Current advances in skin-on-a-chip models for drug testing

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Abstract: Skin-on-a-chip models are highly desirable in drug testing compared to conventional 2D cell culture and animal models as they can replicate organ-specific 3D structural organization and physiological functions at a relatively low cost. To engineer a physiologically relevant skin model, human skin structures have been integrated onto microfluidic platforms to construct skin-on-a-chip systems that can mimic the complex *in vivo* situation. In this mini-review, we first briefly introduce some critical technologies employed to develop *in vitro* skin-on-a-chip models. We then review the applications of the state-of-the-art skin-on-a-chip models in drug testing, with a focus on using models of full-thickness skin equivalents (FTSEs), skin models with additional components such as vasculature, immune cells and hair follicles as well as multi-organ-on-a-chip models. Finally, we discuss some current challenges and future directions of development of complex, and in vivo-like skin-on-a-chip models.

Keywords: Organ-on-a-chip; human skin equivalents (HSEs); skin-on-a-chip; microfluidics; drug testing

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Introduction

Skin is an intricate organ of the human body and serves many essential physiological functions for human survival such as fluid homeostasis, thermoregulation, immune defence, and sensory detection. It forms an efficient physical barrier to protect the body against environmental pathogens, toxic chemicals, mechanical disturbances, and UV radiation (1). Due to its accessibility and large surface contact area, skin is considered as a suitable and vital route for administration of drugs or application of cosmetic products (2,3). Testing of these substances on the skin is thus of crucial importance to assess the dosing and therapeutic efficacy, to identify the potential adverse skin reactions and mode of action, and to analyze human environmental risks (4).

Currently, animal models are extensively used for such drug testing, but they are usually lacking availability, highly time-consuming and costly, ethically questionable and may not represent the physiology, immunity and metabolism of the human skin, resulting in a limited ability to extrapolate to human conditions (5). Thus, human skin equivalents (HSEs) for drug testing using developed *in vitro* skin models are considered valuable tools for studying the molecular basis of cellular responses in skin physiology and pathology (6,7). Conventional two-dimensional (2D) culture models have involved cultures of keratinocytes or co-culture of keratinocytes with immune cells and dermal fibroblasts on petri-dishes or microtiter plates (1). These models are well-established and straightforward to use; however, they fail to reconstitute the complex three-dimensional (3D) cell-cell and cell-matrix interactions found in the body, limiting their accuracy in predicting the complicated effect of drug

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metabolism on the actual skin.

To tackle these limitations, development of 3D skin models with cells cultured in extracellular matrix (ECM)-like materials (e.g., hydrogels) is rapidly gaining significant attention as they can better simulate the architectural and chemical complexity of living tissues (8-10). Typically, a 3D HSE should contain three distinct layers including epidermis, dermis and subcutaneous adipose tissue (10). In addition, cells grown in 3D skin models should form prevalent gap junctions and tight junctions. These subtle cellular structures can enhance the communication of different skin cells, maintain skin tissue integrity and function, and facilitate *in vitro* drug testing (11). Especially in terms of drug diffusion, drugs in 3D culture models need to diffuse across multiple layers of cells to reach the final targets. Formed stratum corneum structures in the 3D models can reduce the drug diffusion rate and significantly reduce the bioavailability of drugs, which resembles human skin's barrier function. However, this barrier function cannot be found under the 2D culture condition as these subtle structures cannot be maintained on rigid culture dish (11). Nevertheless, most of the traditional 3D skin models still have some serious limitations such as weak barrier properties, lack of vasculature and skin appendages (e.g., sweat glands and hair follicles), and thus are incapable of fully recapitulating the multicellular complexity of the human skin tissue (12). Moreover, these 3D skin models cannot offer precise control over spatiotemporal chemical gradients and physical environmental factors (e.g., temperature, mechanical forces, gas), and may pose many technical challenges such as sampling luminal contents for analysis of the adsorption, distribution, metabolism, elimination and toxicity (ADMET) of drugs as well as harvesting cellular components in specific positions for extended biological analysis (8,13). Therefore, there is an urgent need to fabricate more physiologically mimicking and functional skin models for drug testing.

A novel skin model, known as "skin-on-a-chip" model or on-chip skin model, has made considerable advances in the field of skin tissue engineering. It can narrow the gap between traditional 2D culture and the *in vivo* situation, and thus provides the possibility of addressing all these limitations mentioned above. "Skin-on-a-chip" is to culture skin tissues within a microfluidic system, which can control many physical and biochemical parameters such as medium flow, mechanical force and gradients of biochemicals, mimicking the 3D microenvironments of the natural human skin. These models have the potential to create functional skin tissues with controlled 3D organization of skin layers and appendages. Herein, in this paper, we begin by providing an overview of some key technologies that are used to construct skin-on-a-chip models. We then discuss the recent progress on the applications of this type of emerging *in vitro* skin models for drug testing. Finally, the current challenges and future directions in the development of skin-on-a-chip will be highlighted.

Key technologies in the development of skin-on-a-chip models

A skin-on-a-chip model combines skin culture models created by various microfabrication techniques with the microfluidic technology and/or sensing technologies (*Figure S1*) (14,15). The microfabrication techniques such as photolithography, replica molding and 3D printing/bioprinting, allow for fabrication of complex tissue-like structures within the microfluidic chips (15-19). For instance, Lee *et al.* used 3D bioprinting of keratinocytes and fibroblasts to engineer human skin tissues, representing epidermal and dermal layers, respectively (20). Compared with traditional methods to construct skin tissues, 3D bioprinting offered many advantages such as flexibility, reproducibility, high resolution and high-throughput culture. These engineered skin models can be potentially applied in transdermal and topical formulation discovery, and dermal toxicity studies. In another study, the nanoporous alumina mask was used to fabricate a nanogold platform substrate with its surface nanopatterned with the RGD (Arg-Gly-Asp) peptide, and two-photon stereolithography techniques were used to manufacture a three-layer-structured cell chip (21). This cell array system can mimic 3D skin cell growth by seeding skin fibroblasts in such designed structures. Moreover, this device can also achieve high-throughput testing of *in vitro* effects of cosmetic drugs. More detailed information about such microfabrication techniques applied to fabricating tissue models was reviewed by Verhulsel *et al.* (22). Taken together, the microfabrication techniques offer the ability to precisely control cell shape, position and 3D organization of skin layers and appendages in a skin-specific context displaying more realistic functionality.

However, these microfabricated skin models are still deficient in their ability to recapitulate the human skin due to the lack of several essential cellular or structural components (23). For example, the lack of vascular network in most *in vitro* skin models cannot simulate *in vivo* blood

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circulation in their native counterparts, which is responsible for supplying the living cells with nutrients and growth factors (23). Microfluidics is another core technology to tackle this problem, altering the way we study living skin cells in both the 2D and 3D systems and allowing us to develop a more ideal skin-on-a-chip models (24). In general, microfluidic technology can manipulate or process small amounts (10^{-9} to 10^{-18} L) of fluids in hollow microchannels, and because of their small sizes, the fluids in the microfluidic systems exhibit specific physical properties such as the laminar flow (14,23). When different fluids flow beside each other in the same hollow microchannel, they are entirely laminar and virtually do not mix between neighboring fluids (23). This interesting property enables a small sample volume to be analysed, and thus addresses the limited access to the patient-derived samples and reduces the consumption of the chemicals. The decrease in the sample size also reduces the amounts of drugs to be tested and results in a higher sensitivity for detecting biomarkers of skin tissues (23). For instance, Mah *et al.* fabricated a microfluidic system for *in vitro* skin permeation studies, which was able to utilize a small amount of medium (70–200 μL) and a small area of skin tissue (0.283 cm^3) (25). Due to its miniaturization, this device can be useful in conducting extensive pre-formulation studies for expensive new drugs with limited availability. Microfluidics-based platforms also permit precise regulation of the cellular microenvironment in skin-on-a-chip models, such as controlling the dynamic fluid behaviours and external physical factors (e.g., temperature, mechanical force, gas) at the microscale, and providing nutrients and chemical cues to the skin cells (26,27). Additional examples of such a microfluidic technology applied to skin-on-a-chip models are outlined in subsequent sections.

The conventional microfluidic technology combined with the tissue engineering technology has made it possible to engineer more complex skin microsystems. However, when performing drug testing on them, compared with traditional functional measurements such as morphology, viability, and biochemical analyses, measurements of skin-specific functions such as barrier permeability could offer more useful information and produce a more valuable evaluation of drug responses (28). The incorporation of various *in situ* biosensors into the chip is another important step, not only providing non-invasive real-time readouts of skin tissue function but realising detection of its dynamic responses to the pharmaceutical compounds (23,29). Recently, Zhang *et al.* demonstrated that sensing technologies by integrating the physical and chemical biosensors into the microfluidic systems could endow the measuring process with *in situ*, continual, non-invasive and automated capability, which achieved a better monitoring of drug efficacy or toxicity (30). Alexander *et al.* fabricated a non-invasive skin-on-a-chip system integrated with a sensor to monitor the transepithelial electrical resistance (TEER) of reconstructed human epidermis (31). Their *in vitro* diagnosis system was able to monitor metabolic parameters and reveal the change of skin tissues over time, while maintaining constant cultures on chip. Taken together the combination of microfabrication techniques, microfluidic technology and biosensing technologies allows us to construct more sophisticated skin-on-a-chip models and enable parallel, real-time and high-throughput drug testing on them. Such skin-on-a-chip models may help us gain insight into the drug mode of action and provide the data necessary for preclinical evaluation of drug candidates, which will be reviewed in the following sections.

Skin-on-a-chip models for drug testing applications

As discussed above, the physiological relevance of skin models has been improved by various technologies to obtain more accurate and reproducible results of drug research. In the following section, an overview of the latest research efforts for skin-on-a-chip models, their designs, and physiological properties relevant to drug testing is presented. In particular, we highlight FTSE models, skin models incorporating additional skin components and multi-organ-on-a-chip models, which can recapitulate the physiologically relevant structures and functionalities of the normal human skin.

FTSEs comprising epidermal and dermal layers are much more similar to *in vivo* skin in consideration of transport properties when compared to reconstructed single human epidermal equivalents. Thus, they are more extensively used in skin-drug interaction studies (6). For example, Abaci *et al.* prepared FTSEs composed of epidermal and dermal compartments outside the chip and then placed them into a pumpless microfluidics platform after full differentiation, stratification and cornification (32). This chip was carefully designed to have a stable air-liquid interface in a gravity-driven flow system, and a physiological residence time of blood in human skin tissues. Such design managed to support maturation and differentiation of FTSEs and maintenance of their barrier function for 3 weeks. Moreover, it showed the ability to examine the toxic effects of doxorubicin (an anticancer drug) at a clinically

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relevant concentration, which caused a spatial detachment of the basal layer along the epidermal-dermal interface in the FTSEs model. Similarly, in a recent study by Alberti *et al.*, circular pieces of FTSEs were inserted into a designed microfluidic chip to rigorously validate its permeation against the static Franz diffusion cell, a traditional *in vitro* skin permeation testing system (33). The result of caffeine permeation exhibited that this skin-on-a-chip model could reduce the effect of the unstirred water layers that may form in the static Franz diffusion cell and affect the substance transport process. Although these studies suggested that such simple chips could be useful as *in vitro* platforms for skin drug testing, they were not entirely successful because FTSEs were not constructed directly in the microfluidic device and could not benefit from the dynamic culture.

More recently, Wufuer *et al.* designed an *in vitro* human skin-on-a-chip device comprising three polydimethylsiloxane (PDMS) layers and two porous membranes to enable direct co-culture of keratinocytes (epidermal layer), fibroblasts (dermal layer) and vein endothelial cells (endothelial layer) in a microfluidic system (34). The different microfluidic channel systems within the three PDMS layers enabled the perfusion of various kinds of culture medium with different flow rates. The diseased vascularized skin model with inflammation and edema was then obtained by perfusing tumor necrosis factor alpha (TNF-α) through the microfluidic channels, and finally used to test the efficacy of the therapeutic drug (dexamethasone) on reducing TNF-α-induced inflammation and edema. The microfluidic channels within this human skin-on-a-chip device has shown crucial role in mimicking the complex and dynamic microenvironment within native human tissue by introducing perfusing culture medium and biofactors. However, in this work, the researchers adopted the 2D skin cell culture, which lacks the 3D complexity of the human skin. To tackle these problems, Lee *et al.* prepared a skin-on-a-chip model with 3D FTSEs, where human dermal primary fibroblasts were encapsulated in a 3D collagen hydrogel to represent the dermis layer, and HaCaT cells or primary human keratinocytes were cultured on top of the collagen-fibroblast mixture exposed to air-liquid interface in the presence of epidermalization media to represent epidermis layer (35). Microfluidic channel beneath the skin construct was seeded with endothelial cells to construct the vascular structure in the chip. Gravity-induced rocking platform, as a pumpless operation system, was used for the long-term culture of the chip while minimizing bacterial contamination and air bubble formation. This chip with endothelial cell-coated fluidic channels could maintain the growth of human keratinocytes and support their differentiation from air exposure by 10 days. In addition, using a similar chip, the same research group compared the abilities of three different collagens, including rat tail, porcine skin and duck feet collagen, to support the growth and differentiation of primary dermal fibroblasts and human keratinocytes. Among these three collagens, probably due to the different mechanical properties, the rat tail collagen was demonstrated to be the best one as the scaffold biomaterial for HSEs, based on the expression of marker proteins and formation of the dermal and epidermis layers (36). They have also compared the differentiation of 3D FTSEs constructed using a conventional transwell and using a microfluidic chip (37). The authors found that the contraction of the collagen hydrogel was the lowest under the dynamic chip condition possibly due to sufficient convection of the culture medium, which was favourable for drug permeation testing. Overall, these research results provided valuable information for the development of a more physiologically relevant skin-on-a-chip model, especially the ones that can be used for drug testing. In addition, Sriram *et al.*, developed a full-thickness human skin-on-a-chip model integrated with a fibrin-based dermal matrix inside to better recapitulate the structure and functions of human skin (*Figure S2A,B*) (38). Compared with HSEs cultured in standard culture inserts, this device endowed HSEs with an improved epidermal morphogenesis, a more advanced epidermal differentiation, a faster maturation time as well as a tighter dermo-epidermal junction. Furthermore, the result of the TEER and caffeine permeation assay demonstrated that the HSEs within the chip had a superior barrier property, showing the potential ability for cost-effective, high-throughput drug permeability and toxicity testing.

To further generate more reliable *in vitro* skin-on-a-chip models, there is a growing interest in integrating different skin components, such as vasculature, immune cells and hair follicles into the microfluidic devices (1). In one study, Abaci *et al.* utilized the 3D bioprinting technology to build micropatterned vascular networks, and then embedded this vascular pattern into HSEs fabricated using induced pluripotent stem cell (iPSC)-derived endothelial cells (39). These vascularized HSEs had a robust epidermis and were endowed with an endothelial barrier function, which can be further improved by combining with microfluidic devices for application in drug screening. Recently, Mori and co-workers established another novel approach to construct a perfusable

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vasculature in HSEs (40). In this study, nylon wires were used to prepare sacrificial channels in a collagen hydrogel. After removing the wires, the channels were coated with the endothelial cells forming perfusable vascular channels within the dermal compartments. The skin-equivalent was cultured on a dynamic perfusion system, showing nature-mimicking dermal/epidermal morphology and barrier function. The cell distribution of the perfused and non-perfused skin-equivalents were evaluated, revealing that the vascular channels could serve as a nutrition transport pathway to maintain availability of HSEs The permeation of test drug molecules, caffeine and isosorbide dinitrate, from the epidermal layer into the vascular channels under perfusion condition was measured to study the importance of vascular perfusion on percutaneous absorption of HSEs. Their HSEs, as a skin-on-a-chip system, demonstrated promising applications for drug testing and reconstruction of skin substitutes. To develop an immune-competent skin-on-a-chip model, Ramadan and Ting demonstrated a bi-channel microfluidic platform capable of 3D co-culture of keratinocytes and monocytes, which represented components of the epidermis and immune systems, respectively (*Figure S2C*,*D*,*E*) (41). This chip combined the TEER probes and magnetic bead-based immune assay, and it could evaluate the effects of the lipopolysaccharide and UV irradiation on the skin barrier. In another study, Ataç *et al.* described a microfluidics-based system capable of integrating HSEs and hair follicles, while also prolonging the culture and substance testing period of HSEs (*Figure S2F*) (42). However, the hair follicles in this system were not incorporated directly as a part of the skin tissue, and thus cannot significantly influence the substance penetration characteristics. To address this issue in the future, researchers can directly transplant entire functional units of hair follicles or seed hair follicle precursor cells into skin-on-a-chip models (6).

The development of skin-on-a-chip models has highlighted the importance of interactions between different skin cells, and those between cells and ECM. To further extend the level of skin complexity, current studies focus on the tissue-tissue or organ-organ interactions physically separated *in vivo* but connected by metabolites in medium (43,44). To mimic this *in vivo* process, novel multi-organ systems, also known as multi-organ-on-a-chip models, have been successfully developed with different tissue equivalents cultured in separate chambers and linked by microfluidic channels (45). These on-chip models can simulate the process of human metabolism and predict the response to drugs throughout the body. In a study by Wagner *et al.*, a multi-organ microfluidic device was established to integrate human skin biopsies and human primary hepatocytes (46). This two-organ system could maintain a stable long-term performance for up to 28 days, support molecular crosstalk between the tissues and was also suitable for repeatedly testing the responses of the toxic substance troglitazone to the liver at different molecular levels. Likewise, Maschmeyer *et al.* introduced an endothelialised multi-organ chip that also enabled a repeated dose toxicity testing of troglitazone (47). In this chip, human 3D liver equivalents and skin biopsies were cultured simultaneously, while the microfluidic channels were successfully covered with endothelial cells. Moreover, this research group established a four-organ-chip system including human skin, intestine, liver and kidney equivalents to generate an ADME profile of substances (*Figure S2G*) (48). Although these studies can provide some useful information about the systemic drug testing, the multi-organ models are still in their infancy. Due to the complexity of each individual human organ, continued efforts must be made to produce more feasible integrated chips for reproducing these intricate interactions.

Concluding remarks

The recent advancement of microfluidic technologies, microfabrication techniques and sensing technologies have shown great potential in developing a more complex, and *in vivo*-like skin-on-a-chip model for drug testing applications. However, some challenges remain to be addressed. One of the major challenges is the precise control of the skin microenvironment, real-time monitoring and accurate analysis of drug action in a user-friendly manner, which can be achieved through the optimization of the microfluidic systems (e.g., design and fabrication of microfluidic devices) and combination of innovative detection methods (e.g., novel biosensors). Another challenge is the inability to recapitulate the heterogeneous nature of the skin. Currently, most available microfabricated skin models are created using animal cells or specific human cell types such as keratinocytes and fibroblasts rather than various human skin cell types and components. Employing iPSCs that can almost infinitely differentiate into all skin cell types has shown potential to solve this problem (49). Using iPSC-derived skin cells can be highly beneficial for developing a patient-specific skin-on-a-chip model integrated with additional skin components (e.g., immune cells and skin appendages) and

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enhanced barrier and dermal properties. Furthermore, 3D multi-organ-on-a-chip models introduced above hold great promise in replicating the complex physiological interactions that are present in the human body, but they still require further system validation and characterization for use as *in vitro* platforms amenable to drug testing. Overall, to generate a reliable skin-on-a-chip model, biologists, bioengineers, pharmacologists and biostatisticians should collaborate. Their endless efforts in designing, developing, and validating a skin-on-a-chip model will ultimately lead to the reduction of animal experimentation and the acceleration of drug research.

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Footnote

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References

1. Abaci HE, Guo Z, Doucet Y, et al. Next generation human skin constructs as advanced tools for drug development. Exp Biol Med (Maywood) 2017;242:1657-68.
2. Van Gele M, Geusens B, Brochez L, et al. Three-dimensional skin models as tools for transdermal drug delivery: challenges and limitations. Expert Opin Drug Deliv 2011;8:705-20.
3. Küchler S, Strüver K, Friess W. Reconstructed skin models as emerging tools for drug absorption studies. Expert Opin Drug Metab Toxicol 2013;9:1255-63.
4. Ponec M. Skin constructs for replacement of skin tissues for in vitro testing. Adv Drug Deliv Rev 2002;54:S19-30.
5. Flaten GE, Palac Z, Engesland A, et al. In vitro skin models as a tool in optimization of drug formulation. Eur J Pharm Sci 2015;75:10-24.
6. Mathes SH, Ruffner H, Graf-Hausner U. The use of skin models in drug development. Adv Drug Deliv Rev 2014;69-70:81-102.
7. Ågren M. Wound healing biomaterials-Volume 1: therapies and regeneration, Woodhead Publishing, 2016:101-16.
8. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nat Biotechnol 2014;32:760-72.
9. Zhao X, Lang Q, Yildirimer L, et al. Photocrosslinkable gelatin hydrogel for epidermal tissue engineering. Adv Healthc Mater 2016;5:108-18.
10. Yildirimer L, Hobson D, Lin ZY, et al. Tissue-engineered human skin equivalents and their applications in wound healing. In: Hasan A. editor. Tissue engineering for artificial organs: regenerative medicine, smart diagnostics and personalized medicine. Weinheim: Wiley-VCH, 2017:215-41.
11. Polini A, Prodanov L, Bhise NS, et al. Organs-on-a-chip: a new tool for drug discovery. Expert Opin Drug Discov 2014;9:335-52.
12. van den Broek LJ, Bergers LI, Reijnders CM, et al. Progress and future prospectives in skin-on-chip development with emphasis on the use of different cell types and technical challenges. Stem Cell Rev 2017;13:418-29.
13. van Duinen V, Trietsch SJ, Joore J, et al. Microfluidic 3D cell culture: from tools to tissue models. Curr Opin Biotechnol 2015;35:118-26.
14. Huh D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. Trends Cell Biol 2011;21:745-54.
15. Ghaemmaghami AM, Hancock MJ, Harrington H, et al. Biomimetic tissues on a chip for drug discovery. Drug Discov Today 2012;17:173-81.
16. Pati F, Gantelius J, Svahn HA. 3D bioprinting of tissue/organ models. Angew Chem Int Ed Engl 2016;55:4650-65.
17. Wisser F, Schumm B, Mondin G, et al. Precursor strategies for metallic nano-and micropatterns using soft lithography. J Mater Chem C Mater 2015;3:2717-31.
18. Elitaş M, Yüce M, Budak H. Microfabricated tools for quantitative plant biology. Analyst 2017;142:835-48.
19. Au AK, Lee W, Folch A. Mail-order microfluidics: evaluation of stereolithography for the production of microfluidic devices. Lab Chip 2014;14:1294-301.
20. Lee V, Singh G, Trasatti JP, et al. Design and fabrication of human skin by three-dimensional bioprinting. Tissue Eng Part C Methods 2014;20:473-84.
21. Hee AJ, Lee KJ, Kim DH,et al. Skin fibroblast cells on 3D skin cell chip using nanogold platform structures and three-floor structures. Sci Adv Mater 2016;8:2147-52.
22. Verhulsel M, Vignes M, Descroix S, et al. A review of

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microfabrication and hydrogel engineering for micro-organs on chips. Biomaterials 2014;35:1816-32.
23. Mohammadi MH, Heidary AB, Beydaghi V, et al. Skin diseases modeling using combined tissue engineering and microfluidic technologies. Adv Healthc Mater 2016;5:2459-80.
24. Hasan A, Paul A, Vrana NE, et al. Microfluidic techniques for development of 3D vascularized tissue. Biomaterials 2014;35:7308-25.
25. Mah CS, Kochhar JS, Ong PS, et al. A miniaturized flow-through cell to evaluate skin permeation of endoxifen. Int J Pharm 2013;441:433-40.
26. Selimović S, Dokmeci MR, Khademhosseini A. Organs-on-a-chip for drug discovery. Curr Opin Pharmacol 2013;13:829-33.
27. Zheng F, Fu F, Cheng Y, et al. Organ-on-a-chip systems: microengineering to biomimic living systems. Small 2016;12:2253-82.
28. Wang YI, Oleaga C, Long CJ, et al. Self-contained, low-cost body-on-a-chip systems for drug development. Exp Biol Med (Maywood) 2017;242:1701-13.
29. Kilic T, Navaee F, Stradolini F, et al. Organs-on-chip monitoring: sensors and other strategies. Microphysiol Syst 2018;2(3).
30. Zhang YS, Aleman J, Shin SR, et al. Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci U S A 2017;114:E2293-302.
31. Alexander FA, Eggert S, Wiest J. Skin-on-a-chip: transepithelial electrical resistance and extracellular acidification measurements through an automated air-liquid interface. Genes 2018;9:114-23.
32. Abaci HE, Gledhill K, Guo Z, et al. Pumpless microfluidic platform for drug testing on human skin equivalents. Lab Chip 2015;15:882-8.
33. Alberti M, Dancik Y, Sriram G, et al. Multi-chamber microfluidic platform for high-precision skin permeation testing. Lab Chip 2017;17:1625-34.
34. Wufuer M, Lee G, Hur W, et al. Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. Sci Rep 2016;6:37471-2.
35. Lee S, Jin SP, Kim YK, et al. Construction of 3D multicellular microfluidic chip for an in vitro skin model. Biomed Microdevices 2017;19:22-35.
36. Song HJ, Lim HY, Chun W, et al. Fabrication of a pumpless, microfluidic skin chip from different collagen sources. J Ind Eng Chem 2017;56:375-81.
37. Song HJ, Lim HY, Chun W, et al. Development of 3D skin-equivalent in a pump-less microfluidic chip. J Ind Eng Chem 2018;60:355-9.
38. Sriram G, Alberti M, Dancik Y, et al. Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. Mater Today 2017. In Press.
39. Abaci HE, Guo Z, Coffman A, et al. Human skin constructs with spatially controlled vasculature using primary and iPSC-derived endothelial cells. Adv Healthc Mater 2016;5:1800-7.
40. Mori N, Morimoto Y, Takeuchi S. Skin integrated with perfusable vascular channels on a chip. Biomaterials 2017;116:48-56.
41. Ramadan Q, Ting FC. In vitro micro-physiological immune-competent model of the human skin. Lab Chip 2016;16:1899-908.
42. Ataç B, Wagner I, Horland R, et al. Skin and hair on-a-chip: in vitro skin models versus ex vivo tissue maintenance with dynamic perfusion. Lab Chip 2013;13:3555-61.
43. Sung JH, Esch MB, Prot JM, et al. Microfabricated mammalian organ systems and their integration into models of whole animals and humans. Lab Chip 2013;13:1201-12.
44. Luni C, Serena E, Elvassore N. Human-on-chip for therapy development and fundamental science. Curr Opin Biotechnol 2014;25:45-50.
45. Jin H, Yu Y. A review of the application of body-on-a-chip for drug test and its latest trend of incorporating barrier tissue. J Lab Autom 2016;21:615-24.
46. Wagner I, Materne EM, Brincker S, et al. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab Chip 2013;13:3538-47.
47. Maschmeyer I, Hasenberg T, Jaenicke A, et al. Chip-based human liver-intestine and liver-skin co-cultures-A first step toward systemic repeated dose substance testing in vitro. Eur J Pharm Biopharm 2015;95:77-87.
48. Maschmeyer I, Lorenz AK, Schimek K, et al. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab Chip 2015;15:2688-99.
49. Han YF, Tao R, Sun T, et al. Advances and opportunities for stem cell research in skin tissue engineering. Eur Rev Med Pharmacol Sci 2012;16:1873-7.

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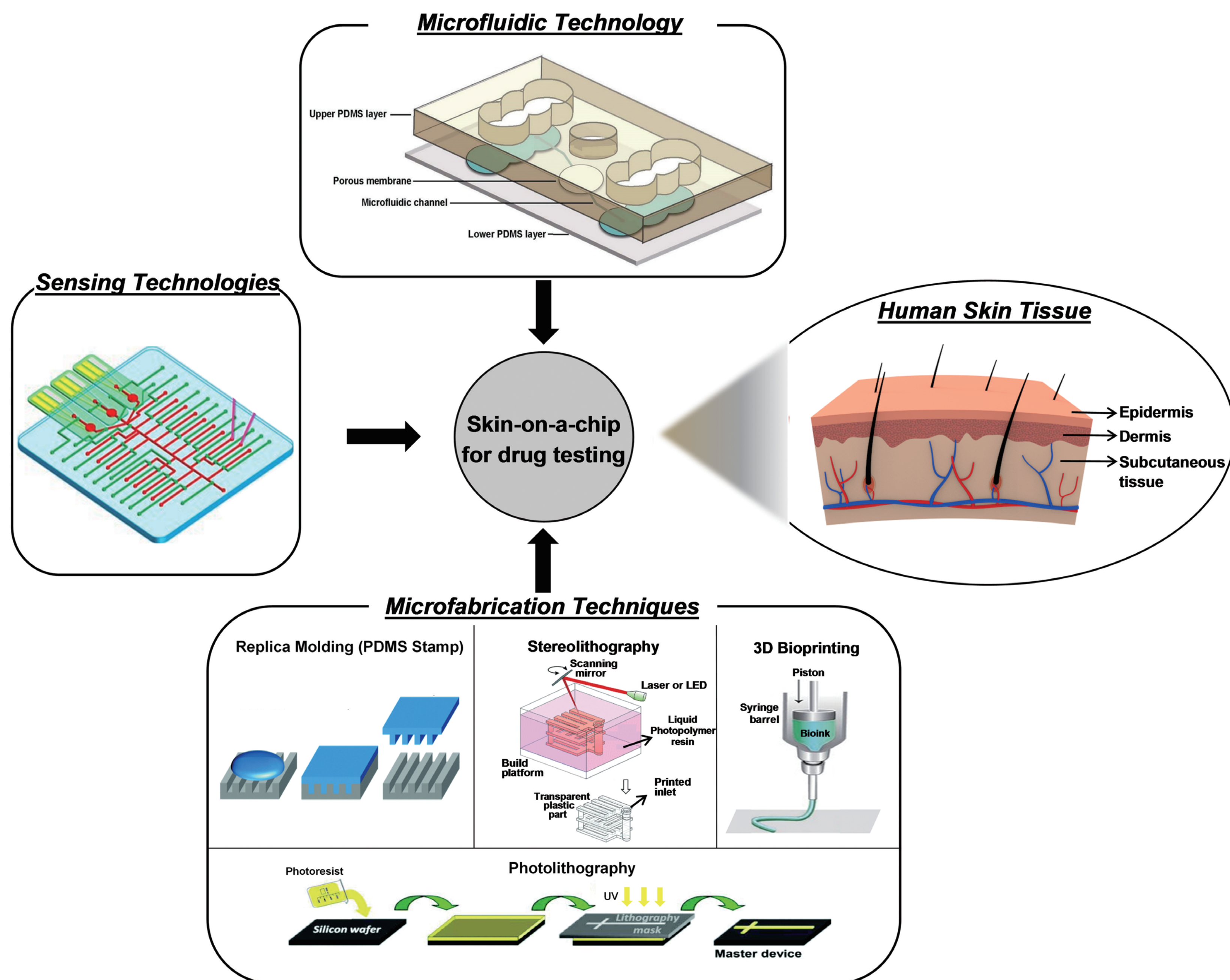

Figure S1 Development of skin-on-a-chip models mimicking native human skin structures for parallel, real-time and high-throughput drug testing using microfluidics, microfabrication and sensing technologies. Images (3D bioprinting, replica molding, photolithography, stereolithography and sensing technologies) were reproduced with permission from Ref. (16-19,30), respectively.

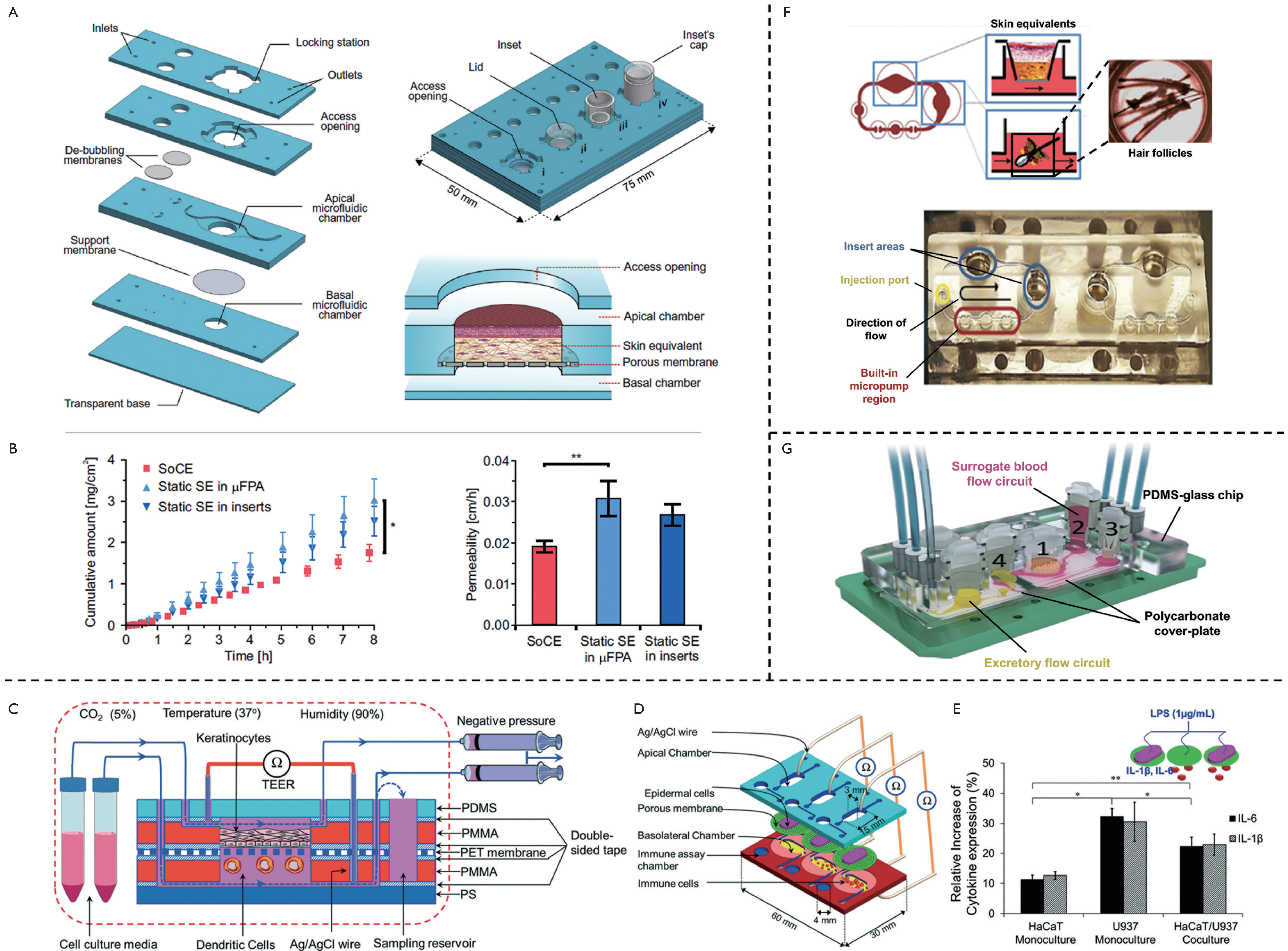

Figure S2 Microengineered skin-on-a-chip models. (A) Conceptual diagram of a full-thickness skin-on-a-chip model and schematic design of a chip device. Five microstructured poly(methyl methacrylate) layers were assembled and thermally bonded together to produce the microfluidic device with the co-culture of human fibroblasts and keratinocytes. (B) Caffeine cumulative amount and permeability coefficient in the full-thickness skin-on-chip equivalent compared to the static skin equivalent in the culture inserts and after insertion into microfluidic permeation array. Reproduced from Ref. (38) with permission. (C) Cross-sectional schematic view of a skin-on-a-chip device with co-culture of keratinocytes and monocytes. (D) 3D schematic view of the device composed of three parallel cell culture compartments and TEER measuring electrodes. (E) Assessment of the effect of lipopolysaccharide on skin barrier. Significant increase in pro-inflammatory cytokines IL-6 and IL-1β secretion was detected in the media of U937 mono-culture i.e. in the absence of the keratinocyte barrier, compared to that of the HaCaT monoculture. A moderate increase in cytokine expression was observed upon treating the HaCaT/U937 co-culture with lipopolysaccharide, which indicates that the keratinocyte layer can form a robust barrier against lipopolysaccharide invasion. Reproduced from Ref. (41) with permission. (F) Schematic view and photograph of a whole tissue perfusion system. The skin equivalents and follicular unit extracts were separately cultured on membrane inserts and interconnected with microfluidic channels. Reproduced from Ref. (42) with permission. (G) 3D schematic view of a four-organ-on-a-chip device. Numbers of the four microfluidic compartments represent for culture of intestine [1], liver [2], skin [3], and kidney [4] equivalents, respectively. Reproduced from Ref. (48) with permission.