

# TheraSkin®

*Real Skin Wound Therapy*

## *TheraSkin® Analysis, Stage 1 Findings: Identification of Key Growth Factors, Cytokines and Collagen in TheraSkin®*

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MEDICAL CENTER AND THE UNIVERSITY  
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## Objective

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

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## 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)<sup>5</sup>. 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.<sup>6</sup>

Samples of TheraSkin were cut into small pieces and extracted in T-PER (Pierce) at a concentration of 1 mL T-PER per 50 mg tissue. The samples were sonicated in a water bath sonicator for 15 min. followed by 22 hrs of gentle shaking at 4C. The samples were then centrifuged at 2000g for 3 min. to remove large cellular debris followed by 10,000g for 5 min. The supernatants were recovered and stored at -80C<sup>7</sup>. The samples were assayed for human IGF1 and VEGF with Quantikine ELISA kits from R&D systems<sup>8</sup>. Results represent the testing of two samples from different donors.

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## Conclusion

TheraSkin provides a broad spectrum of major growth factors, cytokines and collagen recognized to be relevant characteristics in healing wounds.

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## Findings

### GROWTH FACTORS

12 different growth factors were found in TheraSkin, including those from the five major growth factor families and proteins involved in skin wound healing<sup>9</sup>.

### 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.

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## Discussion

It is frequently difficult to determine the biological deficiencies of a given wound. These findings indicate that without expensive diagnostics to determine the particular biological deficiencies of a wound, (i.e., does it need growth factors, collagen or both?) a practitioner can use TheraSkin to provide both a 1) broad spectrum of human growth factors and cytokines, and 2) human collagen in significant quantities to jumpstart wound healing. This differs from the bio-engineered skin substitutes (BSSs) currently on the market which only contribute a limited array of the human biological characteristics relevant for wound healing.

TheraSkin provides a significant layer of human collagen to the wound bed. Apligraf<sup>®</sup> provides bovine rather than human collagen to the wound. Collagen provided by Dermagraft<sup>®</sup> is limited to that expressed by the fibroblasts. These BSSs provide growth factors and cytokines to a wound, but TheraSkin provides a more complete array of relevant biological characteristics of actual human skin associated with wound healing because TheraSkin is human skin.

**TheraSkin provides a wound with the relevant tools it needs to heal.**

Growth Factors	Cytokines	Collagen
PDGFA	TNF	Type I
PDGFD	IL1a	Type III
VEGFD (FiGF)	IL1b	Type IV
VEGFA	IL2	Type V
EGF	IL3	Type VI
IGF	IL4	Type IX
TGFA	IL5	Type X
FGF2	IL6	Type XI
TGFB1	IL12	Type XII
TGFB3	IL13	Type XIV
HGF	IL16	Type XV
BMP7	IL17A	Type XVII
	IL18	Type XVIII
	IL25, 27, 32	Type XX

## Relevant Characteristics Needed for TheraSkin<sup>®</sup> Wound Healing

### Growth Factors

There are five major growth factor families and proteins involved in skin wound healing. As minimally manipulated donated human skin, TheraSkin contains these relevant growth factors and provides an “at ready” supply of these growth factors to promote wound 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.

### 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 most associated with wound healing. TheraSkin’s notably collagen source is human derived, rather than bovine, and therefore contributes human collagen to the wound. TheraSkin provides a significant layer of human collagen to the wound to promote wound healing.

- **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

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## What is TheraSkin?

TheraSkin is a biologically active cryopreserved human skin allograft with both epidermis and dermis layers. TheraSkin jumpstarts wound healing by providing living cells and an “at-ready” supply of growth factors, cytokines and collagen in a fully developed extracellular matrix. TheraSkin has the relevant biological wound healing characteristics of real human skin because it is human skin.

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## References

1. Dr. Qishan Lin: UAlbany Proteomics Facility Director; Center for Functional Genomics, University of Albany, 1 Discovery Dr, 342H, Rensselaer, NY 12144.
2. Dr. Eran Rosines: R&D Staff Scientist, LifeNet Health, 1864 Concert Dr., Virginia Beach, VA 23451.
3. Dr. James Clagett: Chief Scientific Officer, LifeNet Health, 1864 Concert Dr., Virginia Beach, VA 23451.
4. Dr. Brian M. Taylor: Research Supervisor,  $\mu$ Quant Core Facility, Institute of Human Virology, 725 W. Lombard St., Baltimore, MD 21201.
5. 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.
6. This analysis was performed by Dr. Qishan Lin at the UAlbany Proteomics Facility.
7. Tissue extraction performed at LifeNet Health.
8. ELISA testing performed by Dr. Brian M. Taylor at the Institute of Human Virology's  $\mu$ Quant Core Facility.
9. Bryant RA, Nix D. Acute chronic wounds. Current management concepts. 3rd ed. St. Louis: Elsevier Misby: 2007.