Mechanistic Modeling Using a PhysioPD™ Research Platform Facilitates Identification of Key Pathways Involved in Skin Aging and Potential New Anti-aging Technologies

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Introduction

- Despite progress elucidating the molecular mechanisms involved in skin aging, identifying approaches to improve skin appearance remains a challenge
- Combining molecular biology, gene expression, and clinical data with computational mechanistic modeling provides a novel approach to support material identification and evaluation

Objectives

- Increase mechanistic understanding of the dynamic processes involved in skin aging
- Represent key aspects of skin biology and the responses to Standard-of-Care (SOC) anti-aging materials
- Evaluate potential anti-aging technologies
- Develop an asset intended for evaluating material efficacy involved in the reversal and prevention of visible skin aging, and related skin research

Methods

The Skin PhysioPD™ Research Platform is a mechanistic, quantitative model to evaluate skin aging mechanisms and visible effects of anti-aging applications.

- The Skin PhysioPD Platform integrates data and knowledge from numerous sources into a single contextual framework
- Differential equations represent the dynamic processes associated with intrinsic and extrinsic skin aging and response to anti-aging treatments
- Virtual Consumers (VCs) representing clinical and biological variability were created by varying parameters
- Key drivers of treatment response were identified by Sensitivity Analysis (SA)
- Anti-aging potential of new products was evaluated on different VCs

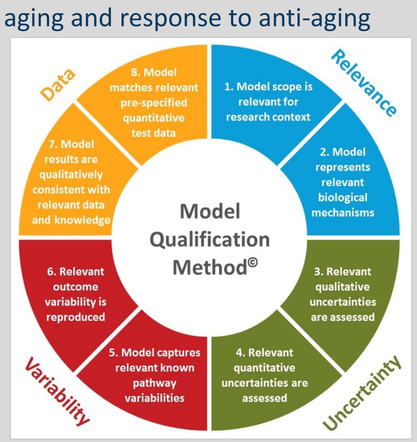


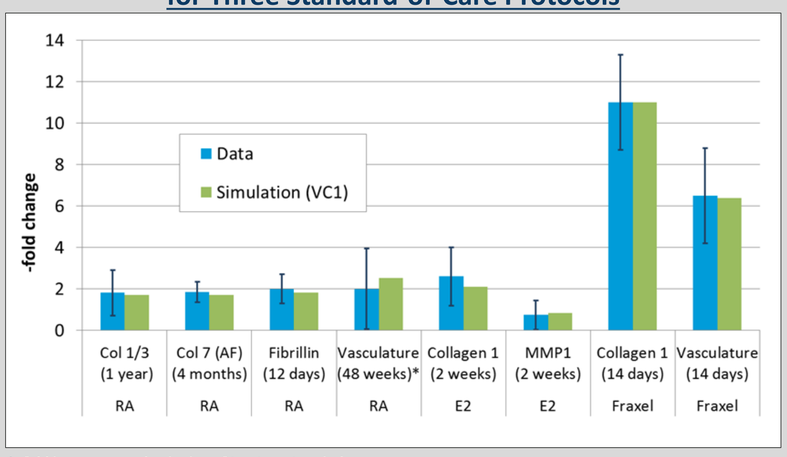
Figure 1. The Platform was qualified according to Rosa's Model Qualification Method[1] (MQM)

Results

The Platform was qualitatively and quantitatively qualified to the expected behaviors based on literature and P&G proprietary data.

- Qualification experiments included topical application of retinoic acid (RA), b-estradiol (E2) over various periods of time, and non-ablative laser resurfacing treatment (Fraxel®)
- Platform qualification was conducted in a moderately photoaged, middle-aged Virtual Consumer, VC1

Outcome Data Compared to Simulation Results for Three Standard-of-Care Protocols



* -fold increase over beginning of treatment period

Figure 2. Data (+/- SD) compared to simulation results

Results

The Skin PhysioPD Research Platform represents biological components involved in skin aging, mechanisms of anti-aging applications, and outcomes.

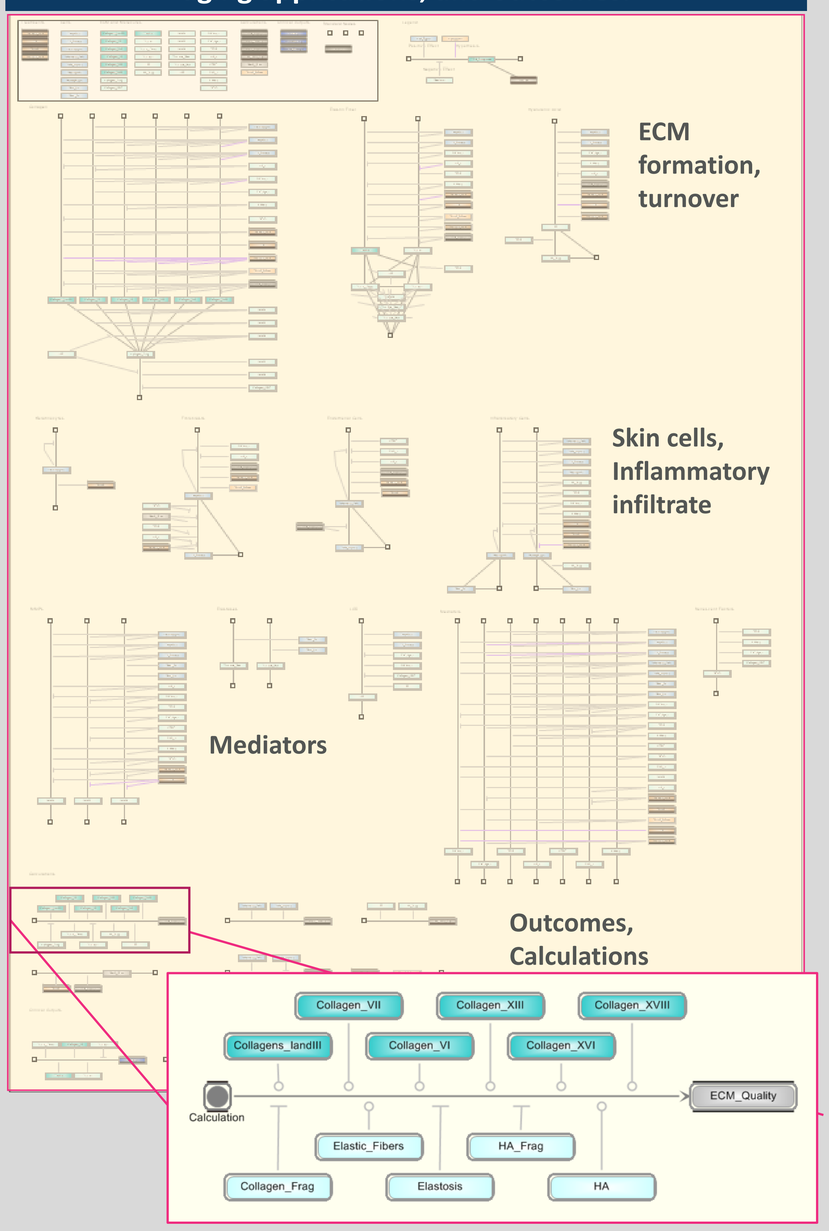


Figure 3. The Skin PhysioPD Research Platform includes representation of extracellular matrix (ECM) components production, turnover, and processing, lifecycle of skin cells, mediators' production and effects (e.g., inflammatory cytokines, ECM processing enzymes, AP-1, NFkB), effect of inflammatory infiltrate, cutaneous vasculature and angiogenesis. Inset: ECM quality, a key outcome, is calculated based upon the density and quality of ECM components e.g., collagens, elastic fibers, and hyaluronic acid (HA)

Cohort of VC responders and non-responders were evaluated for response to qualifying and potential novel anti-aging protocols.

- Five VCs were created by varying mechanistic pathways such as level of AP-1 expression, fraction of senescent FB, and inflammatory response
- VCs facilitate investigation of responses to SOP and novel anti-aging applications for different phenotypes of aged skin
- New application protocols were simulated on all VCs, and the predicted effects on the magnitude and the speed of skin appearance improvements were analyzed, leading to insights supporting prioritization of technologies
  - e.g., Protocol B is predicted to have a potential impact on improvement of ECM quality in all VCs, indicating that it may be a strong anti-aging candidate across diverse population of consumers

Table 1. Responses of VCs with different skin characteristics to anti-aging protocols

Virtual Consumer #	Protocol				
	RA (1 year)	E2 (1 year)	Fraxel (single)	New Protocol A (1 Year)	New Protocol B (1 year)
VC1 moderately photoaged	responder	responder	responder	responder	responder
VC2 intrinsically aged	responder	responder	responder	responder	responder
VC3 severely photoaged	responder	non-responder	non-responder	low-responder	responder
VC4 severely photoaged	responder	non-responder	responder	responder	responder
VC5 severely photoaged	non-responder	non-responder	responder	low-responder	responder

Results

Systematic SA highlighted the key drivers of improvement in skin appearance in response to application protocols.

- To assess the effects of individual mechanisms on the response to anti-aging protocol applications, sensitivity coefficients were calculated using the partial derivative of the ECM Quality output with respect to each model parameter
- The identified key contributors to the ECM Quality for all applications include
  - HA production and turnover
  - ECM components with short half lives
    - e.g., collagen 7
  - AP-1 dependent pathways
  - TGFb production and clearance
  - Recruitment and number of ECM-producing fibroblasts
  - Contact cell inhibition
- Pathways identified as sensitive may be promising approaches for future anti-aging strategies

Drivers most affecting EC Quality after long term E2 application

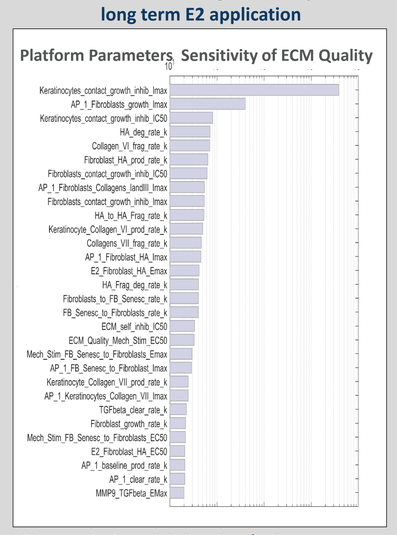


Figure 4. Sensitivity Analysis identifies the pathways with the greatest impact on a selected outcome (here, ECM Quality) under a given protocol (here, E2)

Example Results : Increase in HA after long term topical E2 application may contribute to improvement in visible skin appearance.

- Reported effects of long term E2 application on skin appearance have been inconclusive
- However, it has been observed that short term E2 treatment (2 weeks) leads to upregulated production of ECM components (e.g., collagen 1)[6]
- Simulation results indicate that long term E2 application may lead to improvement in skin appearance, primarily driven by increased production and accumulation of HA
- Results are consistent with empirical data indicating that a major age-related change in aged skin is loss of HA water binding ability, resulting in dehydration, atrophy, and loss of elasticity[8]
- Simulations show best improvement in intrinsically (VC2) and moderately photoaged skin (VC1), which corresponds to younger, less damaged skin phenotype, suggesting that such individuals are best candidates to obtain benefits from long term application of E2

Topical E2 application, once daily, one year

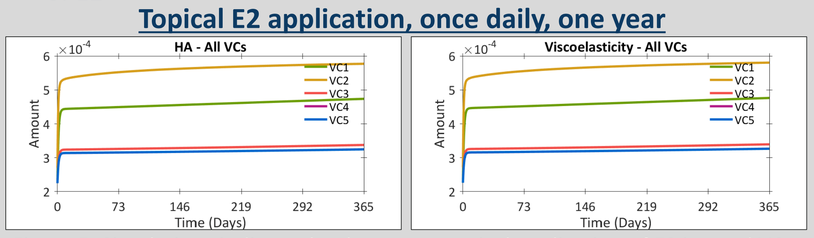


Figure 5. Simulations indicate that long term topical application of E2 in aged VCs will increase HA content (left panel), with concomitant improvement in viscoelasticity (right panel).

Conclusions

- A quantitative, mechanistic model of aged, photodamaged human skin, the Skin PhysioPD Platform, facilitates exploration of mechanisms involved in skin aging and evaluation of approaches that could reverse it
- Platform qualification included testing simulated responses to SOC anti-aging applications (RA, E2, and Fraxel)
- Five VCs representing different phenotypes and mechanisms of aged skin were created to investigate responses to SOP and novel anti-aging applications
- New application protocols were simulated on all VCs, and the predicted effects on the magnitude and the speed of skin appearance improvements were analyzed, leading to insights supporting prioritization of anti-aging applications
- SA highlighted the potential key drivers of improvements in aged skin appearance that can be promising anti-aging approaches (e.g., HA)
- The ability to directly examine individual mechanisms or combinations of pathways involved in the reversal of skin aging using in silico models can be used to improve the success rate for anti-aging applications
- On-going research using the Platform is expected to continue to enhance P&G's ability to make informed development decisions

References

1. Friedrich, C. M. (2016) *CPT: Pharmacometrics & Systems Pharmacology* **5**, 43-53
2. Griffiths, C. E., et al. (1993) *The British journal of dermatology* **129**, 415-421
3. Griffiths, C. E., et al. (1995) *Arch Dermatol* **131**, 1037-1044
4. Woodley, D. T., et al. (1990) *JAMA* **263**, 3057-3059
5. Watson, R. E., et al. (2009) *Br J Dermatol* **161**, 419-42

References (continued)

6. Son, E. D., et. Al. (2005) *J Invest Dermatol* **124**, 1149-1161
7. Orringer, J. S., (2012) *Dermatol Surg* **38**, 1668-1677
8. Stern, R., and Maibach, H. I. (2008) *Clin Dermatol* **26**, 106-122

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