Decellularization of Human Dermis Using Matracell® Technology: Process, Preclinical Studies, and Medical Applications

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Abstract

Decellularized human skin has been used for a variety of medical procedures; primarily wound healing, soft tissue reconstruction, and sports medicine applications. A variety of methods exist to prepare this useful class of biomaterial. In particular, LifeNet Health has introduced a patented decellularization technology, trade-named MatrACELL®, with application for human dermis and designed to result in an allograft scaffold to support host cellular in-growth and revascularization. By using a unique combination of anionic, non-denaturing detergent and endonuclease enzymatic treatments, human dermis is rendered acellular with removal of ≥97% of the donor genetic material, while retaining the biomechanical strength of untreated tissue. In addition, the MatrACELL technology is complemented by terminal sterilization of the final packaged material to yield an allograft with a Sterility Assurance Level of 10^{-6} consistent with implantable medical devices. Using a cytotoxicity assay and *in vivo* mouse models, MatrACELL Dermis was shown to be biocompatible and capable of supporting cellular and vascular in-growth. With respect to other treated human dermis materials, 2 mm thick MatrACELL Dermis exhibited an ultimate tensile strength of 635.4±199.9 N vs. 532.0±154.0 N for 2 mm thick GraftJacket® MaxForce Extreme and suture retention strength of 134.9±55.1 N for MatrACELL Dermis vs. 106.5+/-27.9 N for GraftJacket MaxForce Extreme. Effective decellularization is demonstrated by a residual DNA content of 15.97±4.8 ng/mg of dry weight, in contrast to the higher reported residual DNA levels of 134.6±44.0 and 272.8±168.8 ng/mg dry weight for GraftJacket and Alloderm®, respectively. Taken together, these characteristics indicate the potential utility of MatrACELL Dermis for a variety of wound healing, soft tissue reconstruction, and sports medicine applications.

Introduction

Decellularized human skin has been used for a variety of medical procedures; primarily wound healing, soft tissue reconstruction, and sports medicine applications. In theory, decellularization serves to remove cellular material and provide a clean scaffold for host cellular and vascular in-growth. One reported clinical application is the repair of rotator cuff tears.[1-6] During this procedure, the dermal matrix is typically used to augment the repair as well as support directed healing. Similarly, Achilles and quadriceps tendon augmentation procedures using decellularized human skin are reported.[7-10] Also, soft tissue reconstruction procedures are commonly performed with decellularized human skin including primary, staged, and revision breast reconstruction.[11-13]

In addition, hernia repair using similar materials has been reported[14-17] as well as the treatment of skin wounds such as diabetic foot ulcers.[18-20] While the use of decellularized human skin is especially noted here, other collagen-based materials such as small intestinal submucosa and dermal xenograft can be used for these clinical applications.[21-23] Collagen-based membranes are also used in Guided Tissue/Bone Regeneration (GTR/GBR) to treat dental periodontal intrabony defects and for ridge augmentation procedures.[24-27]

Dermis is not the only human material that is decellularized for clinical applications. Human cardiovascular materials have been decellularized and used in a variety of clinical applications as extensively described.[28-40] One of these particular technologies, trade named MatrACELL™, has been patented[41-44] and applied to human cardiovascular tissue. MatrACELL-treated pulmonary patches received FDA 510k clearance and have been in clinical use since 2009. The same technology is now applied to human dermis with the resultant material referred to as MatrACELL Dermis under the trade names DermACELL®, Oracell®, or ArthroFLEX®, depending on the particular material dimensions and clinical application. The properties and potential applications of MatrACELL Dermis are described here.

Desirable Soft Tissue Properties

In general, desirable properties for soft tissue materials for wound treatment, soft tissue reconstruction, and augmentation procedures include:

- Biocompatibility
- Availability in a range of sizes and thicknesses
- Excellent handling characteristics
- High suture retention
- Low-to-moderate costs
- Capability of cellular in-growth
- Low infectious potential
- Capability of revascularization
- Conformity to desired application

In order to obtain these properties, the scientific rationale utilized to develop an effective decellularization process involved the following:

1. Use of reagents that would leave the remaining extracellular matrix biocompatible and biomechanically sound.
2. Characterization of biocompatibility.
3. Characterization of the biomechanical strength of the resultant extracellular matrix.
4. Validation of manufacturing procedures to reproducibly decellularize the tissue including characterization of minimal DNA residuals.

In response to these challenges, LifeNet Health has developed the MatrACELL™ process designed to yield a decellularized, biocompatible, and biomechanically sound human dermal matrix.

The MatrACELL Decellularization and Sterilization Process

The MatrACELL process was developed to minimize the amount of reagents and reagent contact time required to decellularize bio-implants. MatrACELL-processed tissue is rendered acellular in a solution of non-denaturing anionic detergent (N-Lauroyl sarcosinate, NLS), recombinant endonuclease (Benzonase®), and antibiotics (Polymixin B, Vancomycin and Lincomycin).[45-47] Following decellularization, the tissue is rinsed of the decellularization reagents. The MatrACELL process has been fully validated to reproducibly render human dermis tissue acellular as assessed by ≥97% reduction of DNA content (described below). The bio-implant is also treated to remove and replace the water volume with glycerol[48-49] prior to final packaging in order to allow room temperature storage and rapid preparation time. Finally, the bio-implant is terminally sterilized with validated low temperature, low dose (<20 kGy) gamma irradiation.[50] This final step results in a Sterility Assurance Level of 10^{-6} as anticipated for a medical device and allows tissue allografts to be labelled as sterile, while also inactivating viruses[51] and retaining key mechanical properties.[52]

Preclinical Evaluation of MatrACELL Dermis

MatrACELL decellularized tissue has been assessed via analytical methods, biomechanical testing, and *in vivo* analysis. Representative study results are presented here.

Histological Analysis

The MatrACELL process is designed to remove cellular materials from tissue. As shown in Figures 1 and 2, histological analysis demonstrates removal of cellular and potentially immunogenic components. This is further supported by DNA analysis described in the next section.

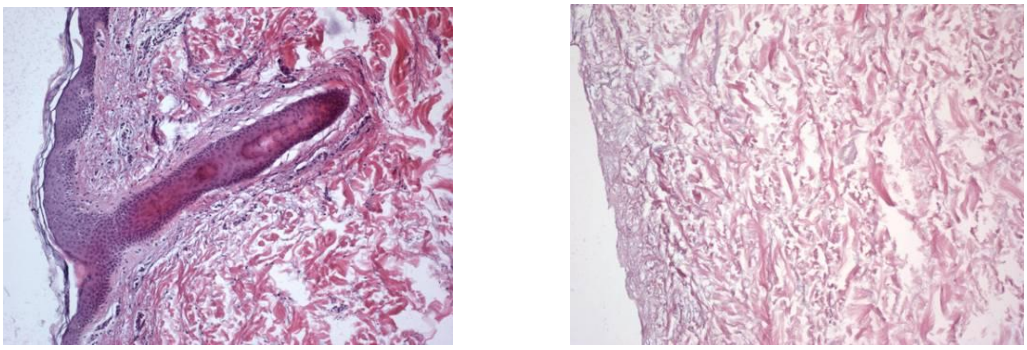

Figure 1. Histological analysis of tissue prior (left) to decellularization and after (right) the MatrACELL process. The staining is Hemotoxylin and Eosin (H&E) to show general cellular remnants. Note the presence of stained cellular material prior to decellularization in contrast to the acellular appearance of the tissue on the right.

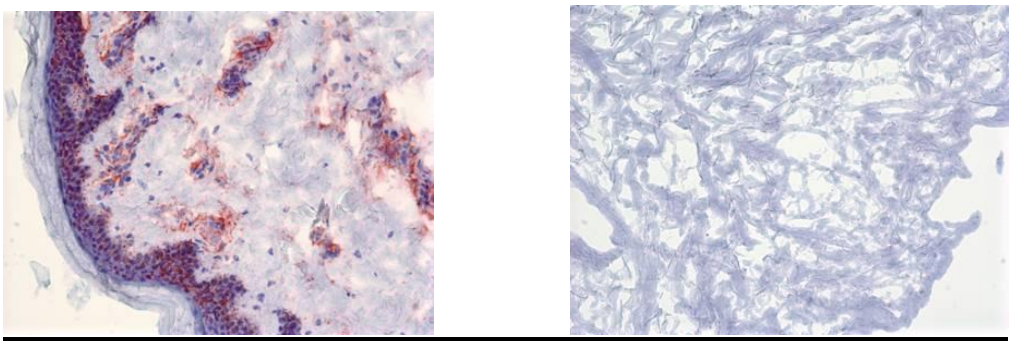

Figure 2. Histological analysis of tissue prior (left) to and after (right) the MatrACELL process. The staining is for Major Histocompatibility Complex 1 (MHC1) to detect cellular material. Note the presence of brick-red stained MHC1 prior to decellularization in contrast to the appearance of the tissue on the right.

Analysis of DNA Residuals

The DNA content of tissue decellularized using the MatrACELL process is reduced by ≥97%. The DNA assay was validated as described in the International Committee on Harmonization document Q2, "Validation of Analytical Procedures: Text and Methodology." The assay utilizes a fluorometric dye, PicoGreen (Invitrogen) that has a lower limit of detection of 0.7 ng DNA/ml and lower limit of quantitation of 2.7 ng DNA/ml. The results of an internal validation process for decellularization are shown in Figure 3. In this study, parameter limits for decellularization of skin were assessed as a function of resultant DNA residuals. Note that all parameters result in an average of >97% DNA reduction.

Processing Validation Parameter	Average DNA preprocessing ng/mg wet weight	Average DNA post-processing ng/mg wet weight	% DNA Reduction
Lower Processing Limit	118.18±19.23	1.67±0.16	98.6%
Target Processing	107.36±13.89	2.78±0.14	97.4%
Upper Processing Limit	132.98±12.24	1.76±0.12	98.7%

Figure 3. DNA content for dermis before and after MatrACELL process decellularization quantified at lower, target, and upper processing parameters. All DNA content results are presented as ng/mg of wet weight of material.[52] Note the substantial DNA reduction at all parameters.

In addition, a comparison of the DNA content of two other commercially available decellularized human tissues, Alloderm and GraftJacket, which are both manufactured by procedures from the same organization is demonstrated in Figure 4. Note that, in limitation, this is not a side-by-side experiment, but rather by comparison to literature data.[54,55] However, all values are represented as ng/mg dry weight of tissue. In the respective publications, the DNA analyses are quoted as follows; GraftJacket: "DNA content was determined with use of the PicoGreen dsDNA Assay (Molecular Probes) according to the manufacturer's instructions…DNA content of GraftJacket averaged 134.6 ± 44.0 ng/mg dry weight." and for Alloderm: "Mean DNA concentration plus or minus standard error was…272.8 +/- 168.8 microg./gm. tissue for…cadaveric dermis". It is unclear why the similar materials GraftJacket and Alloderm differ in these studies since they are prepared by the same process. It could be lot variability or differences in assay methods. Regardless, reported residual DNA values for GraftJacket and Alloderm far exceed those of MatrACELL Dermis.

Decellularized Human Dermis	Residual DNA* (ng/mg dry weight)	Study Reference
MatrACELL Dermis	**15.97±4.8**	Data on file, LifeNet Health.
GraftJacket	**134.6±44.0**	Derwin KA, Baker AR, Spragg RK, Leigh DR, Ianotti JP. Biomechanical, biochemical, and cellular properties commercial extracellular matrix scaffolds for rotator cuff tendon repair. J Bone Joint Surg Am. 2006; 88:2665-2672.
Alloderm	**272.8±168.8**	Choe JM, Bell T. Genetic material is present in cadaveric dermis and cadaveric fascia lata. J Urol. 2001; 166:122-4.

* ***Please note that this data comes from three different sources.***

Figure 4. Residual DNA content for MatrACELL[53], GraftJacket[54], and Alloderm.[55]

Biomechanical Testing

Depending on the intended use, the biomechanical properties of decellularized dermal matrices may be of clinical significance, especially in load-bearing applications. Barber and Aziz-Jacobo (2009)[56] reported the testing of numerous commercially available materials used for soft-tissue augmentation. Using the same methodologies and testing laboratory, 2 and 1.5 mm thicknesses of MatrACELL Dermis were tested and the data compared to that previously published for GraftJacket® (2 and 1.5 mm thick decellularized human dermis, Wright Medical, Arlington, TN), SportsMesh® (0.8 mm thick knitted polyurethane urea fabric, Biomet Sports Medicine, Warsaw, IN) and OrthoAdapt™ (0.5 mm thick equine pericardium, Synovis Orthopedic and Wound Care, Irvine, CA).[57] Suture retention strength was measured as the force needed to pull out a simple vertical stitch of Arthrex No.2 FiberWire passed through the tissue 5 mm from the edge. As previously reported,[57] these results are shown in Figure 5. Ultimate tensile strength was determined by pressure clamping two ends of a single layer of material and elongating to failure. After cyclic loading, a final destructive test was performed and the ultimate load-to-failure was considered the point of final material failure. As previously reported,[57] these results are shown in Figure 6.

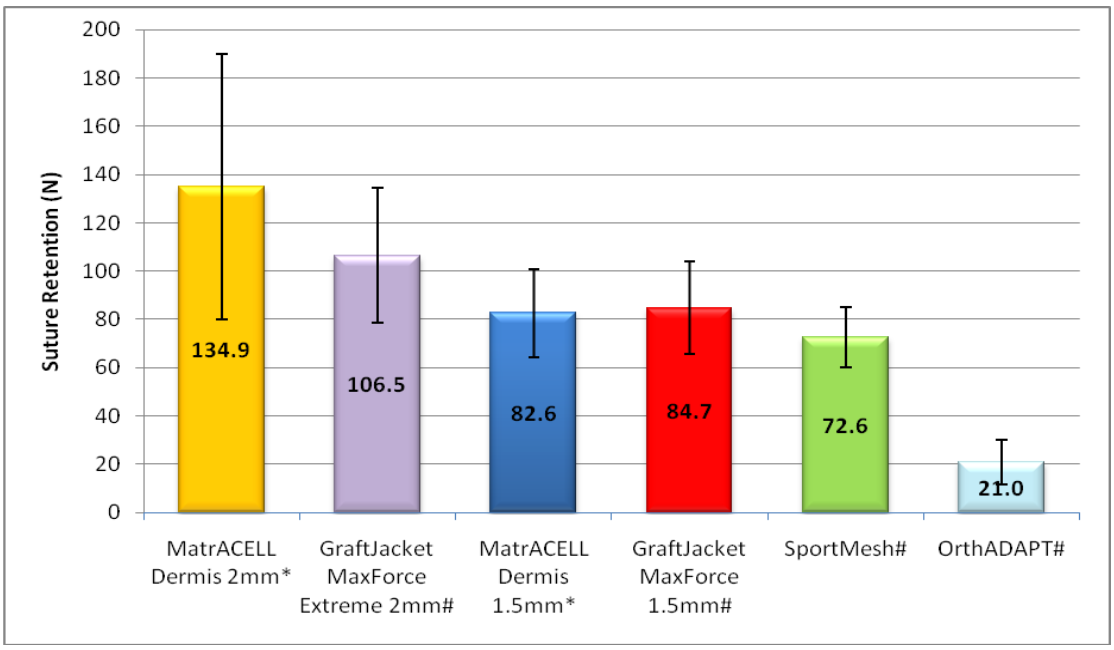

Figure 5. Suture retention strength comparison of medical implant matrices. Testing was performed as described in the text of this Section. Data generated for MatrACELL Dermis(*)[57] and all other materials (#),[56] respectively, was generated at different points in time; however, the exact same methods, fixtures, material testing machine, and facility was used for both studies. The values from Barber and Aziz-Jacobo (#) were selected data from Figure 3 of that paper.

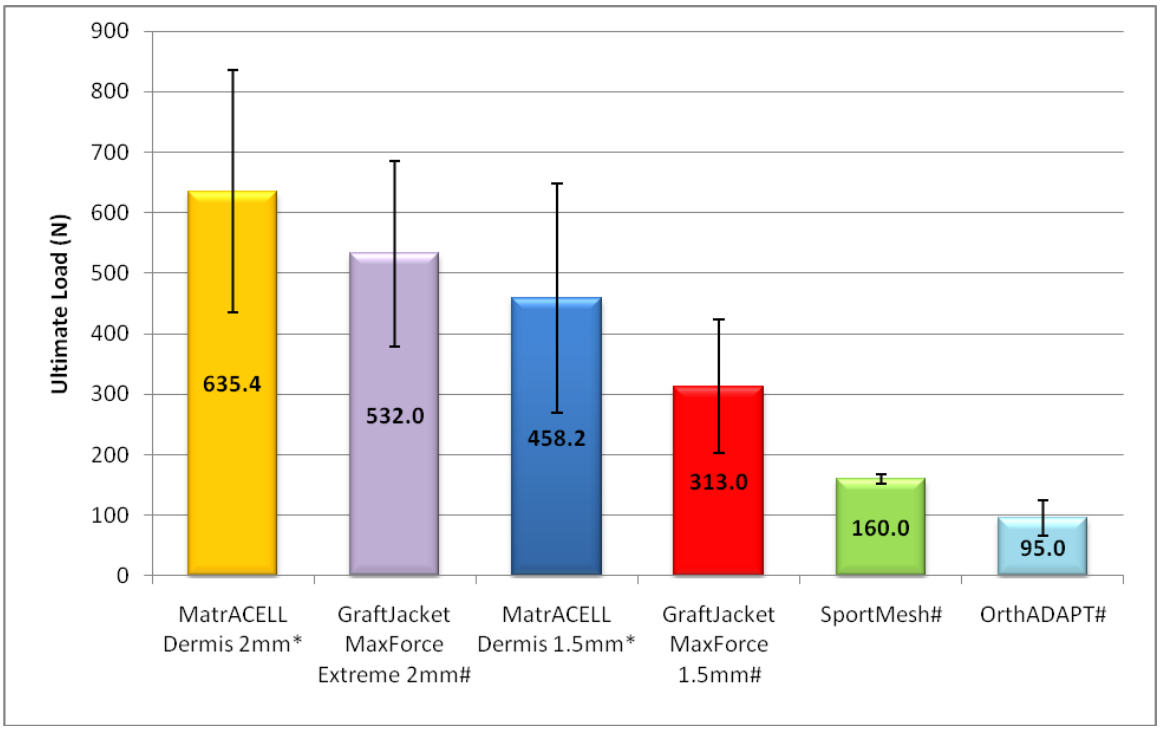

Figure 6. **Ultimate load to failure comparison of medical implant matrices.** Testing was performed as described in the text of this Section. Data generated for MatrACELL Dermis(*)[57] and all other materials (#),[56] respectively, was generated at different points in time; however, the exact same methods, fixtures, material testing machine, and facility was used for both studies. The values from Barber and Aziz-Jacobo (#) were selected data from Table 1 of that paper.

As shown in Figures 5 and 6, the biomechanical integrity of MatrACELL Dermis compares favorably with other materials used in soft-tissue augmentation procedures, including synthetic mesh and equine pericardium.

Small animal study: *in vivo* results

MatrACELL Dermis was tested using a nude mouse skin excisional model. In this study, a portion of skin was excised from the back of a nude mouse and replaced with human MatrACELL Dermis and covered with a dressing. After 16 days, the material was removed and examined histologically. The biocompatibility of the matrix is demonstrated in Figure 7 by in-growth of new blood vessels as well as the appearance of a new cellular layer on the surface of the graft.

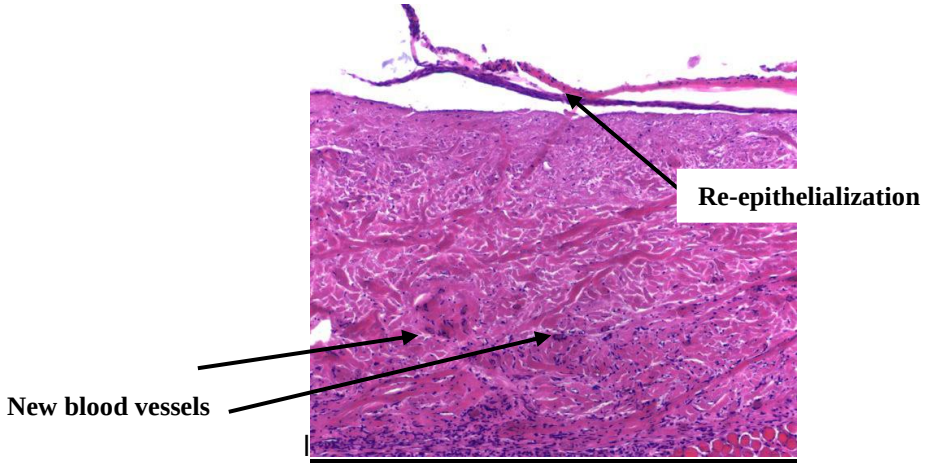

Figure 7. Histological analysis of MatrACELL Dermis explants using nude mouse skin excisional model. The implant was in place for 16 days prior to excision and analysis. The stain is Hematoxylin and Eosin for general cellular features. Note the presence of features indicating new blood vessels and epithelial layer.

MatrACELL Dermis was also tested using a mouse subcutaneous implant model. In this study, MatrACELL Dermis was explanted after 4 weeks and examined histologically. A representative image shown in Figure 8 demonstrates the biocompatible nature of the implanted material again including new vascular in-growth.

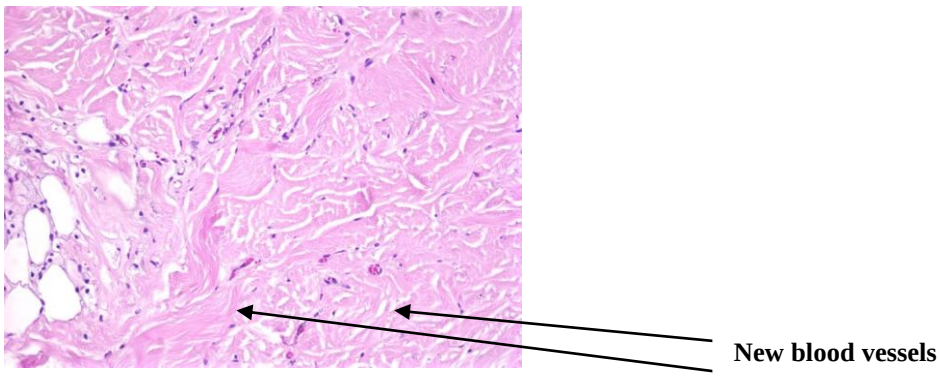

Figure 8. Histological analysis of MatrACELL Dermis explants using mouse subcutaneous implant model. The implant was in place for 4 weeks prior to explant. The stain is Hematoxylin and Eosin for general cellular features. Note the presence of new blood vessels (arrows) and lack of apparent inflammatory response.

Clinical Applications for MatrACELL Dermis

There are many potential clinical applications for MatrACELL Dermis. For these varying needs, numerous dimensions are provided varying in width, length, and thickness. Since introducing the material in 2010, a variety of clinical applications have been identified and explored in the areas of sports medicine surgeries, craniomaxillofacial repairs, breast reconstruction, and wound healing. Some specific applications are noted here. The following Figures demonstrate MatrACELL Dermis in clinical use.

Tendon augmentation: MatrACELL Dermis has application in the augmentation of rotator cuff, Achilles tendon, quadriceps, biceps tendon, and other tendon and ligament structures.

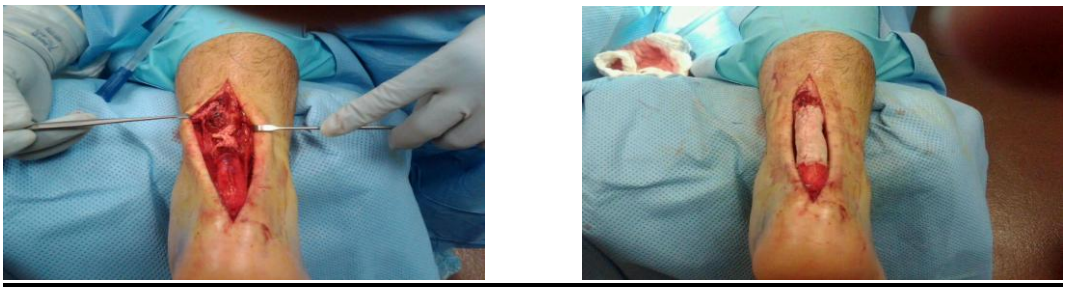

Figure 9. Use of MatrACELL Dermis to augment an Achilles tendon repair.

Figure 10. Use of MatrACELL Dermis to augment a biceps tendon repair.

Craniomaxillofacial Applications: MatrACELL Dermis may have many craniomaxillofacial applications from guided tissue regeneration and ridge augmentation to facial reconstruction procedures. Two such cases are demonstrated in the following Figures.

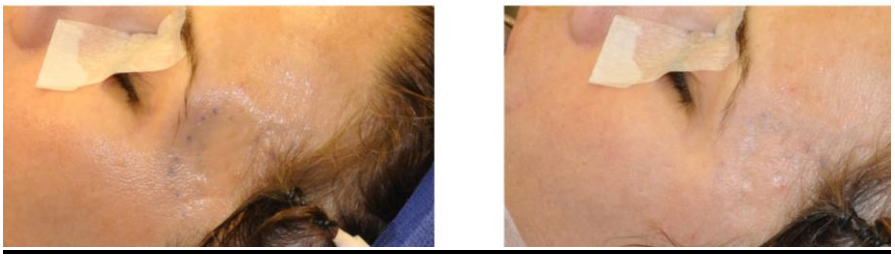

Figure 11: Use of MatrACELL Dermis to repair a temporal depression. The left photo is pre-surgery showing the temporal depression. The second photo demonstrates the depression smoothing following an incision in the hair line and insertion of MatrACELL Dermis.

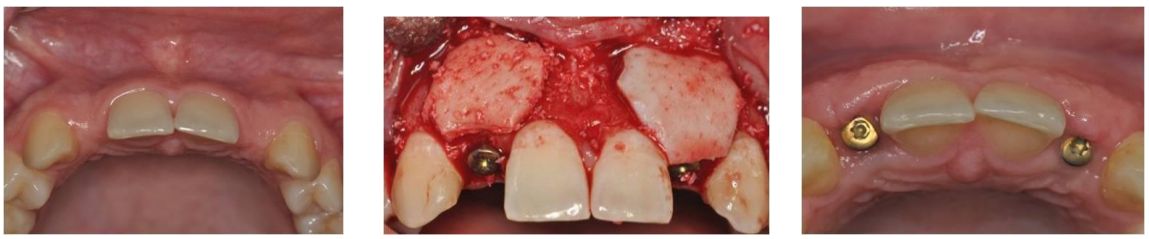

Figure 12: Use of MatrACELL Dermis in conjunction with cortical particulate to correct for the thin bone implant support and to increase the soft tissue profile in these areas. The first photo is pre-surgery showing a thin bone ridge. The second photo shows the application of MatrACELL dermis in conjunction with allograft bone and synthetic implants. The third photo is at 4 weeks post-implant demonstrating increase in tissue profile and a smoothly healed gum line.

Breast Reconstruction: MatrACELL Dermis may also be used in other soft tissue procedures, such as breast reconstruction. Figure 13 demonstrates one such application.

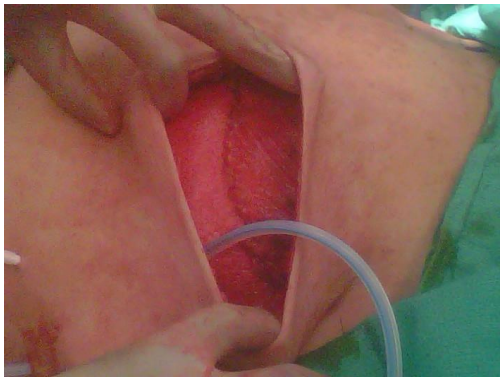

Figure 13: Use of MatrACELL Dermis for breast reconstruction. In this case, 6 x 16 cm piece of MatrACELL Dermis was used in an immediate reconstruction with placement of a tissue expander.

Wound Healing: MatrACELL Dermis may have application in the treatment of chronic wounds such as diabetic foot ulcers. Figure 14 demonstrates one such application.

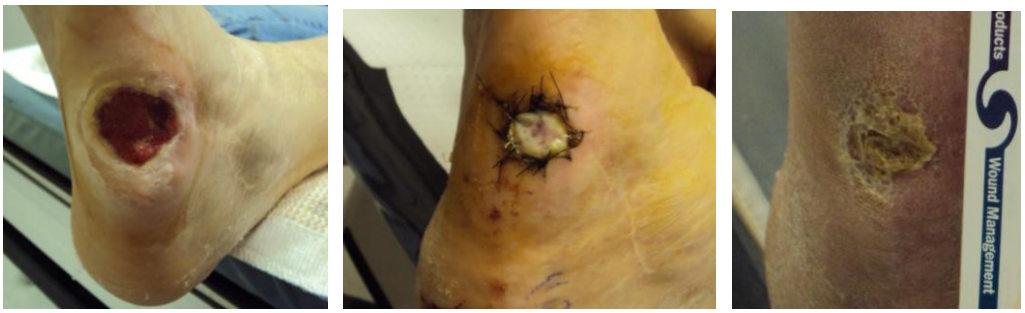

Figure 14: Use of MatrACELL Dermis for wound repair of a diabetic foot ulcer. The first panel shows the debrided wound, the second the wound with a piece of MatrACELL Dermis sown in, and the third panel shows the wound substantially healed at 3 weeks post-treatment.

Conclusions

MatrACELL decellularization of human dermis is a validated process that has been developed to result in effective cellular removal from the dermal matrix with retention of biomechanical properties. Data presented here indicates the process effectively

removes cellular material, including DNA and cellular components, yielding a material that retains biomechanical strength and is biocompatible as demonstrated by *in vivo* models. The material has numerous potential clinical applications, dependant on area and thickness, including tendon augmentation, facial reconstruction, wound healing, breast tissue reconstruction, and dental procedures.

Disclosure: References to published clinical application literature for decellularized human dermis are provided as background information and are not intended to imply cleared indications. Please refer to the manufacturer of any decellularized dermis for proper clinical utilization.

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