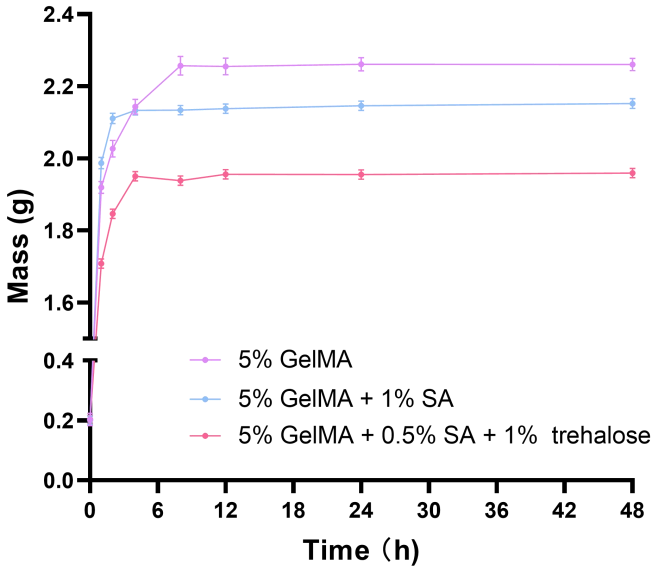
Supplementary Materials

Engineering a keloid-like fibrotic skin-on-a-chip for drug evaluation

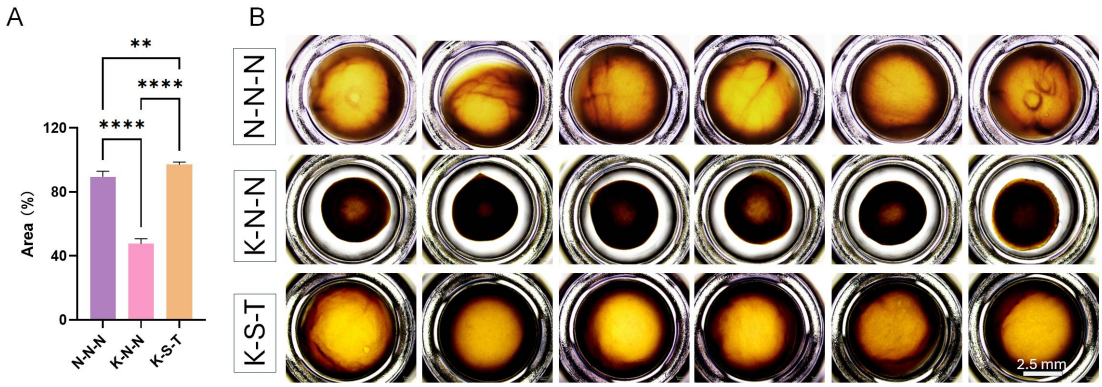
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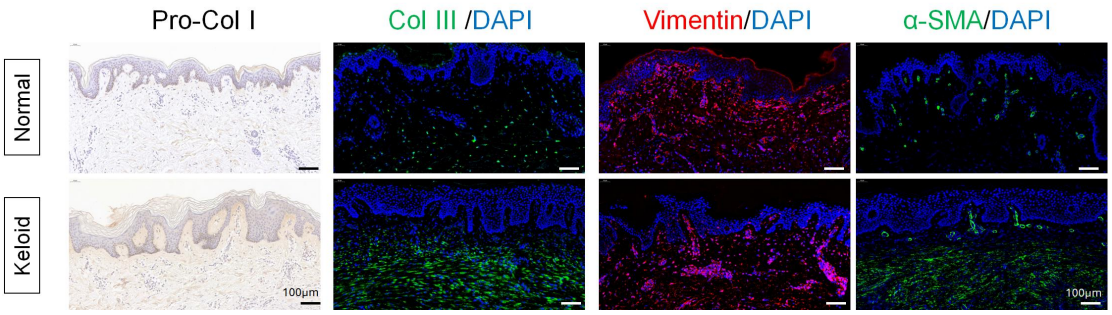
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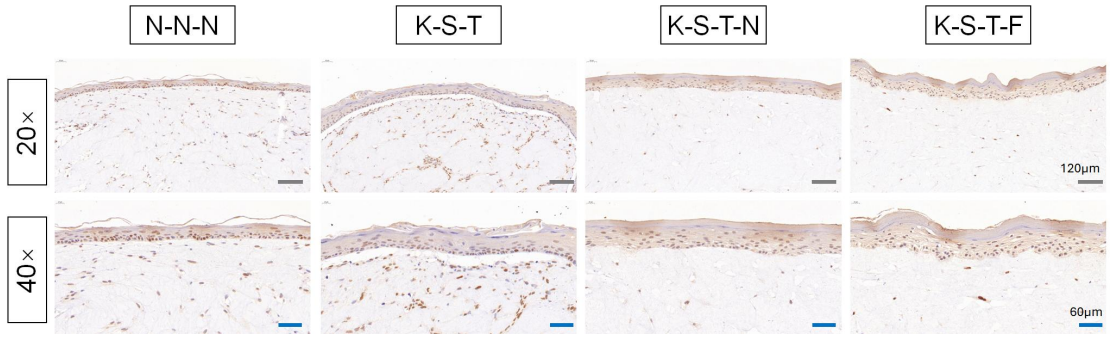
Supplementary Figure 1. Swelling behavior characterization. Time-dependent swelling profiles of 5% GelMA, 5% GelMA + 1% SA, and 5% GelMA + 0.5% SA + 1% trehalose over 48 h. n = 3 technical replicates.



Supplementary Figure 2. Construct contractility assays. Skin models constructed with normal-cell in soft hydrogel (N-N-N), keloid-cell in soft hydrogel (K-N-N), and keloid-cell in stiff hydrogel with TGF-β (K-S-T) were analyzed on day 20. (A) Quantitative analysis of the percentage of model area relative to the chamber bottom area. (B) Bright-field images captured at 2× magnification. n = 6 replicate wells. Scale bar = 2.5 mm (white). **P < 0.01, ****P < 0.0001.



Supplementary Figure 3. Immunohistochemical staining of Pro-Col I in normal skin tissue and keloid skin tissue, and immunofluorescence staining for Col III (green), Vimentin (red), α-SMA (green), and nuclei counterstained with DAPI (blue). Scale bar: 100 μm.



Supplementary Figure 4. Ki67 immunohistochemistry of the skin models at 20× and 40× magnifications. Scale bars: 120 μm (gray); 60 μm (blue).

Supplementary Table 1. Comparison of *in vitro* keloid models across key physiological dimensions

	Transwell skin equivalent	**Spheroid model**	**skin-on-a-chip**	**This work**
Cell source	keloid patient derived fibroblast and keratinocytes, normal human fibroblast and keratinocytes	keloid patient derived fibroblast and keratinocytes, human cell lines	human cell lines or cells from human juvenile prepuce	keloid patient derived fibroblast and keratinocytes
Architecture	full-thickness epidermal-dermal	cell spheroid	cell layer	full-thickness epidermal-dermal
Pathological biomechanical stiffness	No	No	No	Yes
Dynamic fluid culture	No	No	Yes	Yes
Drug evaluation Index	MTT assay	Histology, cytokine, protein expression, gene analysis	Histology, inflammation cytokine, protein expression, metabolic and gene analysis	Histology, inflammation cytokine, fibrotic protein expression, transcriptomic analysis
Reference	10	13, 14	19, 20, 22, 24, 25	

Supplementary Table 2. Characteristics of donors providing primary skin cells used for model construction

Characteristic	Normal Control Donor	Keloid Donor
Sex	Male	Male
Age (years)	45	46
Ethnicity	Han Chinese	Han Chinese
Tissue source	Expanded non-keloid skin	Keloid tissue
Anatomical site	Neck	Back
Clinical condition	Non-keloid	Keloid
Disease duration	N/A	4 years
Symptoms	N/A	Pruritus
Clinical history	Tissue obtained during reconstructive surgery following tissue expander implantation	Developed after excision of a cutaneous mass; progressively enlarged

REFERENCES

10. Lim, I.J.; Phan, T.T.; Song, C.; Tan, W.T.; Longaker, M.T. Investigation of the influence of keloid-derived keratinocytes on fibroblast growth and proliferation in vitro. Plast Reconstr Surg 2001, 107, 797-808, doi:10.1097/00006534-200103000-00022.
13. Choi, Y.; Jang, H.-S.; Shim, J.; Yeo, E.; Kim, M.-H.; Noh, H.; Oh, S.; Park, J.-H.; Lee, D.; Lee, J.H. 3D keloid spheroid model: Development and application for personalized drug response prediction. Communications Biology 2024, 7, 1470, doi:10.1038/s42003-024-07194-2.
14. Dirand, Z.; Tissot, M.; Chatelain, B.; Viennet, C.; Rolin, G. Is Spheroid a Relevant Model to Address Fibrogenesis in Keloid Research? Biomedicines 2023, 11, 2350, doi:10.3390/biomedicines11092350.
19. Kim, K.; Kim, H.; Sung, G.Y. An Interleukin-4 and Interleukin-13 Induced Atopic Dermatitis Human Skin Equivalent Model by a Skin-On-A-Chip. Int J Mol Sci 2022, 23, doi:10.3390/ijms23042116.
20. Sun, S.; Jin, L.; Zheng, Y.; Zhu, J. Modeling human HSV infection via a vascularized immune-competent skin-on-chip platform. Nat Commun 2022, 13, 5481, doi:10.1038/s41467-022-33114-1.
22. Abdo, D.; Zhao, Y.; Okhovatian, S.; Jiménez Vargas, L.F.; Wagner, K.T.; Shakeri, A.; Vosoughi, D.; Radisic, M. A dermis-on-a-chip model for compound screening. Materials Today Bio 2025, 34, 102111, doi: 10.1016/j.mtbio.2025.102111.
24. Biglari, S.; Le, T.Y.L.; Tan, R.P.; Wise, S.G.; Zambon, A.; Codolo, G.; De Bernard, M.; Warkiani, M.; Schindeler, A.; Naficy, S.; et al. Simulating Inflammation in a Wound Microenvironment Using a Dermal Wound-on-a-Chip Model. Advanced Healthcare Materials 2019, 8, 1801307, doi: 10.1002/adhm.201801307.
25. Matei, A.-E.; Chen, C.-W.; Kiesewetter, L.; Györfi, A.-H.; Li, Y.-N.; Trinh-Minh, T.; Xu, X.; Tran Manh, C.; van Kuppevelt, T.; Hansmann, J.; et al. Vascularised human skin equivalents as a novel in vitro model of skin fibrosis and platform for testing of antifibrotic drugs. Annals of the Rheumatic Diseases 2019, 78, 1686-1692, doi: 10.1136/annrheumdis-2019-216108.