SimpliDerm™
Hydrated
Acellular Dermal Matrix

A More Natural, More Intact Dermal Matrix

Summary

SimpliDerm™ by Aziyo® Biologics is a pre-hydrated human acellular dermal matrix allograft derived from human skin that has been aseptically processed and terminally sterilized to preserve the native collagen microstructure while removing potential immunogenic cells and epidermis.*

SimpliDerm™ is designed to maintain a more structurally intact extracellular matrix that is as close to nature as possible. Similar to the native dermis, SimpliDerm™ contains the key extracellular matrix components such as collagen, elastin, and glycosaminoglycan (GAG), which provide an optimal microenvironment for tissue remodeling during regenerative medicine applications[1].

Furthermore, SimpliDerm™ preserves the basement membrane that prevents adhesion formation and a basement membrane complex of blood vessels that promotes angiogenesis[2].

SimpliDerm™ also provides similar mechanical strength, collagen integrity, and bioactive components to that of native dermis.†

Aziyo has developed an exclusive proprietary methodology for processing and sterilizing grafts by using low-dose precision gamma irradiation to achieve a Sterility Assurance Level (SAL) of 10^{-6}. As a sterile graft, SimpliDerm™ provides an extra measure of safety critical for the success of surgical procedures by mitigating the risk of adverse immune response[3] or capsular formation[4]. Furthermore, Aziyo's proprietary processing is able to preserve the key characteristics of the native dermal matrix which ensures that SimpliDerm™ is as close to nature as possible. The matrix properties of SimpliDerm™ are reviewed through the ***in vitro*** and ***in vivo*** studies described here.*,†

* *Instructions for Use*
† Aziyo® Biologics data on file.

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Preservation of the Native Extracellular Matrix (ECM)

The extracellular matrix components and their natural structure in the native dermal tissue are integral for driving proper cell-cell interaction and overall tissue function[1]. To promote healthy tissue remodeling and regeneration process in surgical applications by using acellular dermal matrix allograft, it is integral that the acellular dermal matrix preserves the intact extracellular matrix structure at their native state.

SimpliDerm™ processing utilizes Aziyo's proprietary methods which avoids harsh chemicals that could alter the microstructure, whether by weakening it through disrupting the collagen network, or by cross-linking the tissue[5]. Using histology, immunohistochemistry, transmission electron microscopy, and gel electrophoresis, the matrix component and structure of SimpliDerm™ were compared to that of a native skin tissue to evaluate the extent of preservation of the extracellular matrix.†

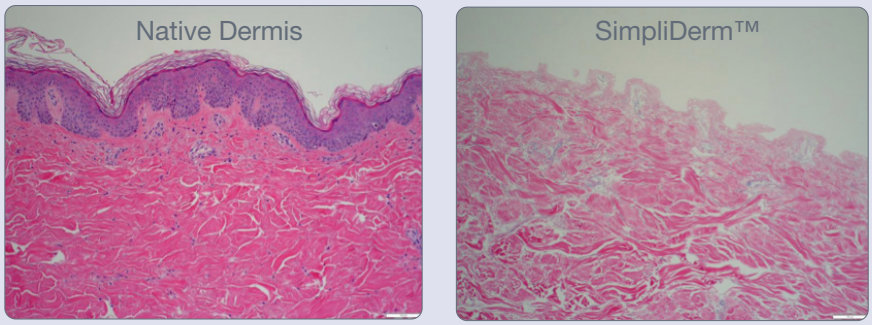
As native dermal matrix is primarily composed of collagen, the collagen structures in native human skin and SimpliDerm™ were compared and evaluated using histology. Hematoxylin and eosin (H&E) stained sections of the native human skin and SimpliDerm™ ***(Figure 1A)*** show a similar collagen structure in terms of density and orientation (depicted in pink) between the two samples. In addition, SimpliDerm™ also effectively preserves the dermal papillae. In contrast, a distinct absence of cellular components (blue punctate nuclear staining) and the epidermis layer (purple layer on top of papillae) is demonstrated in the SimpliDerm™ as compared to the native human skin. This demonstrates Aziyo's proprietary process is effective in removing potential immunogenic cellular components while preserving the extracellular matrix close to that of the native dermis.†

Verhoeff-Van Gieson (VVG) stained sections in ***Figure 1B*** shows the elastin fibers present in the native

H&E Staining†

Fig. 1A

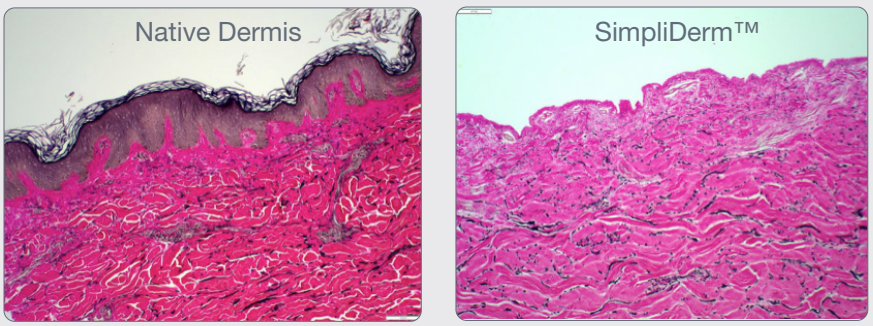
H&E stained images of native human skin and SimpliDerm™ that shows **similar collagen structure and papillae.** Note the absence of cellular components (blue nuclear stain) and epidermis layer in SimpliDerm™.



Verhoeff-Van Gieson Staining†

Fig. 1B

VVG stained images of native human skin and SimpliDerm™ that shows the presence of **similar elastin fiber structure** (dark grey/black stains).



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human skin and SimpliDerm™. Elastin is another key component of the human dermis and contributes to its natural flexibility and compliance. No differences were observed between the native human skin and SimpliDerm™.†

In addition, the immunohistochemistry staining against collagen type IV in the native human skin and SimpliDerm™ ***(Figure 2)*** demonstrates that SimpliDerm™ preserves significant levels of collagen type IV that exists in the native dermis. In particular, collagen type IV is present in the basement membrane complex at the epidermis-dermis junction and surrounding the blood vessel[1,2]. This basement membrane complex of blood vessels supports angiogenesis or new blood vessel formation.†

Transmission Electron Microscopy (TEM) images in ***Figure 3*** shows the ultrastructural features of the collagen fibrils of the native human skin and SimpliDerm™. Intact collagen fibrils were observed in both the native human skin and SimpliDerm™, which suggests that Aziyo's proprietary process effectively preserves the ultrastructural architecture of the collagen matrix.†

Immunohistochemistry Staining Against Collagen Type IV†

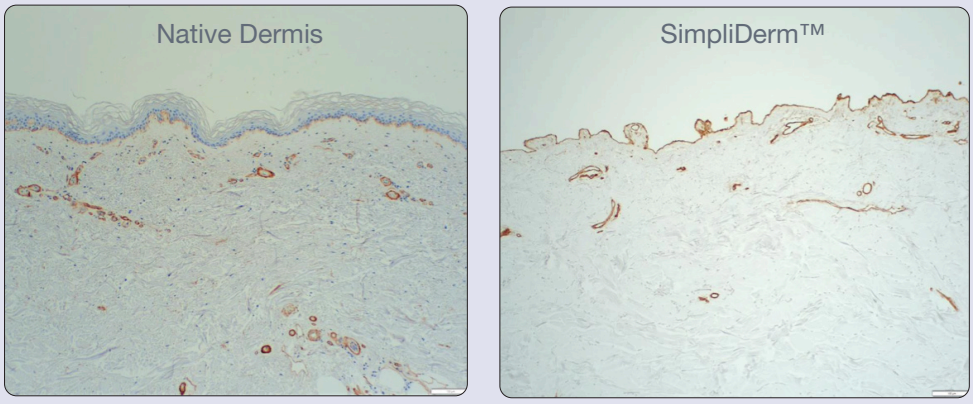

Fig. 2

Immunohistochemistry staining against collagen type IV in the native human skin and SimpliDerm™.

Collagen type IV is significantly present in the basement membrane at the epidermis-dermis junction and around blood vessels.

Transmission Electron Microscopy†

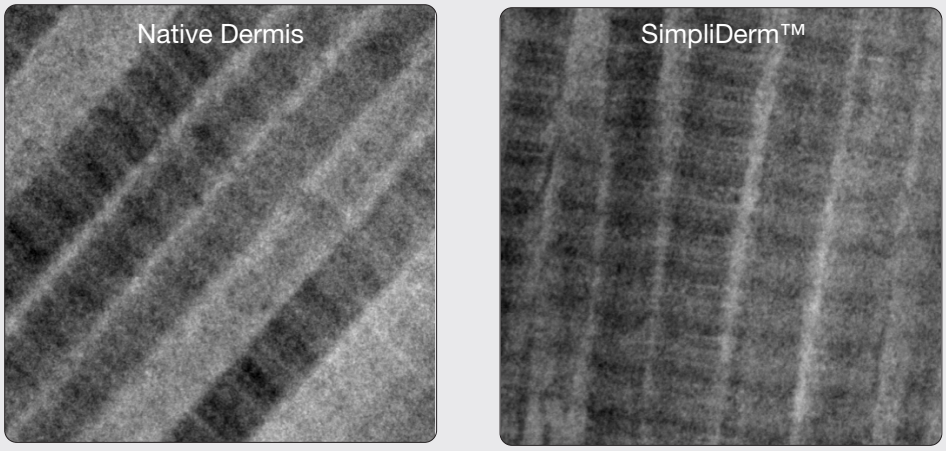

Fig. 3

Transmission Electron Microscopy (TEM) images.

Similar collagen fibril structures are evident in native human skin and SimpliDerm™.

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Along with collagen fibers, glycoasaminoglycans (GAGs) are one of the main structural components of the extracellular matrix in skin tissue[1]. GAGs interact with other extracellular matrix proteins, including the collagen network, and regulate the mechanical properties of the tissue (i.e. stiffness)[6]. GAGs also play an important role in various biological function such as cell growth and proliferation, adhesion, stem cell niche formation, and tissue hydration[7]. GAGs are classified into four groups: 1) chondroitin sulfate/dermatan sulfate, 2) heparan sulfate, 3) keratan sulfate, and 4) hyaluronic acid. In order to evaluate the different types of GAG present in the native human skin, SimpliDerm™ and other commercial products (AlloDerm® RTU and DermACELL®), GAGs were isolated and separated on agarose gel electrophoresis ***(Figure 4).*** Figure 4 shows that SimpliDerm™ closely matches the GAG components in the Native Dermis. The GAG content in SimpliDerm™ was highly comparable to that of AlloDerm® RTU, and both SimpliDerm™ and AlloDerm® RTU preserves the hyaluronic acid component of the dermis that is absent in DermACELL®.†

Matrix Integrity Closer to Nature

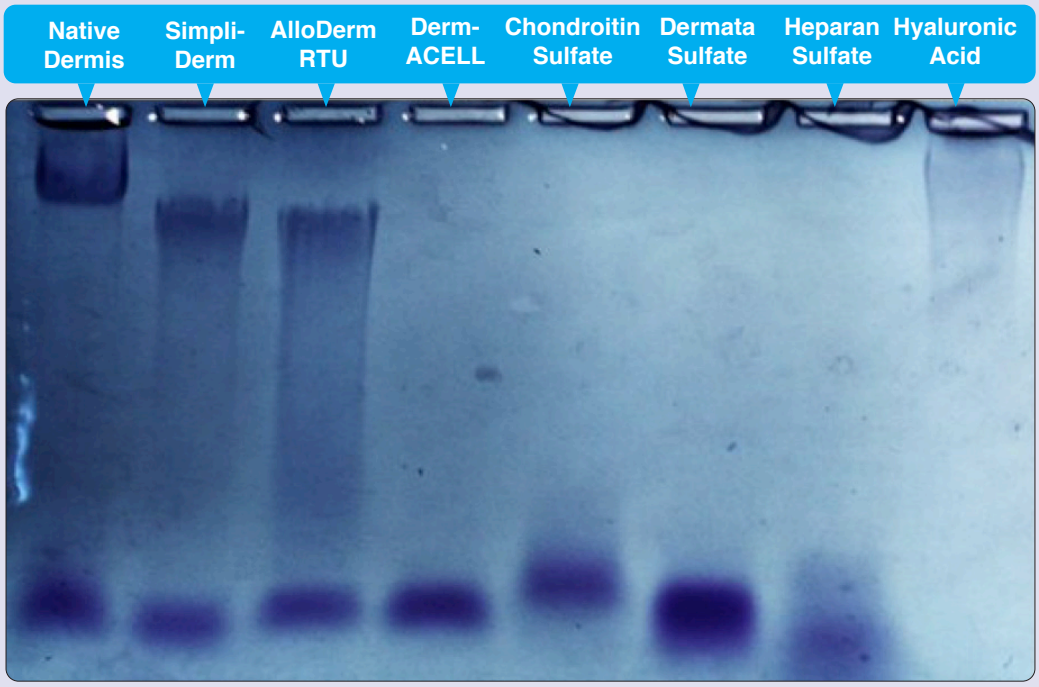
Preserving the intact structure of the extracellular matrix is critical as the function of a biological material is directly dependent on its structure[8]. This is especially the case for proteins that function according to their three-dimensional structure and their interaction with other biological macromolecules[8]. Various test methods that can denature the collagen protein structure were applied to SimpliDerm™ and other human ADMs to evaluate whether each product's matrix integrity was intact and therefore functional after processing.†

Agarose Gel Electrophoresis Separation of GAGs†

Fig. 4

Agarose gel electrophoresis separation of GAGs isolated from the Native Dermis, SimpliDerm™, AlloDerm® RTU and DermACELL® shown in comparison to the four GAG standards (Chondroitin Sulfate, Dermatan Sulfate, Heparan Sulfate, and Hyaluronic Acid).

Figure 4 shows that SimpliDerm™ closely matches the GAG components in the Native Dermis.



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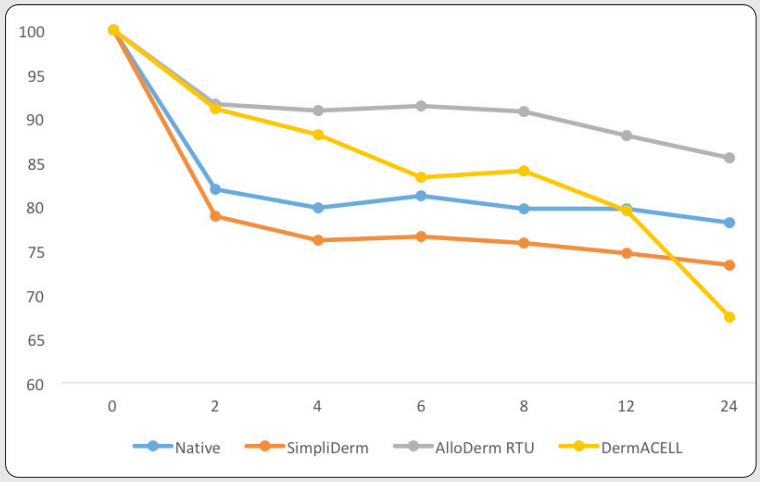
Collagen Stability

Collagen type I is one of major types of collagen that exists in the extracellular matrix of human dermis[1]. The matrix stability of SimpliDerm™ was evaluated by assessing its enzyme susceptibility to collagenase type I. Our results demonstrate that SimpliDerm™ most closely resembled the Native Dermis (by average of 4.18% difference) in terms of the enzymatic digestion profile among the three hydrated acellular dermal matrix products. AlloDerm® RTU showed an average of 9.61% lesser digestion than that of the Native Dermis throughout all time points. This could be due to the presence of higher collagen crosslinking post-processing. DermACELL® showed a gradual digestion trend, but the digestion amount after 24 hours was lower than that of the Native Dermis by 10.67%. This could be due to a change in collagen structure during processing that allows for more rapid digestion.†

The matrix stability of SimpliDerm™ in comparison to that of the native dermis was also compared by measuring the percentage of acid-soluble collagen content ***(Figure 5).*** Damage to the collagen matrix during processing will increase the presence of denatured or unraveled collagen, which can be easily hydrolyzed by strong acid such as hydrochloric acid. Thus, an increase in acid-soluble collagen content when compared to that of the native dermis is an indicator of altered collagen integrity. Our results show that SimpliDerm™ has the lowest acid-soluble collagen content among three acellular matrix products and was closest in value to that of the native dermis ***(Figure 6).***†

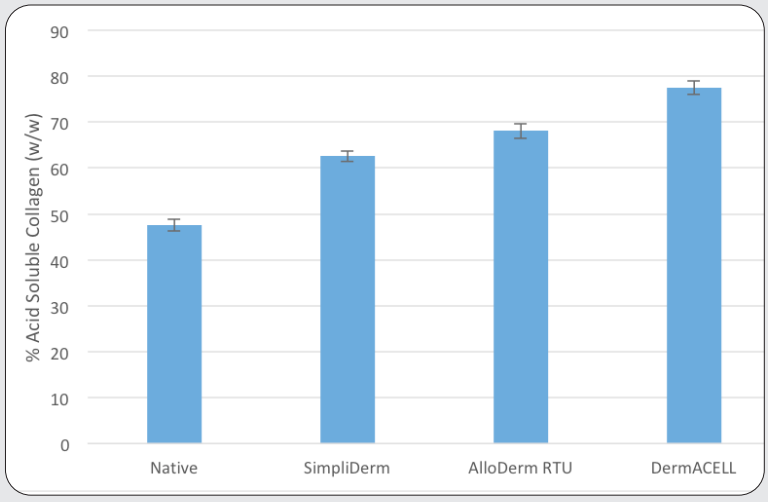
In conclusion, the collagen stability of SimpliDerm™ was the closest to that of the native dermis when compared to other commercial products AlloDerm® RTU and DermACELL®.†

Fig. 5 **Enzymatic Degredation Profile†**



Enzymatic degradation profile using collagenase type I. The collagen stability of the Native Dermis, SimpliDerm™, AlloDerm® RTU and DermACELL® was evaluated by measuring the matrix digestion over time (0-24 hours) through percent dry mass remaining.

Fig. 6 **Acid-Soluble Collagen Content†**



The percentage of acid-soluble collagen content in Native Dermis, SimpliDerm™, AlloDerm® RTU and DermACELL® indirectly obtained by measuring the hydroxyproline content.

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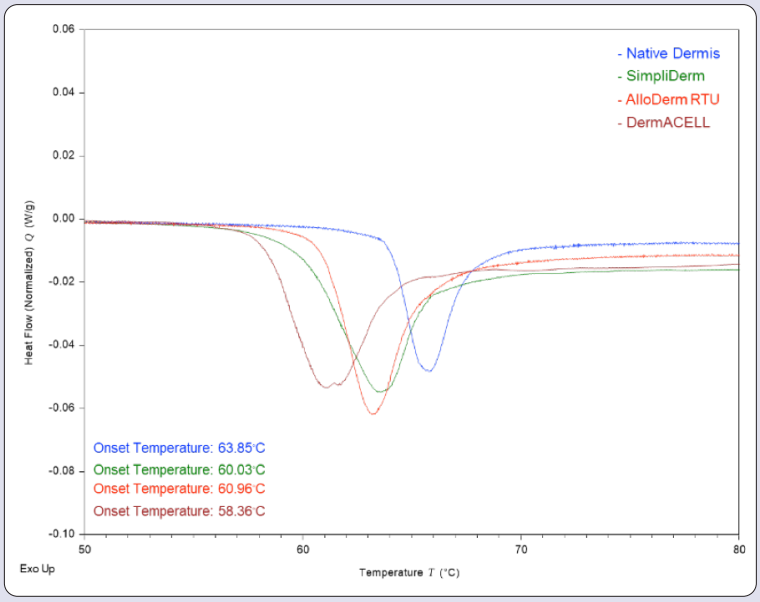
Thermal Stability

Differential Scanning Calorimetry (DSC) is a thermal analysis method often used to study the thermal transitions of a material and evaluate the factors that contribute to the stability of the biomolecules[9]. DSC was utilized to measure the onset melting temperature of the dermal matrix and evaluate the thermal stability of native human skin, SimpliDerm™ and other commercial products (AlloDerm® RTU and DermACELL®).†

The DSC analysis showed that the onset melting temperature of SimpliDermTM was closer to that of the native dermis than DermACELL® and was similar to AlloDerm® RTU ***(Figure 7).***†

Fig. 7 **Differential Scanning Calorimetry†**



Thermal curve showing the onset collagen melting temperature of Native Dermis (63.85°C), SimpliDerm™ (60.03°C), AlloDerm® RTU (60.96°C) and DermACELL® (58.36°C) (N=1, n=3).

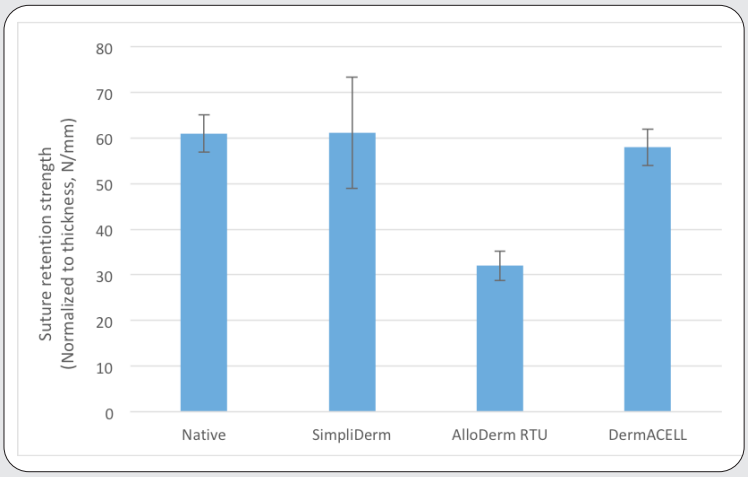
Biomechanical Properties

To evaluate the mechanical properties of SimpliDerm™, samples were subjected to suture retention testing on the Instron testing system using custom-designed grips. The suture retention strength of the native dermis, SimpliDerm™, AlloDerm® RTU, and DermACELL® are shown in ***Figure 8.***†

The mechanical properties of SimpliDerm™ measured by the suture retention strength was 61.14 ± 12.22 N/mm. This value was not significantly different from that of the Native Dermis (61.02 ± 4.12 N/mm) and DermACELL® (57.93 ± 3.97 N/mm).†

In addition, the suture strength of AlloDerm® RTU (32.02 ± 3.25 N/mm) was significantly different from that of the Native Dermis, SimpliDerm™, and DermACELL®.†

Fig. 8 **Suture Retention Strength†**



Suture retention strength of the Native Dermis, SimpliDerm™, AlloDerm® RTU, and DermACELL®. **SimpliDerm™ retained mechanical properties measured by suture retention strength closest to that of Native Dermis.**

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In vivo study:
Abdominal Repair in Non-Human Primate Model

In order to investigate the tissue remodeling and regeneration process of SimpliDerm™ upon surgical implantation, an animal study involving a well-established non-human primate abdominal wall repair model was conducted. SimpliDerm™ measuring 3 x 7 cm was implanted into a full thickness abdominal wall defect created in African Green monkey and the explant time points for evaluation were 2 weeks, 4 weeks, and 3 months.†

Immunohistochemistry staining against CD68, a macrophage marker, for sections obtained from samples at 4-week time point indicated that SimpliDerm™ demonstrated lower inflammatory response than the commercial product AlloDerm® RTU ***(Figure 9).***†

At 3 months, SimpliDerm™ showed prominent new collagen deposition, cellular infiltration by fibroblasts and mild vasculature formation ***(Figure 10A/B),*** which are all indicative of healthy tissue regeneration process. †

In addition, throughout the time point there were no significant adverse reactions associated with the implant such as fibrin, necrosis, mineralization, osseous metaplasia or cavity/pocket (e.g., seroma).†

Overall, SimpliDerm™ demonstrated proper tissue repair and regeneration *in vivo* as evident by low host immune response, neovascularization and new collagen formation.†

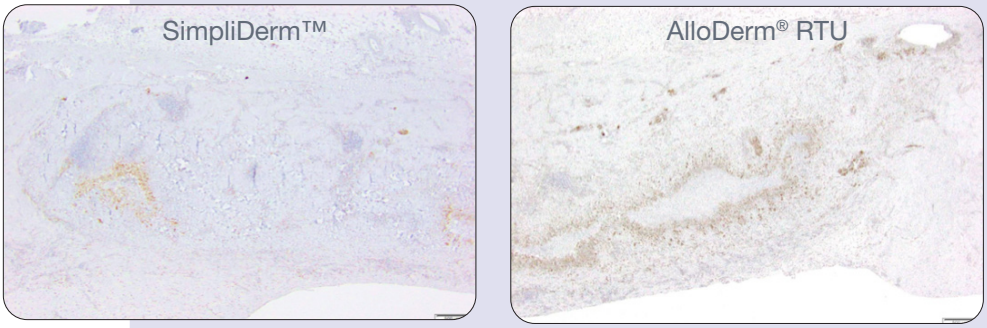

Fig. 9

Immunohistochemistry staining against CD68, a macrophage marker for sections from animals with SimpliDerm™ and AlloDerm® RTU implants at 4-week time point. **SimpliDerm™ shows pockets of inflammatory cell concentration,** while AlloDerm® RTU shows significant confluent bands of inflammatory cell infusion.†

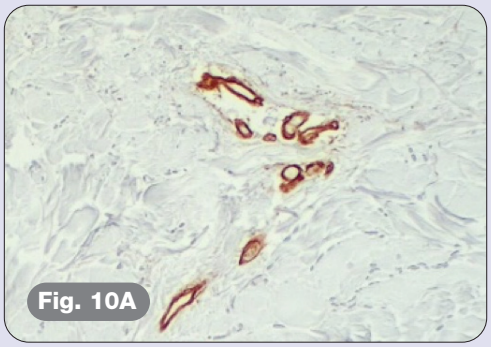

Fig. 10A

Immunohistochemistry staining against collagen IV that shows the basement complex of blood vessels in pre-implant SimpliDerm™.†

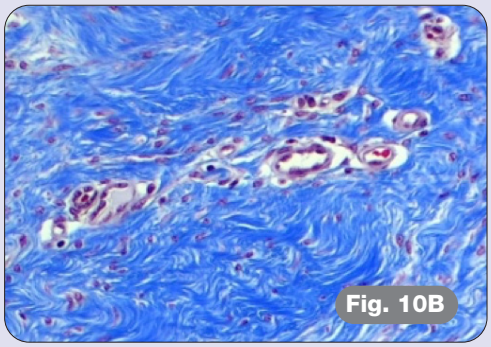

Fig. 10B

Masson's Trichrome staining from animal with SimpliDerm™ implant at 3 months. **New collagen formation (light blue stains) and neovascularization (dark red stains) are evident.†**

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Conclusion

The preclinical studies involving SimpliDerm™ matrix characterization and *in vivo* animal study demonstrate that SimpliDerm™ is designed and processed to preserve the natural matrix as close as possible to native dermis for better tissue repair and regeneration.†

Using Aziyo's proprietary processing SimpliDerm™ allografts achieve a Sterility Assurance Level (SAL) of 10^{-6} while maintaining a structurally intact matrix that retains similar collagen integrity, mechanical strength, and bioactive components of the native dermis.†

All of these properties together demonstrate that SimpliDerm™ is closest to native dermis and allows for lower inflammatory response and better tissue regeneration during regenerative medicine applications that other human ADMs including AlloDerm® RTU and DermACELL®.†

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