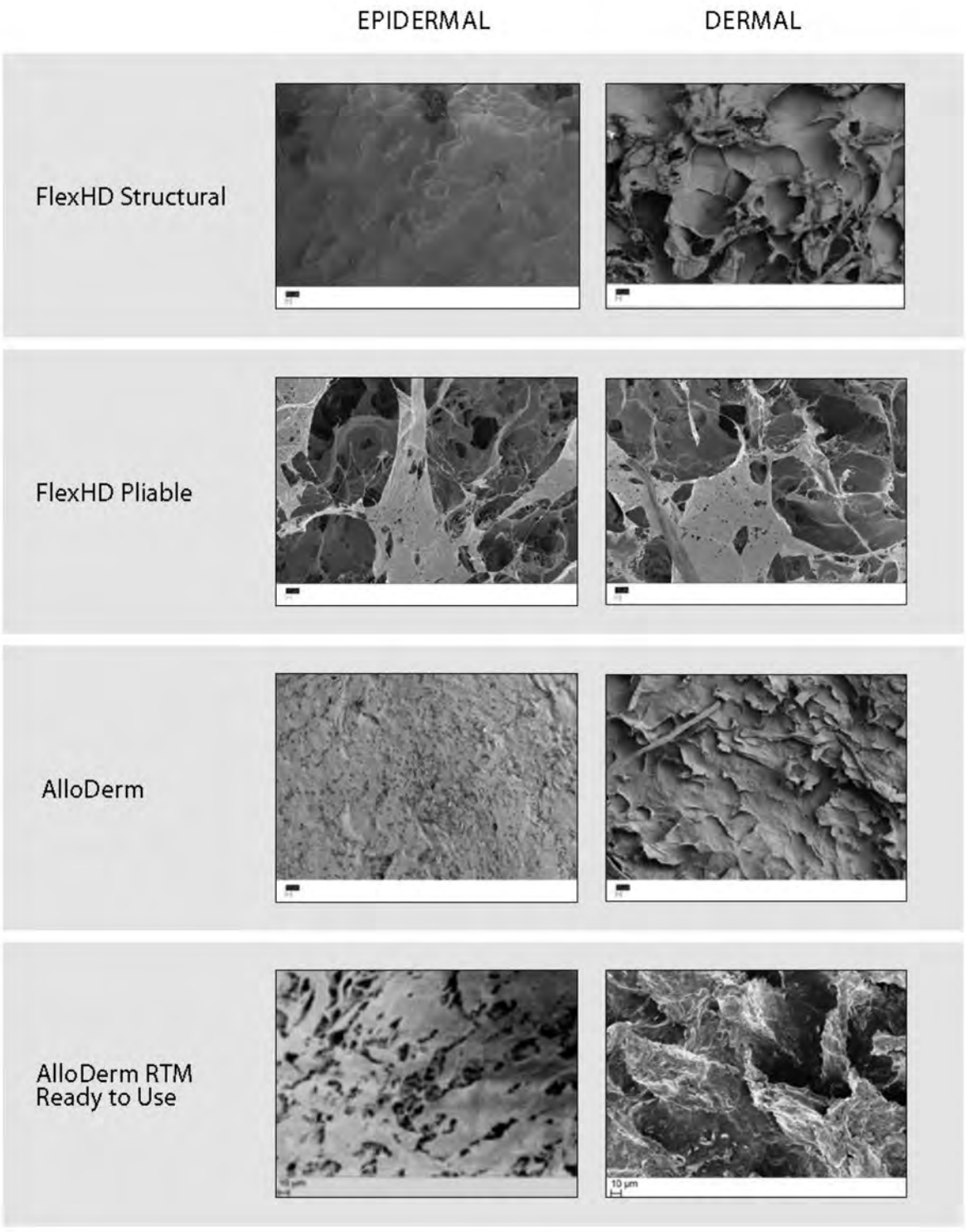

Figure 3:

Scanning electron microscopy (SEM) of the epidermal and dermal sides of various ADMs

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In vitro Fibroblast Attachment				
	No.of Donors	No.of Samples	Cells	Statistical Grouping
FlexHD Structural				
Epidermis	8	60	2620/270	D
Dermis		60	6047/242	BC
FlexHD Pliable				
Epidermis	8	78	7246/359	AB
Dermis		77	8379/308	A
AlloDerm				
Epidermis	6	42	1548/379	DE
Dermis		42	4568/476	C
AlloDerm RTM Ready to Use				
Epidermis	5	36	1039/278	E
Dermis		36	2028/259	DE

Table 1:

Data presented as fluorescence units: Mean/standard error of the mean, SEM. Statistically similar groups as determined by the Bonferroni Method (95% Confidence); means that do not share a letter are statistically different.

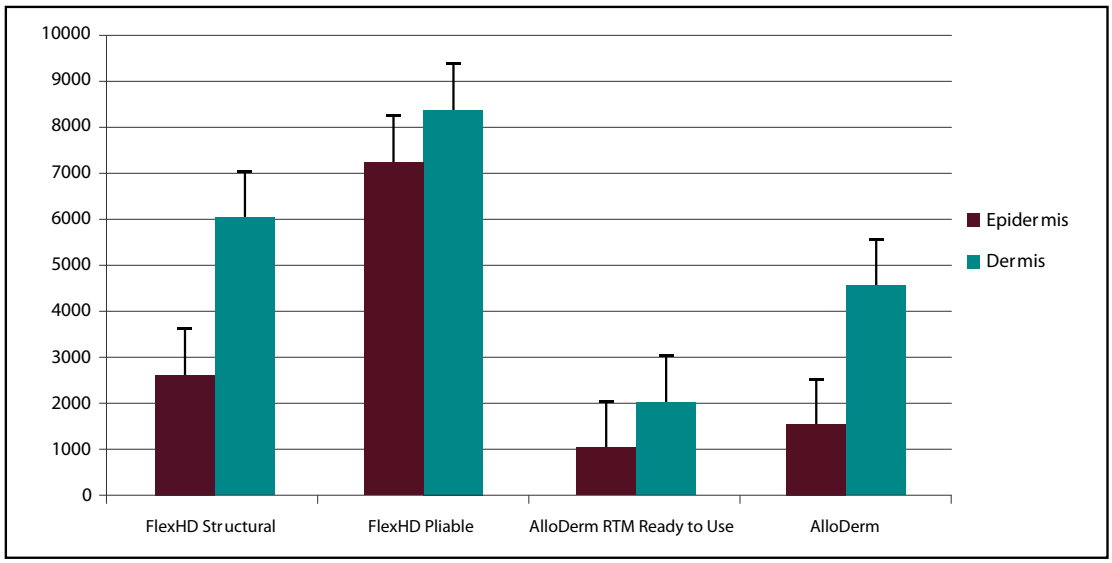

Figure 4:

In vitro fibroblast attachment. Values represent cell fluorescence units. Error is represented as standard error of the Mean.

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Tensile properties

As a result of the more open structure and greater porosity of FlexHD Pliable as contrasted with FlexHD Structural, FlexHD Pliable has an expected reduction in tensile strength: 15.36 vs. 10.97MPa. However, FlexHD Pliable has a significantly higher tensile strength than that of either AlloDerm or AlloDerm RTM Ready to Use: 10.97 vs. 9.22 and 9.46MPa. See Table 2, Figure 5.

Modulus of elasticity is a measure of flexibility. The greater the modulus of elasticity, the stiffer the material. The modulus of FlexHD Pliable is 38% lower than that of FlexHD Structural: 7.30 vs. 10.14MPa, rendering a more flexible material. The modulus of elasticity of FlexHD Pliable is statistically equivalent to that of AlloDerm: 7.30 vs. 6.98MPa. AlloDerm RTM Ready to Use, however, demonstrates a higher modulus of elasticity, or greater stiffness, than either AlloDerm or FlexHD Pliable. These differences are statistically significant. See Table 2, Figure 6.

Based on the modulus of elasticity results, AlloDerm RTM Ready to Use is 19% stiffer than the original AlloDerm.

Tensile Properties*

Tissue	No. of Donors	No. of Samples	Ultimate Tensile Strength mean/SEM (MPa)	Grouping**	MODULUS mean/SEM (MPa)	Grouping	Elongation-At-Break mean/SEM (%)	Grouping
Flex HD Structural	5	154	15.36/0.34	A	10.14/0.25	A	1.73/0.04	A
Flex HD Pliable	6	300	10.97/0.21	B	7.30/0.13	C	1.62/0.02	AB
AlloDerm	11	88	9.22/0.54	C	6.98/0.38	C	1.48/0.05	B
AlloDerm RTM Ready to Use	6	100	9.46/0.22	C	8.31/0.22	B	1.22/0.02	C

* Data presented as mean/standard error of the mean, SEM.
** Statistically similar groups as determined by the Bonferroni Method (95% Confidence); means that do not share a letter are statistically different.

Table 2: Tensile Properties

** Data presented as mean/standard error of the mean, SEM.*

*** Statistically similar groups as determined by the Bonferroni Method (95% Confidence); means that do not share a letter are statistically different.*

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Elongation at break is a measure of the amount of stretch before tensile failure. For this parameter, FlexHD Pliable (1.73mm/mm) is statistically equivalent to both FlexHD Structural and AlloDerm (1.62 and 1.48mm/mm). AlloDerm RTM Ready to Use (1.22mm/mm), however, has a statistically lower elongation at break as compared to all other tissue types. See Table 2, Figure 7.

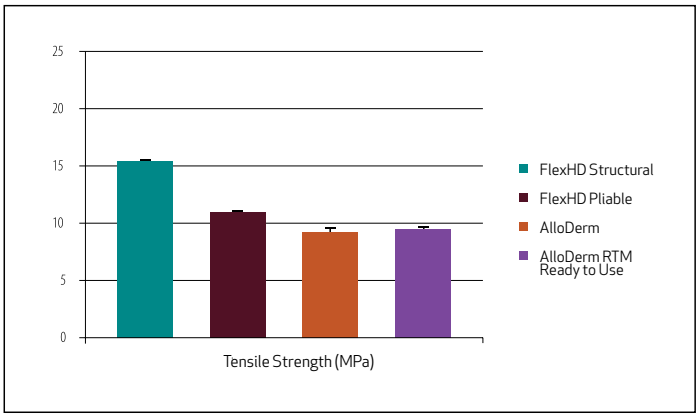

Figure 5:
Ultimate tensile strength. Error is represented as standard error of the mean.

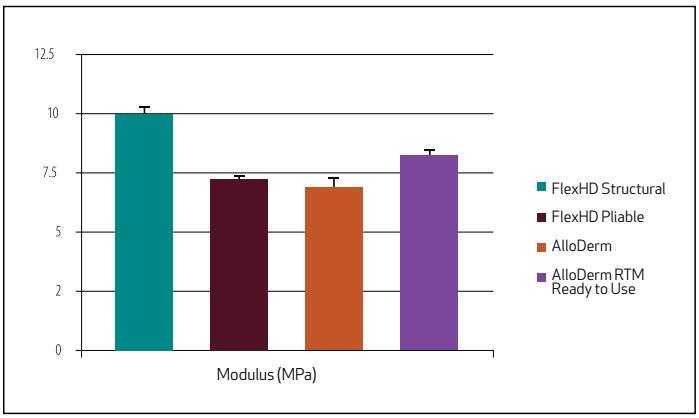

Figure 6:
Modulus of elasticity. Error is represented as standard error of the mean.

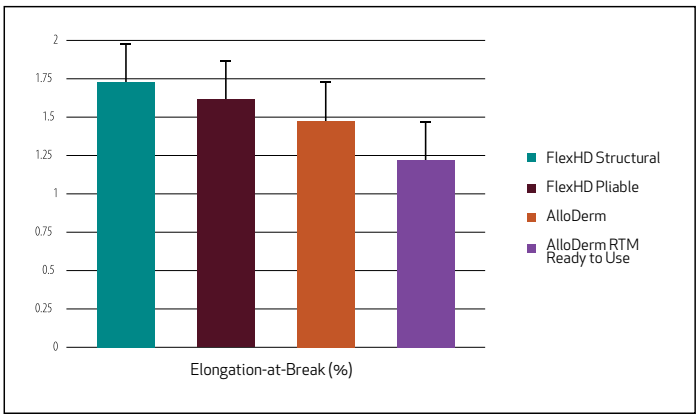

Figure 7:
Elongation at break. Error is represented as standard error of the mean.

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Suture Retention

The suture retention strength of FlexHD Pliable is statistically higher than for AlloDerm (4.1 vs. 3.2MPa). FlexHD Structural and AlloDerm are equivalent in suture retention strength property; 3.4 vs. 3.2MPa. AlloDerm RTM Ready to Use has not yet been tested for this parameter.

Tissue	No.of Donors	No.of Samples	Suture Retention Strength (MPa) Mean/SEM	Statistical Grouping
FlexHD Structural	40	709	3.40/0.03	B
FlexHD Pliable	9	214	4.10/0.07	A
AlloDerm	10	121	3.20/0.9	B

Table 3: Suture retention strength

Data presented as mean/standard error of the mean, SEM. Statistically, similar groups as determined by the Bonferroni Method (95% Confidence); means that do not share a letter are statistically different.

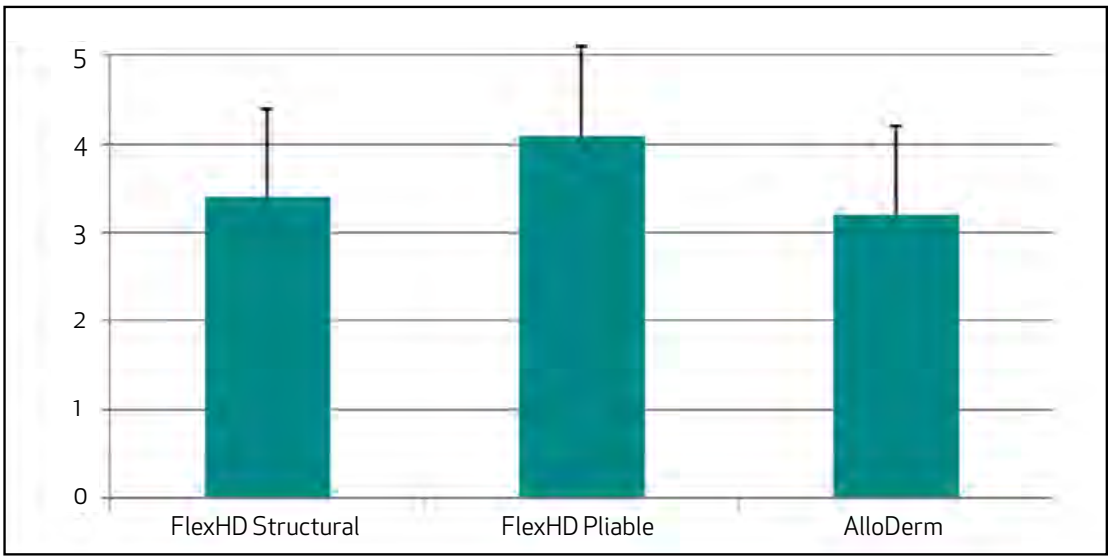

Figure 8: Suture retention strength (MPa)

Complaint monitoring prior and post-implementation of endotoxin testing

Complaints of sterile inflammation expressed as non-infectious erythema (blue) as well as overall medical complaint occurrence rates were monitored from January 2017 to June 2019. After implementation of endotoxin testing in January 2018, no sterile inflammation/non-infectious erythema complaints were recorded until the end of the monitoring period [10,11].

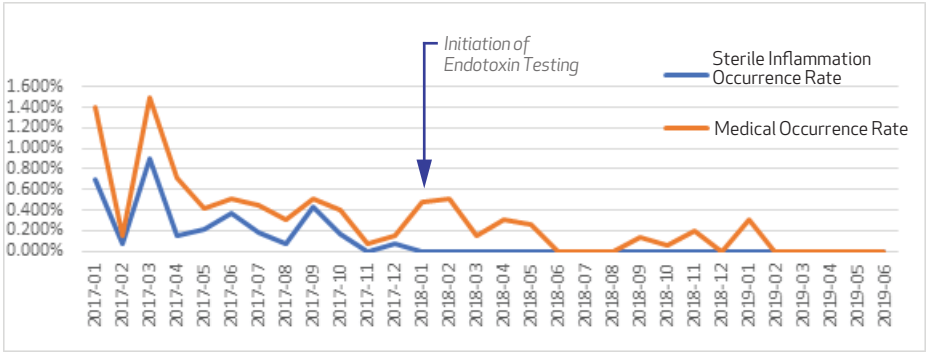

Figure 9: Sterile Inflammation and overall medical complaint occurrence rates for FlexHD and BellaDerm acellular dermal matrix by allograft packaging date, MTF Biologics, Jan 2017 – June 2019.

DISCUSSION

FlexHD Pliable is a novel ADM designed specifically for Plastic and Reconstructive Surgery applications. The tissue is derived from a deeper cut in the human dermis, relative to that used for FlexHD Structural. The porosity of this tissue increases deeper into the dermis layer. Accordingly, the interconnected channels are larger and the pores are more uniform at the two surfaces of the tissue. That differentiates FlexHD Pliable from FlexHD Structural and other competitive human ADM offerings.

Open and porous tissue structure of the reticular dermis layer supports quick cell incorporation:

The deeper cut used for FlexHD Pliable results in a different microstructure as compared to FlexHD Structural. High magnification imaging clearly shows the more open and porous structure of the Pliable tissue form. Both the dermal and epidermal sides are very similar. This uniformity leads to a greater balance of cell attachment between the epidermal and dermal sides of the ADM when compared to FlexHD Structural. An overall increase in fibroblast attachment results from the more porous structure.

Both AlloDerm and AlloDerm RTM Ready to Use demonstrate less fibroblast attachment when compared to FlexHD Pliable or FlexHD Structural. On the epidermal side of the graft, FlexHD Pliable demonstrated over six-times the fibroblast attachment compared to the epidermal side of AlloDerm RTM Ready To Use.

Deeper reticular layer is more pliable and conformable, yet sufficiently strong for Plastic and Reconstructive Surgery applications:

The initial goals of developing FlexHD Pliable were to generate a tissue form that provided a more supple, flexible graft when compared to FlexHD Structural, and to retain clinically relevant strength. Because the porosity of the tissue in FlexHD Pliable is substantially greater than FlexHD Structural, the tensile strength was expected to be lower; this difference was confirmed.

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However, FlexHD Pliable still demonstrated significantly higher tensile strength when compared to AlloDerm and AlloDerm RTM Ready to Use.

The modulus of elasticity is a measure of a material's stiffness or flexibility. The higher the modulus of elasticity, the stiffer the material, the less "conformable" the material becomes. The modulus of elasticity is lower for FlexHD Pliable relative to FlexHD Structural, suggesting a more flexible graft.

Interestingly, FlexHD Pliable and AlloDerm have statistically similar modulus of elasticity measurements, thereby demonstrating similar flexibility profiles. However, AlloDerm RTM Ready to Use, the next generation AlloDerm tissue form, shows a significant increase in modulus of elasticity, rendering the material significantly stiffer than both FlexHD Pliable and the original AlloDerm. The processing and sterilization methods which differ in AlloDerm RTM Ready to Use when compared to AlloDerm appear to have reduced the flexibility of the graft, and substantially reduced the ability of the tissue to maintain fibroblast attachment (49% lower fibroblast attachment on the epidermal side of AlloDerm RTM Ready To Use as compared to the same side of AlloDerm). This is not entirely surprising and confirms that material properties can be significantly altered when tissue processing and/or sterilization changes occur.

Additional safety testing:

Endotoxin is a component of lipopolysaccharides (LPS) found primarily in the cell wall of gram-negative bacteria. It is not infectious but elicits a pyrogenic reaction that may manifest as fever, redness, inflammatory response and varies widely depending on the organism it originates from, the site of patient exposure and the individual immune response. An additional layer of safety was added for FlexHD Pliable in January 2018 when endotoxin release testing was implemented for every donor lot that was processed in order to minimize potential reactions in patients. After identification of what appeared to be sterile inflammation expressed as non-infectious erythema in some patients, MTF Biologics implemented endotoxin testing for every lot of dermal tissue produced. As reported in Nguyen et al[10,11], since endotoxin screening of ADM donor lots began in January 2018, no cases of sterile inflammation had been reported from screened units through December 31, 2018.

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CONCLUSION

MTF has developed two distinct ADM tissue forms, each tailored for different applications. We believe FlexHD Pliable is a more appropriate ADM for applications where pliability is desirable given its strength, flexibility and porosity profile. FlexHD Pliable is stronger than AlloDerm and AlloDerm RTM Ready to Use, maintains flexibility similar to AlloDerm but is significantly more flexible than AlloDerm RTM Ready to Use, while demonstrating substantially greater cell attachment than both AlloDerm tissue forms.

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Figures and Tables
FlexHD Structural and FlexHD Pliable vs. AlloDerm and AlloDerm RTU

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MICROSTRUCTURE SKIN ILLUSTRATION

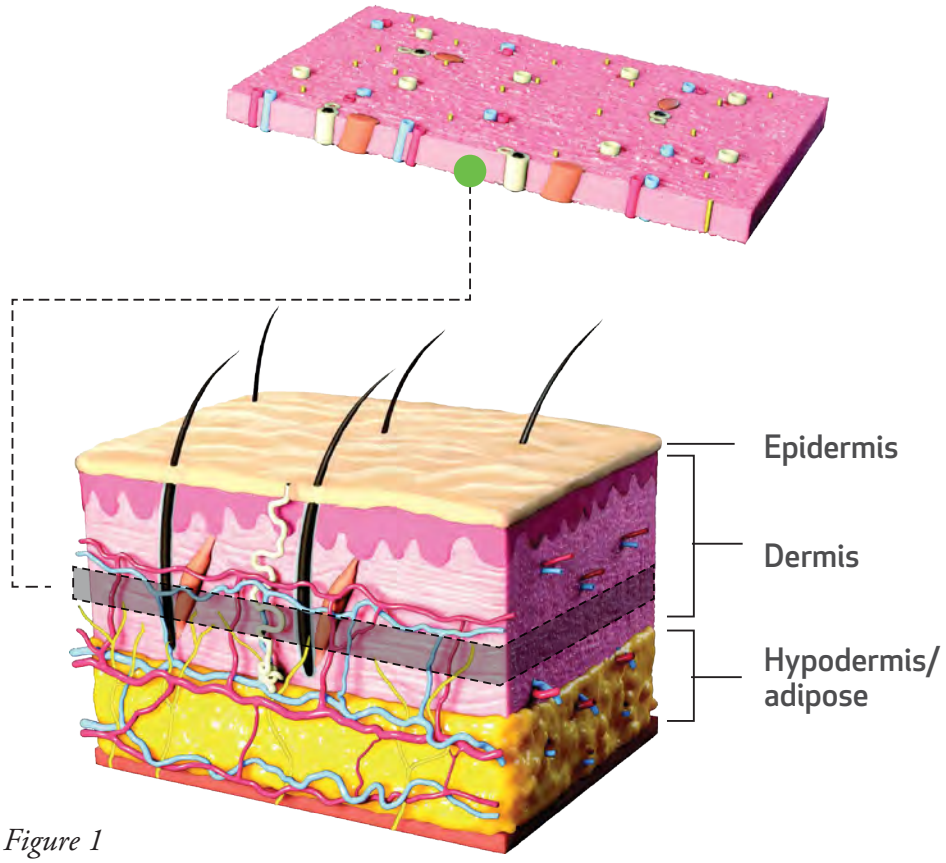
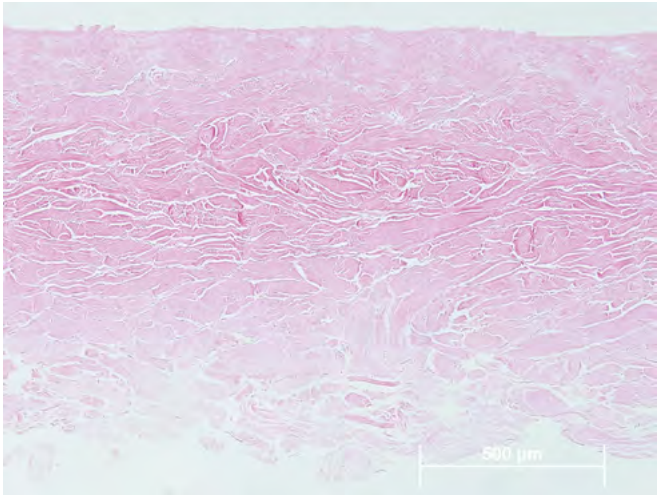


Figure 1

HISTIOLOGY IMAGES

FlexHD Structural



FlexHD Pliable

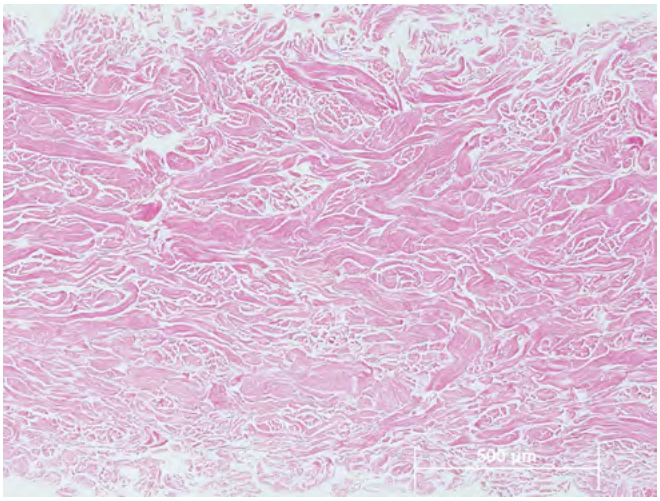


Figure 2

SEM IMAGES

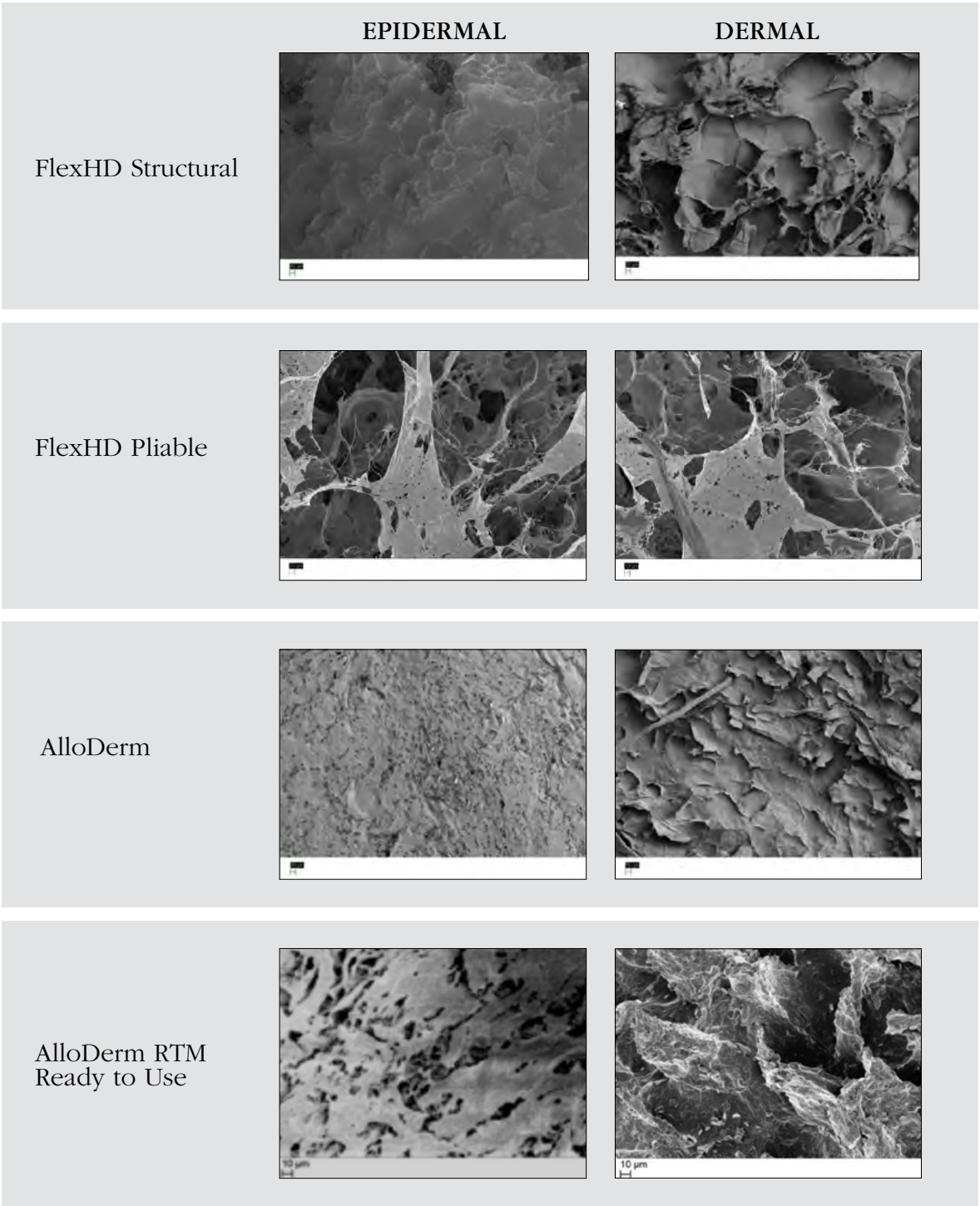


Figure 3

IN VITRO FIBROBLAST ATTACHMENT CHART

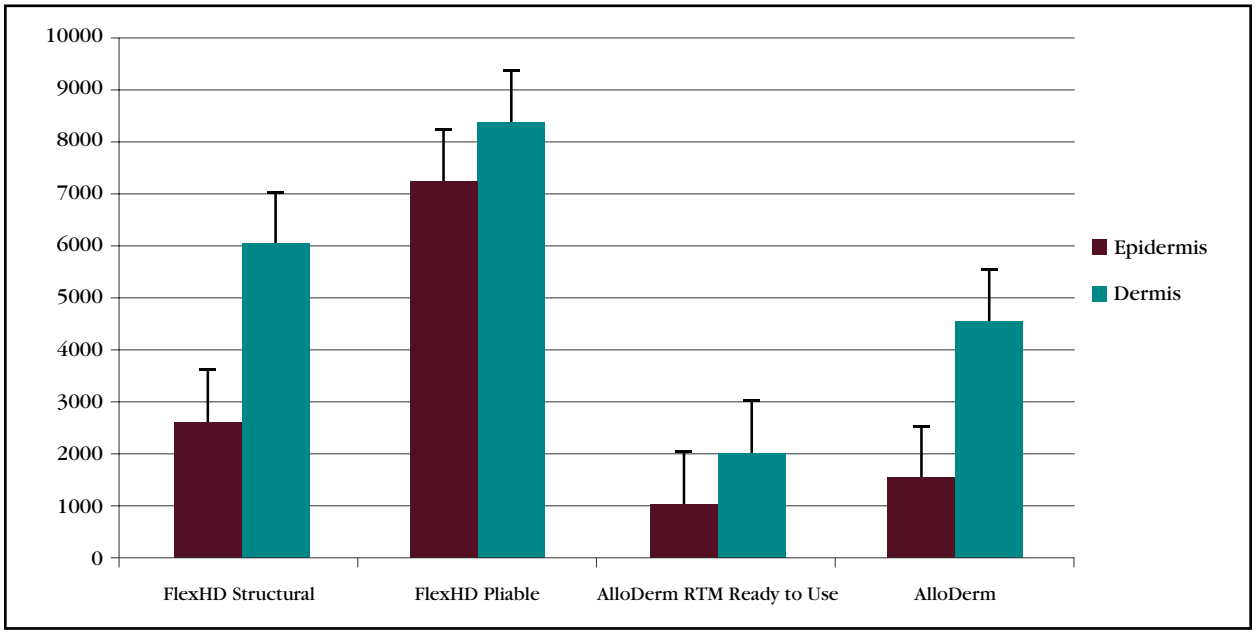


Figure 4:

In vitro fibroblast attachment. Values represent cell fluorescence units. Error is represented as standard error of the mean.

PREVIOUS
FIGURES & TABLES
NEXT

TENSILE STRENGTH CHART

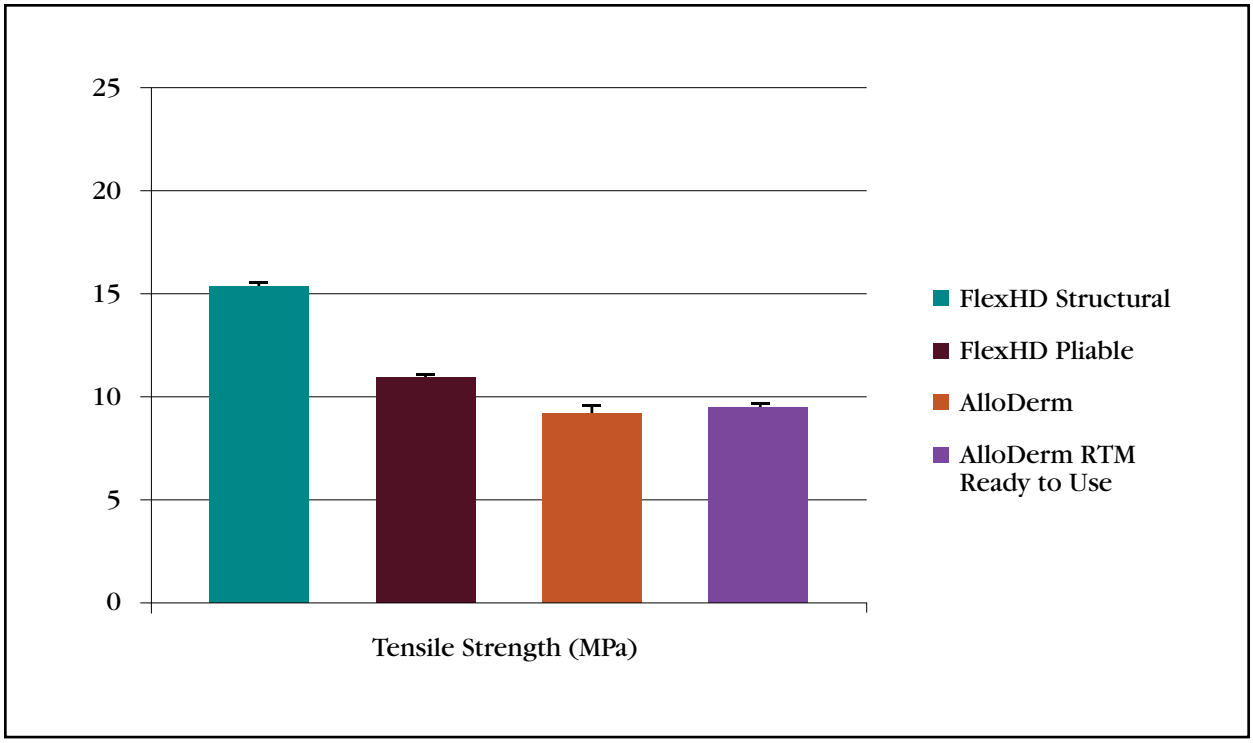


Figure 5:
Ultimate tensile strength. Error is represented as standard error of the mean.

MODULUS OF ELASTICITY CHART

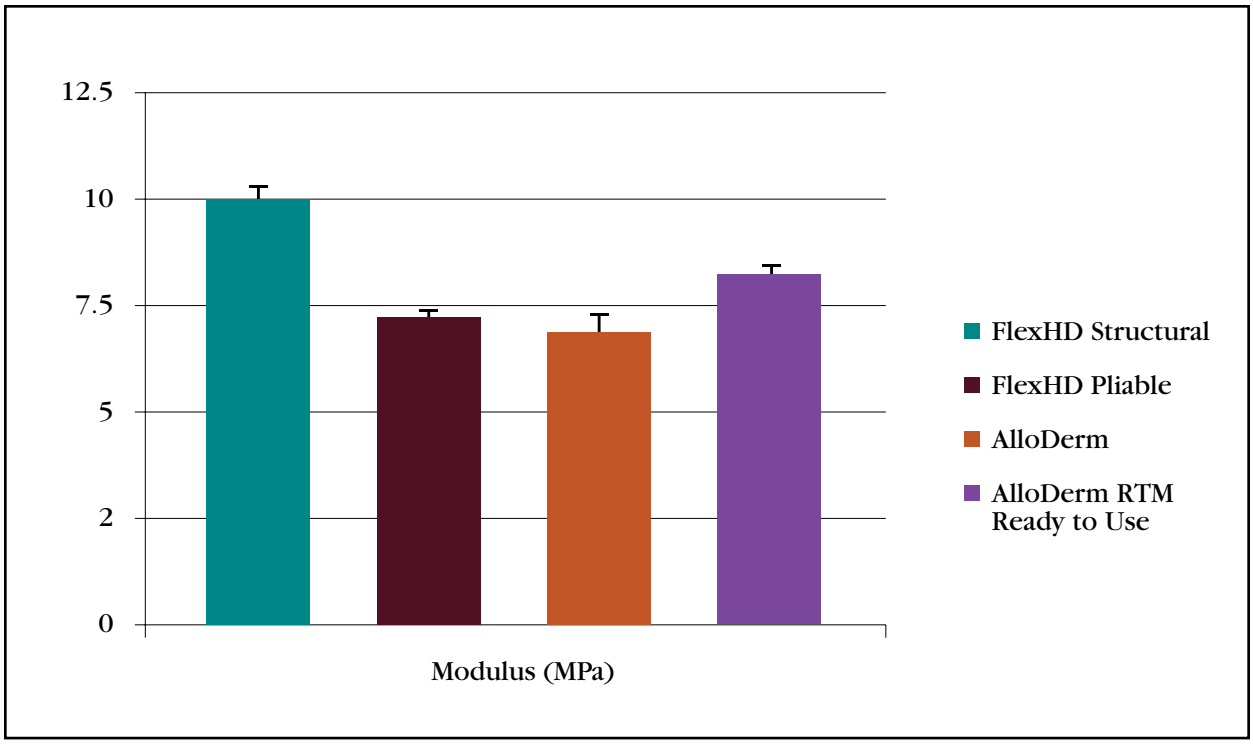


Figure 6:
Modulus of elasticity. Error is represented as standard error of the mean.

ELONGATION AT BREAK CHART

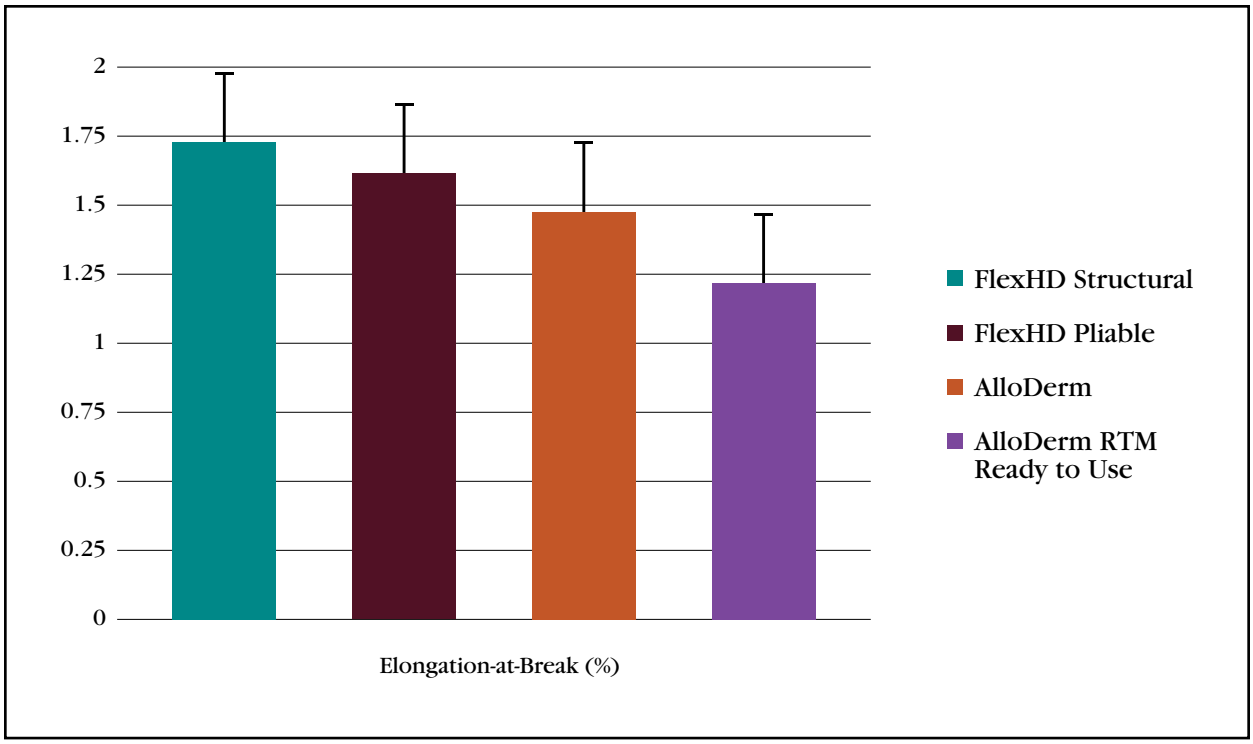


Figure 7:
Elongation at break. Error is represented as standard error of the mean.

SUTURE RETENTION STRENGTH CHART

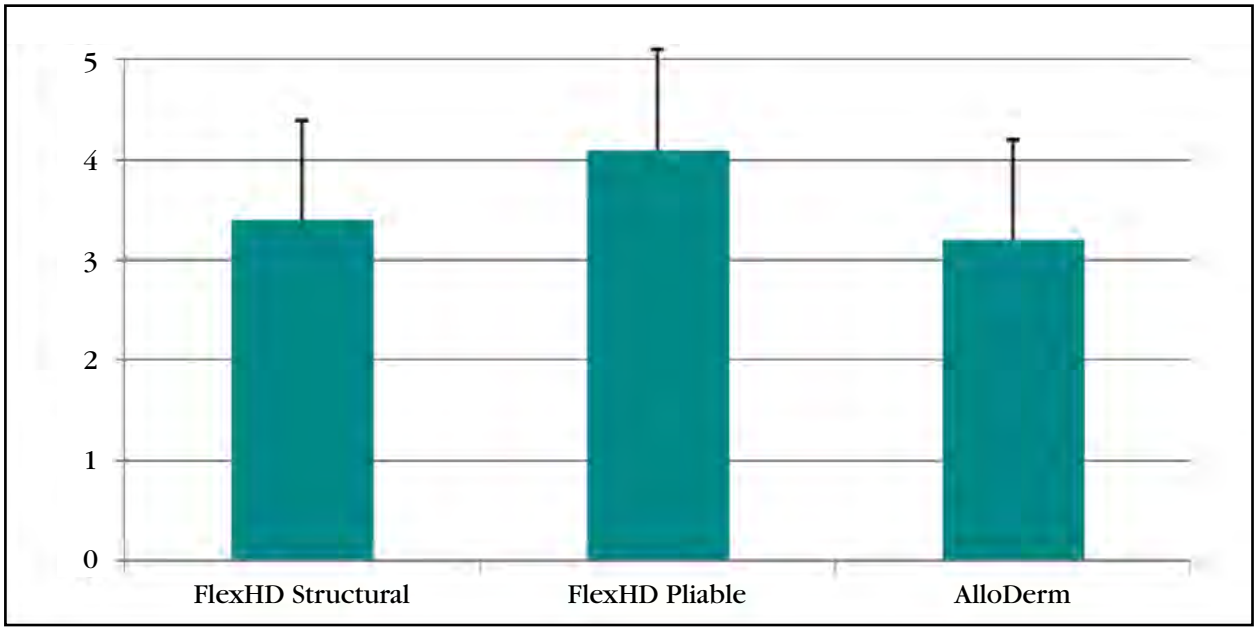


Figure 8:
Suture retention strength (MPa)

STERILE INFLAMMATION OCCURANCE

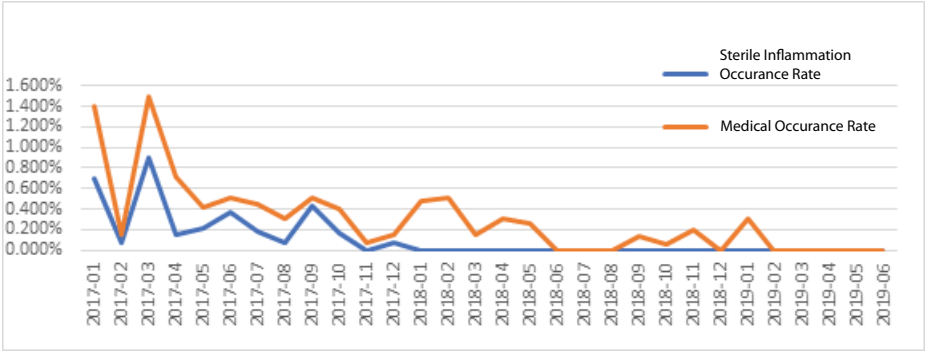


Figure 9:

Sterile Inflammation and overall medical complaint occurrence rates for FlexHD and BellaDerm acellular dermal matrix by allograft packaging date, MTF Biologics, Jan 2017 – June 2019.

IN VITRO FIBROBLAST ATTACHMENT

In vitro fibroblast attachment				
	No. of Donors	No. of Samples	Cells	Statistical Grouping
FlexHD Structural				
Epidermis	8	60	2620/270	D
Dermis		60	6047/242	BC
FlexHD Pliable				
Epidermis	8	78	7246/359	AB
Dermis		77	8379/308	A
AlloDerm				
Epidermis	6	42	1548/379	DE
Dermis		42	4568/476	C
AlloDerm RTM Ready to Use				
Epidermis	5	36	1039/278	E
Dermis		36	2028/259	DE

Table 1:

Data presented as fluorescence units: mean/standard error of the mean, SEM.
Statistically similar groups as determined by the Bonferroni Method (95% Confidence);
means that do not share a letter are statistically different.

TENSILE PROPERTIES

Tensile Properties*

Tissue	No. of Donors	No. of Samples	Ultimate Tensile Strength mean/SEM (MPa)	Grouping**	MODULUS mean/SEM (MPa)	Grouping	Elongation-At-Break mean/SEM (%)	Grouping
Flex HD Structural	5	154	15.36/0.34	A	10.14/0.25	A	1.73/0.04	A
Flex HD Pliable	6	300	10.97/0.21	B	7.30/0.13	C	1.62/0.02	AB
AlloDerm	11	88	9.22/0.54	C	6.98/0.38	C	1.48/0.05	B
AlloDerm RTM Ready to Use	6	100	9.46/0.22	C	8.31/0.22	B	1.22/0.02	C

* Data presented as mean/standard error of the mean, SEM.
** Statistically similar groups as determined by the Bonferroni Method (95% Confidence); means that do not share a letter are statistically different.

Table 2:

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SUTURE RETENTION STRENGTH

Tissue	No. of Donors	No. of Samples	Suture Retention Strength (MPa) Mean/SEM	Statistical Grouping
FlexHD Structural	40	709	3.40/0.03	B
FlexHD Pliable	9	214	4.10/0.07	A
AlloDerm	10	121	3.20/0.9	B

Table 3:

Data presented as mean/standard error of the mean, SEM. Statistically, similar groups as determined by the Bonferroni Method (95% Confidence); means that do not share a letter are statistically different.