DermACELL®
Advanced Decellularized Dermis

Analysis of the Acellular Matrix, Growth Factors, and Cytokines Present in DermACELL® Advanced Wound Management

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Findings

Fragments of the following proteins were found by LC-MS/MS to be present in DermACELL:

Collagens	GF-binding ECM	Additional ECM	Matrikines	Growth Factors	Cytokines
Type I	Heparan Sulfate Proteoglycan (HSPG)	Elastin	Tenascin-C	BMP6	IL1a
Type III	Chondroitin Sulfate Proteoglycan (CSPG)	Nidogen (Entactin)	Laminins	CTGF	IL1b
Type IV	Perlecan (HSPG2)	Keratin	Decorin	EGF	IL2
Type V	Aggrecan		Endostatin	HGF	IL5
Type VI	Lumican		Pentastatin	PDGFD	IL9
Type VII	Versican		Tumstatin	TGFB1	IL22b
Type VIII	Glypican		Elastokines	VEGFA	IL25
Type XII	Syndecan			VEGFD (FIGF)	IL27
Type XIV	Tenascin (C & N)				IL32
Type XVII	Thrombospondin 2				TNF
Type XVIII	Dermatopontin				
Type XX	Decorin				
Type XXI	Vitronectin				
Type XXIII	Laminin (α1-5, β1-3,γ1&3)				
Type XXVII	Fibrinogen (Fibrin precursor)				
Type XXVIII					

Objective

Identify the extracellular matrix (ECM) components, growth factors, and cytokines in DermACELL, a decellularized sterile human dermal allograft.

Introduction

Human skin is a complex tissue containing various extracellular matrix molecules, growth factors, and cytokines[3]. While healthy human skin is capable of repairing damaged areas and replacing all components, chronic wounds display an inability to properly synthesize many components and rebuild healthy skin[3-5]. The purpose of this study was to ensure that DermACELL, a minimally manipulated human skin product, retains the components of healthy human skin known to be lacking in chronic woulnds and known to support the repair of damaged skin.

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Methodology

Samples were solubilized in a detergent solution assisted by mechanical homogenization followed by a protein separation based on molecular size. Subsequently, the separated proteins were enzymatically fragmented, after which the amino acid sequence of each fragment was determined by liquid chromatography with tandem mass spectrometry (LC - MS/MS)[1]. The amino acid sequences of each fragment were then compared against a database containing the sequences of known proteins to determine the corresponding protein for each fragment. From this comparison, a list of proteins in each sample was generated[2]. This list was mined for extracellular matrix (ECM) components, growth factors, and cytokines to create a table of proteins whose fragments were found in the DermACELL sample. Additionally, some components were further verified or identified by immunohistochemical staining and by enzyme-linked immunosorbent assay (ELISA).

Conclusion

The results of this study indicate that DermACELL retains ECM components, matrikines, growth factors, and cytokines consistent with healthy human skin and relevant to the natural repair of damaged skin. Additionally, DermACELL provides structural integrity to damaged skin by supplying human ECM that chronic wounds (such as diabetic wounds) lack and are unable to properly synthesize[5].

Discussion

The environment of the chronic wound is highly destructive characterized by high levels of proteases and abnormalities in both biochemical structure of ECM and the expression of growth factors[3,5]. The findings of this study show that the processing of DermACELL preserves many of the structural components, growth factors, and cytokines present in healthy human skin. Applying DermACELL to the chronic wound environment can act to replace the damaged and abnormal skin wth a minimally manipulated human dermis containing the same wound repairing factors present in natural healthy skin.

Using LC-MS/MS, DermACELL was found to contain ECM components present in the native dermis ECM including collagens, proeoglycans, and elastin. ECM provides the substrates for the high levels of proteases known to inhibit wound healing in chronic wound environments[3,5]. Additionally, DermACELL provides human ECM that chronic wounds (such as diabetic wounds) are unable to properly synthesize[5] yet are required to create structural integrity for new skin and a scaffold for wound repairing cells[3].

When healthy human skin is damaged, the structual components of the ECM are broken down and internalized[3]. It has been demonstrated in the scientific literature that the ECM cleavage products can act as signaling molecules (matrikines) and are capable of encouraging vital wound repair processes[6,7]. Some examples of matrikines include Tenascin-C and Laminin which are known to promote cell migration from the leading edges of healing wounds6. The elastikines (cleavage products of elastin) and collagen cleavage products of tumstatin, pentastatin and endostatin are known promoters of cell proliferation and angiogenesis[6,7].

While cell-secreted growth factors are an essential component of wound repair activity, they are not designed to work in isolation but rather intended to be sequestered, regulated, and enhanced by ECM[5]. In harsh wound environments, increased growth factor expression alone often does not result in increased functional activity[5]. A multitude of the human skin ECM proteins known to regulate growth factor activity and known to be important in wound repair (including HSPG, CSPG, Thrombospondin, Tenascin, Dermatopontin, and numerous proteoglycan core poroteinds[3,5]) were preserved in the DermACELL processing.

DermACELL Provides a Human Skin Replacement

The findings suggest that DermACELL retains a broad array of extracellular matrix components, matrikines, growth factors, and cytokines present in healthy human skin and can provide the structural integrity lacking in chronic wounds. Only DermACELL provides the natural human ECM with greater than 97% of the DNA removed and minimizes the risk of infection with a 10^{-6} sterility assurance level for closure of damaged skin.

References

1. LC-MS/MS is an analysis whereby the fragmented proteins present in a solution are separated by molecular weight. Then, each separated fragment is further broken into smaller components and the molecular weights of those smaller components is determined. From the molecular weights of the smaller components, the amino acid sequence of the orginal fragment can be resolved.
2. This analysis was performed by Dr. Qishan Lin at the UAlbany Proteomics Facility.
3. Bryant RA, Nix D. *Acute Chronic Wounds. Current Management Concepts*. 3rd ed. St. Louis: Elsevier Misby: 2007.
4. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. "Growth factors and cytokines in wound healing" Wound Repair Regen. 2008 Sep-Oct; 16(5):585-601
5. Schultz GS, Wysocki A. "Interactions between extracellular matrix and growth factors in wound healing: Wound Repair Regen. 2009 Mar-Apr; 17(2): 153-62
6. Tran KT, Lamb P, Deng JS. "Matrikines and matricryptins: Implications for cutaneous cancers and skin repair: J Dermatol Sci. 2005 Oct; 40(1): 11-20
7. Ricard-Blum S, Ballut L. "Matricryptins derived from collagens and proteoglycans: Front Biosci. 2011 Jan 1; 16:674-97

What is DermACELL?

DermACELL is biocompatible decellularized human dermal allograft with an intact acellular framework.
DermACELL retains native ECM components, matrikines, growth factors, and cytokines while providing a scaffold for recipient cell proliferation and migration for wound closure.
DermACELL is an effective natural barrier to help control infection and assist in the promotion of granulation tissue and epithelialization for wound closure.

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