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TheraSkin®
Real Skin Wound Therapy

Quantitative Analysis of Collagens in TheraSkin®, Apligraf® and Dermagraft®

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Objective

Quantify the presence of type I, III, and IV collagens in TheraSkin, Apligraf, and Dermagraft. (See "Note" on choice of collagens.)

Methodology

Samples of TheraSkin, Apligraf, Dermagraft, and human skin (assayed within 1 day of recovery) were cut into small pieces and extracted in 0.5M acetic acid with 1mg/mL pepsin. Samples were placed in a shaker for 24hrs at room temperature. Samples were then centrifuged at 2000g for 3 min. to remove large cellular debris followed by 10,000g for 10 min. The supernatants were recovered and stored at -80˚C. The samples were assayed for human type I collagen by an ELISA kit from Cosmo Bio, human type IV collagen by ELISA kit from Echelon Biosciences, and type III collagen by indirect ELISA with an anti-type III collagen monoclonal antibody from Abcam. All results represent the average of two samples.

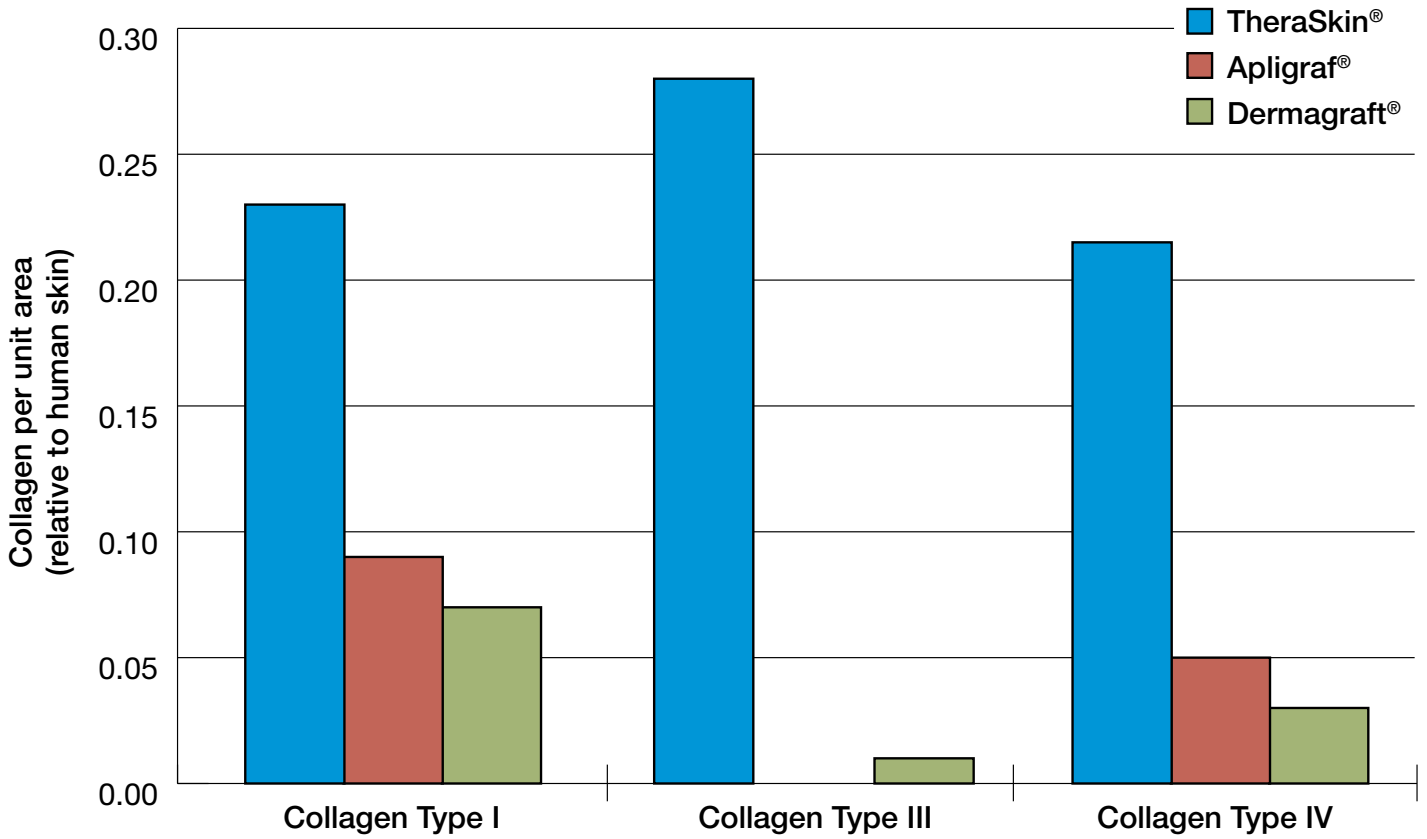
Findings

COLLAGEN QUANTIFICATION

TheraSkin possessed ~2.5 times as much human type I collagen as Apligraf and ~3 times that of Dermagraft, 50 times more human type III collagen than Dermagraft with Apligraf being below the detectable limit, and nearly 5 times more human type IV collagen than both Apligraf and Dermagraft.

COLLAGEN TYPE I TO TYPE III RATIO

The ratio of type I to type III collagen for TheraSkin was determined to be approximately the same as that of real human skin, while the ratio for Dermagraft is 49, or 15 times that of human skin. The ratio is not determinable for Apligraf, since type III levels were below the detectable limit of the methodology.



Discussion

The extracellular matrix of human skin performs multiple functions during wound healing including structural support for cells, direct cell signaling, and controlled growth factor binding/release. The collagens are the most abundant extracellular molecules in human skin accounting for more than 70-80% of the dry weight[2]. In human skin, types I and III account for more than 95% of total collagen and represent the two most important collagen types in skin in relation to normal architecture and wound healing.

TheraSkin provides more type I and type III human collagen than either Apligraf or Dermagraft. Of the three products tested, TheraSkin provides a more complete profile of human collagens, including type IV.

Furthermore, of the three biologic wound care products tested only TheraSkin provides the proper ratio of type I to type III collagen that is characteristic of healthy human skin. In a healthy person, higher collagen type III production is evident in the early wounding state and necessary to progression. Overproduction of type I and elevated type I to type III ratios have been associated with keloids[7]. Persistent treatment of skin with topical glucocorticoids can result in a dramatic change in type I to type III ratios, leading to induced skin atrophy[6]. These and similar studies on cardiac, muscle, eye, and kidney tissue suggest that 1.) the presence of both type I and type III collagen, and 2.) the proper ratio of the two collagens, are important to the healing process.[3, 4, 5, 6, 8, 9, 10, 11]

Ratio of Type I to Type III Collagen			
TheraSkin®	Apligraf®	Dermagraft®	Human Skin
3.1	N/A	49	3.3

Conclusion

Compared to Apligraf and Dermagraft, TheraSkin provides both the largest quantity of human collagen and the most complete profile of human collagens characteristic of healthy human skin.

Study Notes

This study was supervised by Dr. Eran Rosines, Research and Development Staff Scientist of LifeNet Health, Dr. Yu Jing, Research and Development Intern of LifeNet Health and Dr. James Clagett, Chief Scientific Officer of LifeNet Health[1].

Choice of collagen types studied

Collagen is the major structural protein found in the dermis and is secreted by dermal fibroblasts as tropocollagen. Collagen gives the skin its tensile strength and is necessary for wound healing. Normal human dermis is primarily composed of type I collagen which is the most abundant collagen type in the human body. It is present in scar tissue, the end product of tissue repair. Type III is the collagen of granulation tissue, and is produced by young fibroblasts before type I collagen is synthesized.

Types I and III are fiber-forming collagens. Type IV collagen is found primarily in the basal lamina (basement membrane) and serves as part of the filtration system in capillaries.

References

1. R&D Staff Scientist, LifeNet Health, 1864 Concert Dr., Virginia Beach, VA 23451; 2. R&D Intern, LifeNet Health, 1864 Concert Dr., Virginia Beach, VA 23451; 4. Chief Scientific Officer, LifeNet Health, 1864 Concert Dr., Virginia Beach, VA 23451.

2. Brinckmann, J., Bodo, M., Brey, M., Wolff, HH , Müller, PK (1994). Analysis of the age-related composition of human skin collagen and collagens synthesized by fibroblast culture. Arch Dermatol Res, Vol. 286(7), 391-395.http://www.ncbi.nlm.nih.gov/pubmed/7818281

3. Fleischmajer, R., Perlish, JS, Burgeson, RE, Shaikh-Bahai, F., & Timpl, R (1990). Type I and Type III Collagen Interactions During Fibrillogenesis. New York Academy of Science. Vol. 580, 161-175.

4. Friedman, DW, Boyd, CD, Machenzi, JW, Norton, P., Olson, RM & Deak, SB (1993). Regulation of Collagen Gene Expression in Keloids and Hypertrophic Scars. J. Surg Res, Vol. 55 (2), 214-222.

5. Junge, Karsten, Klinge, Uwe, Rosch, Raphael, Mertens, Peter R., Kirch, Jochen, Klosterhalfen, Bernd, Lynen, Petra & Schumpelick, Volker (2004). Decreased Collage Type I/III Ration in Patients with Recurring Hernia After Implantation of Alloplastic Prostheses. Langenbeck's Archives of Survery, Vol. 389, pp. 17-22.

6. Kuo, KW, Sheu, HM, Shen, TC, Yu, HS & Chang, CC (1990). A Study of Changes in the Ratio of Collagen Types in Skin After Long-term Treatment with Topical Glucocorticoids. Gaoxiong Yi Xue Ke Xue Za Zhi, Vol6 (5),224-230.

7. Lim, Ivor J., Phan, Toan-Thang, Bay, Boon-Huat, Qi, Robert, Huynh, Hung, Tan, Walter Tiang-Lee, Lee, Seng-Teik, & Longaker, Michale Thornton (2002). Fibroblasts Cocultured with Keloid Keratinocytes: Normal Fibroblasts Secrete Collagen in a Keloid Like Manner. AJP –Cell Physiology. Vol. 283 (issue 1), C212-C222.

8. Low, RB, Stirewalk, WS, Hultgren, P., Low, ES, & Starcher, B (1989). Changes in Aollagen and Elastin in a Rabbit Right-ventricular RressureOverload. Biochem. J., Vol. 263, 709-713.

9. Naitoh, M., Hosokawa, N., Kubota, H., Tanaka, T., Shirane, H., Sawada, M., & Nishimu, Y (2001). Upregulation of HSP47 and Collagen Type III Fibrotic Disease, Keloid. Biochem Biophys Res Commun. Vol. 280, 1316-1322.

10. Pauschinger, MD, Matthias, Knopf, PhD, Dagmar, Petschauer, MS, Simone, Doerener, PhD, Andrew, Poller, MD, Wolfgang, Schwimmbeck, MD, Peter, Kuhl, PhD, Uwe, Schulthesiss, MD, Heinz-P. (1999). Dilated Cardiomyopathy Is Associated with Significant Changes in Collagen Type I/III Ratio. American Hearth Associatin, Inc., Vol 99, 2750-2756.

11. Uitto, Jouini, Perejda, Andrew J., Abergel, r. Patrick, Chu, Mon-li, & Ramirez, Francesco (1985). Altered Steady-state Ratio of Type I/III Procollagen mRNAs Correlates with Selectively Increased Type I Procollagen Biosynthesis in Cultured Keloid Fibroblasts. National Academy Science, USA, Vol. 82, 5935-5939.

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