

REFERENCES

- Aydin, S. (2015). A short history, principles, and types of Elisa, and our laboratory experience with peptide/protein analyses using Elisa. *Peptides*, 72, 4–15. <https://doi.org/10.1016/j.peptides.2015.04.012>
- Bernauer, U., Bodin, L., Chaudhry, Q., Coenraads, P., Dusinska, M., Gaffet, E., Panderi, I., Rousselle, C., Stepnik, M., Wijnhoven, S., De Jong, W., Goetz, N. (2021). SCCS OPINION ON Gold (nano), Colloidal Gold (nano), Gold Thioethylamino Hyaluronic Acid (nano) and Acetyl heptapeptide-9 Colloidal gold (nano) - SCCS/1629/21 - Final Opinion.
- Cameron, G. J., Alberts, I. L., Laing, J. H., & Wess, T. J. (2002). Structure of type I and type III heterotypic collagen fibrils: An X-ray diffraction study. *Journal of Structural Biology*, 137(1-2), 15–22. <https://doi.org/10.1006/jsbi.2002.4459>
- Choudhury, S., & Das, A. (2021). Advances in generation of three-dimensional skin equivalents: Pre-clinical studies to Clinical Therapies. *Cytotherapy*, 23(1), 1–9. <https://doi.org/10.1016/j.jcyt.2020.10.001>
- Costin, G.E., Raabe, H., Curren, R. (2009) In vitro safety testing strategy for skin irritation using the 3D reconstructed human epidermis. *Rom. J. Biochem.* 46, 165–186.
- Duval, K., Grover, H., Han, L., Mou, Y., Pegoraro, A., Fredberg, J., & Chen, Z. (2017). Modeling Physiological Events in 2D vs. 3D Cell Culture. *Physiology*, 32(4), 266-277. doi: 10.1152/physiol.00036.2016
- Fleischer, J. G., Schulte, R., Tsai, H. H., Tyagi, S., Ibarra, A., Shokhirev, M. N., Huang, L., Hetzer, M. W., & Navlakha, S. (2018). Predicting age from the transcriptome of human dermal fibroblasts. *Genome Biology*, 19(1). <https://doi.org/10.1186/s13059-018-1599-6>
- Fleischmajer, R., Douglas MacDonald, E., Perlish, J. S., Burgeson, R. E., & Fisher, L. W. (1990). Dermal collagen fibrils are hybrids of type I and type III collagen molecules. *Journal of Structural Biology*, 105(1-3), 162–169. [https://doi.org/10.1016/1047-8477\(90\)90110-x](https://doi.org/10.1016/1047-8477(90)90110-x)
- Gold Particles and MVP Golden Collagenine - Infinitec. (2022). Retrieved from <https://infinitec.es/technology/mvp-golden-collagenine/>
- Gupta, A., Mishra, P., Pandey, C. M., Singh, U., Sahu, C., & Keshri, A. (2019). Descriptive statistics and normality tests for statistical data. *Annals of Cardiac Anaesthesia*, 22(1), 67. https://doi.org/10.4103/aca.aca_157_18
- Henriksen, K., & Karsdal, M. A. (2019). Type I collagen. *Biochemistry of Collagens, Laminins and Elastin*, 1–12. <https://doi.org/10.1016/b978-0-12-817068-7.00001-x>
- ISO 10993-5. (2009). Biological Evaluation of Medical Devices. Part 5: Tests for in Vitro Cytotoxicity.
- Johnson, W., Bergfeld, W., Belsito, D., Hill, R., Klaassen, C., & Liebler, D. et al. (2018). Safety Assessment of Tripeptide-1, Hexapeptide-12, Their Metal Salts and Fatty Acyl Derivatives, and Palmitoyl Tetrapeptide-7 as Used in Cosmetics. *International Journal Of Toxicology*, 37(3_suppl), 90S-102S. doi: 10.1177/1091581818807863
- Jones, R., Castelletto, V., Connon, C., & Hamley, I. (2013). Collagen Stimulating Effect of Peptide Amphiphile C₁₆-KTTKS on Human Fibroblasts. *Molecular Pharmaceutics*, 10(3), 1063-1069. doi: 10.1021/mp300549d
- Kazanci, A., Kurus, M., & Atasever, A. (2016). Analyses of changes on skin by aging. *Skin Research and Technology*, 23(1), 48–60. <https://doi.org/10.1111/srt.12300>
- Kim, M., & Park, H. J. (2016). Molecular mechanisms of Skin aging and rejuvenation. *Molecular Mechanisms of the Aging Process and Rejuvenation*. <https://doi.org/10.5772/62983>
- Klicks, J., von Molitor, E., Ertongur-Fauth, T., Rudolf, R., & Hafner, M. (2017). In vitro skin three-dimensional models and their applications. *Journal of Cellular Biotechnology*, 3(1), 21–39. <https://doi.org/10.3233/jcb-179004>

- Kuete, V., Karaosmanoğlu, O., & Sivas, H. (2017). Anticancer activities of African medicinal spices and vegetables. *Medicinal Spices and Vegetables from Africa*, 271–297. <https://doi.org/10.1016/b978-0-12-809286-6.00010-8>
- Kular, J. K., Basu, S., & Sharma, R. I. (2014). The extracellular matrix: Structure, composition, age-related differences, tools for analysis and applications for tissue engineering. *Journal of Tissue Engineering*, 5, 204173141455711. <https://doi.org/10.1177/2041731414557112>
- Makrantonaki, E., & Zouboulis, C. C. (2007). Molecular mechanisms of skin aging: State of the art. *Annals of the New York Academy of Sciences*, 1119(1), 40–50. <https://doi.org/10.1196/annals.1404.027>
- Mondon, P., Hillion, M., Peschard, O., Andre, N., Marchand, T., & Doridot, E. et al. (2015). Evaluation of dermal extracellular matrix and epidermal-dermal junction modifications using matrix-assisted laser desorption/ionization mass spectrometric imaging, in vivo reflectance confocal microscopy, echography, and histology: effect of age and pepti. *Journal Of Cosmetic Dermatology*, 14(2), 152-160. doi: 10.1111/jocd.12135
- Nahm, F. S. (2016). Nonparametric statistical tests for the continuous data: The basic concept and the practical use. *Korean Journal of Anesthesiology*, 69(1), 8. <https://doi.org/10.4097/kjae.2016.69.1.8>
- Neupane, R., Boddu, S. H. S., Renukuntla, J., Babu, R. J., & Tiwari, A. K. (2020). Alternatives to biological skin in permeation studies: Current trends and possibilities. *Pharmaceutics*, 12(2), 152. <https://doi.org/10.3390/pharmaceutics12020152>
- Qin, Z., Fisher, G., & Quan, T. (2013). Cysteine-rich Protein 61 (CCN1) Domain-specific Stimulation of Matrix Metalloproteinase-1 Expression through α V β 3 Integrin in Human Skin Fibroblasts. *Journal Of Biological Chemistry*, 288(17), 12386-12394. doi: 10.1074/jbc.m112.424358
- Quan, T., He, T., Shao, Y., Lin, L., Kang, S., Voorhees, J., & Fisher, G. (2006). Elevated Cysteine-Rich 61 Mediates Aberrant Collagen Homeostasis in Chronologically Aged and Photoaged Human Skin. *The American Journal Of Pathology*, 169(2), 482-490. doi: 10.2353/ajpath.2006.060128
- Quan, T., Qin, Z., Xu, Y., He, T., Kang, S., Voorhees, J., & Fisher, G. (2010). Ultraviolet Irradiation Induces CYR61/CCN1, a Mediator of Collagen Homeostasis, through Activation of Transcription Factor AP-1 in Human Skin Fibroblasts. *Journal Of Investigative Dermatology*, 130(6), 1697-1706. doi: 10.1038/jid.2010.29
- Quan, T., & Fisher, G. (2015). Role of Age-Associated Alterations of the Dermal Extracellular Matrix Microenvironment in Human Skin Aging: A Mini-Review. *Gerontology*, 61(5), 427-434. doi: 10.1159/000371708
- Reilly, D. M., & Lozano, J. (2021). Skin collagen through the lifestages: Importance for skin health and beauty. *Plastic and Aesthetic Research*, 2021. <https://doi.org/10.20517/2347-9264.2020.153>
- Riss, T. L., Moravec, R. A., & Niles, A. L. (2011). Cytotoxicity testing: Measuring VIABLE Cells, dead cells, and Detecting mechanism of cell death. *Methods in Molecular Biology*, 103–114. https://doi.org/10.1007/978-1-61779-108-6_12
- Sakamoto, S., Putalun, W., Vimolmangkang, S., Phoolcharoen, W., Shoyama, Y., Tanaka, H., & Morimoto, S. (2017). Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. *Journal of Natural Medicines*, 72(1), 32–42. <https://doi.org/10.1007/s11418-017-1144-z>
- Schagen, S. (2017). Topical Peptide Treatments with Effective Anti-Aging Results. *Cosmetics*, 4(2), 16. doi: 10.3390/cosmetics4020016
- Shin, J.-W., Kwon, S.-H., Choi, J.-Y., Na, J.-I., Huh, C.-H., Choi, H.-R., & Park, K.-C. (2019). Molecular mechanisms of dermal aging and antiaging approaches. *International Journal of Molecular Sciences*, 20(9), 2126. <https://doi.org/10.3390/ijms20092126>
- Stunova, A., & Vistejnova, L. (2018). Dermal fibroblasts—a heterogeneous population with regulatory function in wound healing. *Cytokine & Growth Factor Reviews*, 39, 137–150. <https://doi.org/10.1016/j.cytogfr.2018.01.003>

van Meerloo, J., Kaspers, G. J., & Cloos, J. (2011). Cell sensitivity assays: The MTT assay. *Methods in Molecular Biology*, 237–245. https://doi.org/10.1007/978-1-61779-080-5_20

Zhang, S., & Duan, E. (2018). Fighting against skin aging. *Cell Transplantation*, 27(5), 729–738. <https://doi.org/10.1177/0963689717725755>

APPENDICES

Appendix A. Supplementary Data for MTS Cytotoxicity Assay

Supplementary Table 1. MTS Absorbance Data of HDF Cells Treated with Product.

Product Concentration (%)	A570	%Cell Viability
	0.97	88.48
0.25	0.98	88.91
	1.06	101.89
	1.29	137.60
0.5	1.19	121.48
	1.07	103.57
	0.96	86.00
1	0.95	84.39
	1.21	125.65
	0.93	81.15
2	0.96	85.89
	0.93	82.36

Supplementary Table 2. MTS Absorbance Data of HDF Cells Treated with Base.

Base Concentration (%)	A570	%Cell Viability
.	1.08	104.70
0.25	0.98	89.85
	0.99	90.38
.	0.93	81.43
0.5	0.98	90.02
	1.11	109.80
.	1.00	93.05
1	0.97	87.62
	0.99	91.43
.	0.99	90.70
2	0.97	87.42
	0.91	79.29

Supplementary Table 3. MTS Absorbance Data of HDF Cells Treated with Comparator.

Comparator Concentration (%)	A570	%Cell Viability
.	1.12	110.56
0.25	1.10	108.04
	1.10	107.66
.	1.07	103.45
0.5	1.23	128.65
	1.29	137.15
.	1.14	114.07
1	1.05	99.71
	1.17	119.09
.	0.88	74.61
2	0.97	88.40
	1.15	116.44

Supplementary Table 4. MTS Absorbance Data of HDF Cells Treated with Combination.

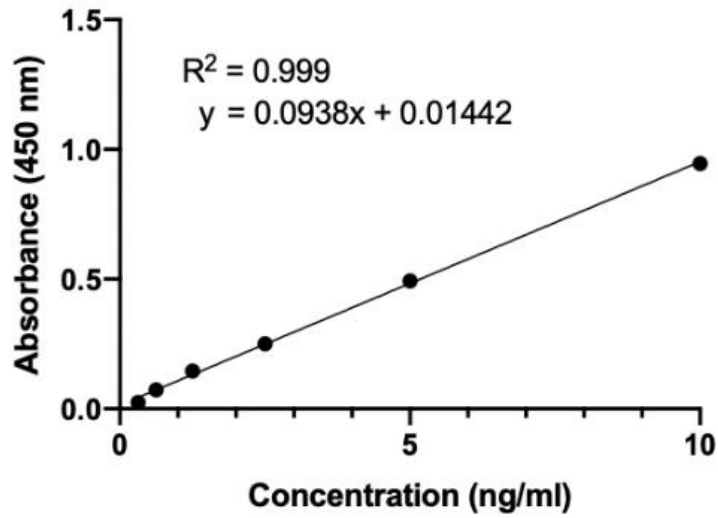
Combination Concentration (%)	A570	%Cell Viability
	0.89	74.90
0.25	1.08	104.22
	1.04	99.20
	1.01	93.60
0.5	1.03	96.50
	0.98	89.55
	1.01	93.62
1	0.94	83.65
	0.97	88.60
	0.95	85.21
2	1.00	92.21
	0.94	83.84

Supplementary Table 5. MTS Absorbance Data of Untreated HDF Cells Control and Blank.

	A570	Average	%Cell Viability
	0.29		
Blank	0.47	0.40	0.00
	0.44		
	1.10		
Control	1.05	1.05	100.00
	0.99		

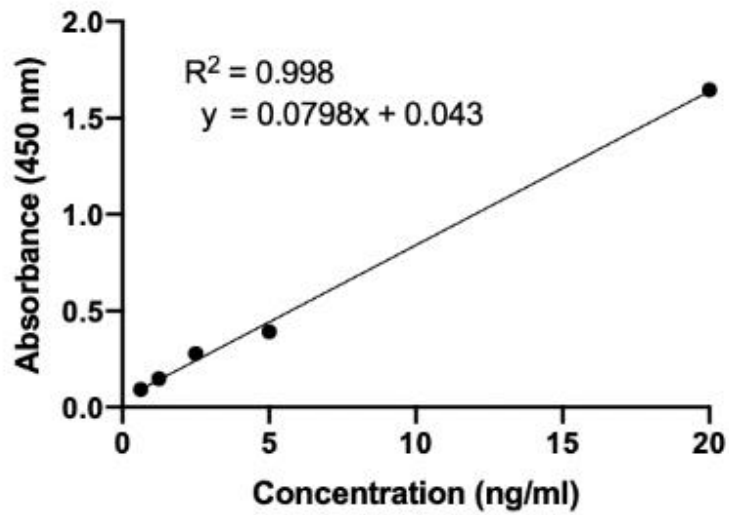
Appendix B. Standard Curves for ELISA

COL1 Standard Curve



Supplementary Figure 1. Standard Curve of Type I Collagen ELISA.

COL3 Standard curve



Supplementary Figure 2. Standard Curve of Type III Collagen ELISA.

Appendix C. Supplementary Data for ELISA

Supplementary Table 6. Type I Collagen ELISA Analysis.

Treatment	OD450	Relative OD450	Concentration (pg/mL)
	0.07	0.03	122.60
Product	0.08	0.03	164.18
	0.08	0.03	156.72
	0.08	0.03	181.24
Base	0.08	0.03	184.43
	0.10	0.05	404.05
	0.10	0.05	390.19
Comparator	0.10	0.05	363.54
	0.08	0.03	215.35
	0.08	0.03	192.96
Combination	0.08	0.03	189.77
	0.08	0.03	158.85
Control	0.07	0.02	83.16
Blank	0.05	0.00	-184.43

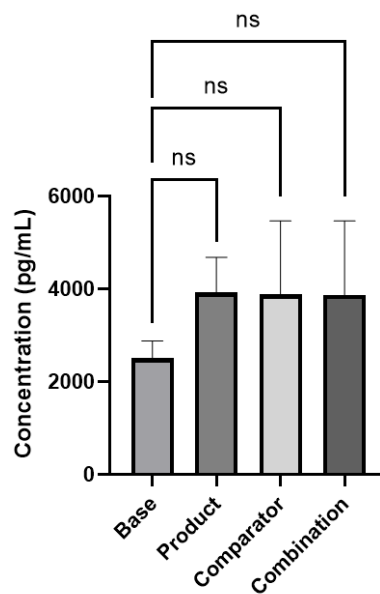
Supplementary Table 7. Type III Collagen ELISA Analysis.

Treatment	OD450	Relative OD450	Concentration (pg/mL)
Product	0.37	0.31	3095.47
	0.49	0.43	4613.22
	0.45	0.39	4050.18
Base	0.31	0.25	2359.85
	0.30	0.24	2216.65
	0.35	0.30	2929.01
Comparator	0.30	0.24	2243.57
	0.44	0.38	3974.30
	0.56	0.50	5421.05
Combination	0.28	0.22	2015.91
	0.52	0.46	4932.68
	0.49	0.44	4647.49
Control	0.13	0.07	186.05
Blank	0.06	0.00	-689.11

Supplementary Table 8. Shapiro-Wilk Test of Type III Collagen Expression.

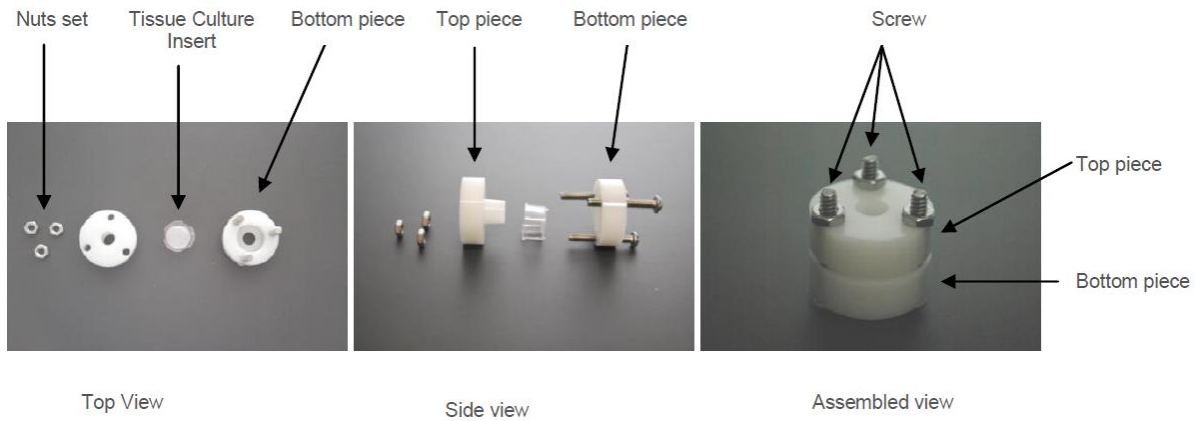
Test for normal distribution					
	Control	Base	Product	Comparator	Combination
Shapiro-Wilk test					
W	Invalid input data	0.8911	0.9782	0.9974	0.8217
P value		0.3576	0.7173	0.903	0.1674
Passed normality test (alpha=0.05)?		Yes	Yes	Yes	Yes
P value summary		ns	ns	ns	ns
Number of values	3	3	3	3	3

Type III Collagen Expression of Treated HDF Cells



Supplementary Figure 3. Type III Collagen Expression of Treated HDF Through EpiDerm™ Skin Model After Treatment With Product, Base, Comparator and Combination Compared to Base. Value of ns $P > 0.05$ indicates a non-significant difference to Base. (n=3, ANOVA).

Appendix D. Supplementary Figure for ELISA



Supplementary Figure 4. MatTek Permeation Fixtures (EPI-200-FIX) Parts and Assembly.