

THERASKIN® ANALYSIS, STAGE 1 FINDINGS:

Identification of Key Growth Factors, Cytokines and Collagen in TheraSkin

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OBJECTIVE

Identify the specific growth factors, cytokines and collagen in TheraSkin, a cryopreserved human skin allograft.

METHODOLOGY

Samples were solubilized in a detergent solution assisted by mechanical homogenization followed by 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)⁴. 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 present in each sample was generated.⁵

CONCLUSION

TheraSkin provides the broad spectrum of major growth factors, cytokines and collagen recognized to be important elements in healing wounds.

FINDINGS

GROWTH FACTORS

10 different growth factors were found in TheraSkin, including those from the five major growth factor families and proteins involved in skin wound healing.

CYTOKINES

16 different cytokines were found in TheraSkin, including several pro-inflammatory and anti-inflammatory cytokines necessary to regulate interactions between cells that participate in the immune response of healing.

COLLAGEN

14 types of human collagen were found in TheraSkin, including three major types (I, III and IV) necessary to promote healing.

Growth Factors	Cytokines	Collagen
PDGFA	TNF	Type I
PDGFD	IL1a	Type III
VEGFD (FIGF)	IL1b	Type IV
EGF	IL2	Type V
TGFA	IL32	Type VI
FGF2	IL4	Type IX
TGFB1	IL5	Type X
TGFB3	IL6	Type XI
HGF	IL12	Type XII
BMP7	IL13	Type XIV
	IL16	Type XV
	IL17A	Type XVII
	IL18	Type XVIII
	IL25	Type XX
	IL27	
	IL32	

DISCUSSION

It is frequently difficult to determine the biological deficiencies of a given wound. These findings indicate that without expensive diagnostics to determine the exact needs of a wound, a practitioner may use TheraSkin to provide a broad spectrum of the growth factors, cytokines and collagen types that have the best chance of meeting the wound's needs. This differs from the bioengineered products currently on the market, known as Living Skin Equivalents (LSEs), which only contribute some of the human biological components necessary for healing.

TheraSkin also provides a thick layer of human collagen to the wound bed. Apligraf® delivers bovine rather than human collagen to the wound. Collagen provided by Dermagraft® is limited to that expressed by the fibroblasts. These and other LSE's provide growth factors and cytokines to a wound, but fall short of delivering the more complete array of biological components of real human skin.

TheraSkin provides a wound with tools it needs to heal.

CELLULAR AND METABOLICALLY ACTIVE COMPONENTS OF THERASKIN®

GROWTH FACTORS

There are five major growth factor families and proteins involved in skin wound healing. Processed from donated human skin, TheraSkin contains each of these critical growth factors and provides them to the wound to promote epithelialization and healing.

PDGF, Platelet-Derived Growth Factors (PDGF, VEGF) – a chemoattractant and mitogen primarily for fibroblasts. PDGFs stimulate formation of extracellular matrix and granulation tissue.

EGF, Epidermal Growth Factors (EGF, TGF-alpha) – mediates action of TGF-Bs on collagen synthesis. TGF-alpha primarily responsible for normal maintenance and turnover of epithelial cells.

FGF, Fibroblast Growth Factor (FGF) – important regulators of wound healing, mitogens for fibroblasts.

TGF-B, Transforming Growth Factor-Beta – newly-discovered GFs stimulate wound healing

IGF, Insulin-Like Growth Factor – IGFs and growth hormone act synergistically to promote wound healing

CYTOKINES

There are two important types of cytokines known to influence wound healing, and TheraSkin contains both types to regulate the interactions between cells that participate in the immune response. Most notably, the **TNF-alpha (Tumor Necrosis Factor-alpha)** stimulates collagen synthesis and provides metabolic substrate, and **IL-1 (Interleukin-1)** stimulates collagen synthesis as well as fibroblast and keratinocyte chemotaxis, all critical elements of skin wound healing.

Pro-inflammatory cytokines – TNF-alpha, IL-1, IL-2, IL-6

Anti-inflammatory cytokines – IL-4

COLLAGEN

Collagen forms the mechanical infrastructure necessary to heal wounds, and TheraSkin contains all three major types of human collagen necessary to promote healing. TheraSkin is notably processed from human tissue, rather than bovine, and contributes human collagen to the wound. TheraSkin provides a thick layer of collagen to the wound bed.

Type I Collagen – tissue distribution to dermis, bone, tendon, ligaments and cornea

Type III Collagen – tissue distribution to skin, vessel wall, reticular fibers of most tissues

Type IV Collagen – tissue distribution to basement membranes

WHAT IS THERASKIN?

TheraSkin is a cryopreserved human skin allograft with both epidermis and dermis layers. TheraSkin is cellular and provides a supply of growth factors, cytokines and collagen to promote wound healing. TheraSkin is an effective natural barrier to help control infection and assist in the promotion of granulation tissue and epithelialization.

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 4. 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 original fragment can be resolved.
 5. This analysis was performed by Dr. Qishan Lin at the UAlbany Proteomics Facility.