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TOPICAL REVIEW

Skin-on-a-chip technologies towards clinical translation and commercialization

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Abstract

Skin is the largest organ of the human body which plays a critical role in thermoregulation, metabolism (e.g. synthesis of vitamin D), and protection of other organs from environmental threats, such as infections, microorganisms, ultraviolet radiation, and physical damage. Even though skin diseases are considered to be less fatal, the ubiquity of skin diseases and irritation caused by them highlights the importance of skin studies. Furthermore, skin is a promising means for transdermal drug delivery, which requires a thorough understanding of human skin structure. Current animal and *in vitro* two/three-dimensional skin models provide a platform for disease studies and drug testing, whereas they face challenges in the complete recapitulation of the dynamic and complex structure of actual skin tissue. One of the most effective methods for testing pharmaceuticals and modeling skin diseases are skin-on-a-chip (SoC) platforms. SoC technologies provide a non-invasive approach for examining 3D skin layers and artificially creating disease models in order to develop diagnostic or therapeutic methods. In addition, SoC models enable dynamic perfusion of culture medium with nutrients and facilitate the continuous removal of cellular waste to further mimic the *in vivo* condition. Here, the article reviews the most recent advances in the design and applications of SoC platforms for disease modeling as well as the analysis of drugs and cosmetics. By examining the contributions of different patents to the physiological relevance of skin models, the review underscores the significant shift towards more ethical and efficient alternatives to animal testing. Furthermore, it explores the market dynamics of *in vitro* skin models and organ-on-a-chip platforms, discussing the impact of legislative changes and market demand on the development and adoption of these advanced research tools. This article also identifies the existing obstacles that hinder the advancement of SoC platforms, proposing directions for future improvements, particularly focusing on the journey towards clinical adoption.

1. Introduction

The skin is the largest organ in the human body, with a total surface area of around 1.8 m^2 [1]. The most critical role of the human skin is the formation of a formidable barrier, shielding the human organism from pathogenic threats, physical/chemical damages, and exposure to allergens as well as irritants present in the surrounding environment [2]. Being the outermost layer of the human body, the skin is

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also susceptible to injuries and dermatological diseases. Despite low fatality, skin diseases cause severe symptoms, such as persistent itching and disfigurement, and may lead to complications, including extra-cutaneous infections and damage to internal organs [3]. In addition, side effects of drugs, allergies, and systemic inflammatory diseases commonly manifest themselves as dermatological symptoms due to the high immunologic activity of the skin (the skin is a reservoir of around 20 billion T cells, almost twice the number present in the blood) [4–6]. Apart from causing physical damage, dermatological disorders have a heavy mental burden, affecting the self-esteem of afflicted patients and posing a threat of mental illness [7–9]. Moreover, skin cancer represents one of the most prevalent malignancies affecting skin tissue. Present data suggest that approximately 20% of Americans will be diagnosed with skin cancer over the course of their lifetime [10]. Therefore, considering the ubiquity of skin diseases and their severity, there is an urgent need to develop more efficient skin-targeted diagnostic and therapeutic methods.

The importance of skin studies is beyond skin-related diseases and their treatment. The skin offers a non-invasive route for the transdermal delivery of systemic medications (i.e. treatment affecting the body as a whole) [11]. Transdermal delivery has advantages over oral administration, such as avoiding the first-pass metabolism and the gastrointestinal environment (e.g. enzymes and stomach acid), ultimately achieving less systemic toxicity with more controlled drug delivery [12, 13]. However, the barrier nature of the skin precludes administration of most of the medications, resulting in meager blood concentrations of transdermally applied drug formulations [12, 14]. Therefore, *in vitro* skin models that recapitulate the complexity of the epidermal barrier offer invaluable insights into the molecular dynamics of skin barrier functions. This approach enables the advancement of targeted therapies and the development of innovative strategies to enhance drug penetration through the skin barrier.

Preclinical drug screening has been mostly dependent on animal models and two-dimensional (2D) cell culture assays [15]. 2D cell culture models can be easily reproduced and are simple in use due to the well-established protocols developed for their handling [2]. However, rigid surfaces of culture flasks do not recapitulate the three-dimensional (3D) environment of the skin extracellular matrix (ECM), thus preventing cells from establishing physiologically relevant cell–cell and cell-matrix interactions [16, 17]. On the other hand, animal models are potent at predicting the systemic effects of drug candidates but suffer from ethical restrictions and low reproducibility [18, 19]. Most importantly, results obtained in animal studies often fail to translate to humans due to the subtle yet critical interspecies differences [20–22]. Although rat and porcine skins serve as gold-standard animal models for drug screening, they have a weaker barrier function, resulting in higher drug permeability [23]. Moreover, rodent models have a limited applicability for human wound healing studies due to the significant differences in their wound healing mechanisms. While murine skin has a higher wound healing capacity and heals by contraction without scarring, the human skin heals through re-epithelialization and granulation, leading to the scar formation [24]. The above-mentioned limitations of animal and 2D cell culture models for the development of pharmaceuticals indicate a pressing need for more predictive models of the human skin.

3D *in vitro* skin models were initially developed as powerful alternatives to conventional animal and 2D cell culture models. By surrounding cells in a tissue-engineered model, 3D matrices emulate the structural and chemical complexity of the native ECM, improving cells' differentiation, metabolism, and toxicity responses. Commercially available 3D reconstructed full-thickness skin models involve seeding keratinocytes on fibroblast-dispersed collagen matrices. The 3D culture enabled by these models enhances the maturation of the epidermis and its barrier function by promoting keratinocyte–fibroblast interactions and facilitating the growth of engineered tissues at the air–liquid interface [25]. Despite significant advantages, 3D *in vitro* models of skin have low reproducibility and a limited culture time due to the donor-to-donor variations in the source of skin cells and limited proliferation potential of basal keratinocytes [26, 27]. The lifespan of these constructs is also constrained by the weak mechanical properties and rapid contraction of hydrogels commonly utilized in skin tissue engineering [28]. Another critical deficiency of engineered skin equivalents is the lack of functional, perfusable vasculature, essential for the continuous supply of nutrients to skin cells and the removal of metabolic by-products [29]. The presence of vascularization is particularly important for overcoming the medium diffusion limitation inherent in matrix-rich skin equivalents [30]. Furthermore, avascular skin models have a limited utility for transdermal drug permeation studies and disease modeling, since the vasculature is closely involved in mediating skin inflammatory response and delivering immune cells to the site of infection and injury [31]. Additionally, engineering an immunocompetent skin model integrating innervation and skin appendages, such as sweat glands, sebaceous glands, and hair follicles remains a formidable challenge [30].

Organ-on-a-chip (OoC) technology holds a huge potential for addressing shortcomings of *2D in vitro* assays and reducing the usage of preclinical animal models [20]. These systems not only offer unprecedented control over experimental variables but also bring a new level of biomimicry and complexity to research [32]. The recent legislative changes by FDA

Modernization Act 2.0 further enhance the translational potential of OoC technology by authorizing the use of these microphysiological systems as alternatives to animal testing in investigating drug efficacy and safety [33]. This approval serves as a critical milestone in OoC adoption, presenting a legislative shift towards more human-relevant methods in pharmaceutical industry.

This technology has also been particularly transformative in skin research, leading to the development of advanced biomimetic skin models, known as skin-on-a-chip (SoC). SoC platforms address the inherent limitations of static *in vitro* skin models, significantly improving the culture duration and physiological relevance of reconstructed skin tissues, thereby providing enhanced fidelity and broader applicability for modeling skin diseases and testing pharmaceuticals. In this review article, we start by providing a foundational understanding of skin anatomy and its vital functions. We further delve into the main considerations essential for designing SoC models, including the key components that are important in the fabrication of these advanced platforms. In addition, we discuss engineering approaches directed at enhancing the complexity of SoC platforms. This review further examines recent literature across various application areas, offering insights into how SoC platforms can be utilized for disease modeling, drugs and cosmetics testing, and beyond. Furthermore, the patent analysis section presents a comprehensive analysis of SoC patents, while the market analysis section provides insights into the evolving market of *in vitro* skin models. Apart from technological advancements, we also outline the current challenges currently impeding the progress of SoC platforms and highlight future directions in which this technology can be improved, with a particular emphasis on their path towards clinical translation.

2. OoC technology

OoC microfluidic devices are dynamic culture systems that enable the recapitulation of tissue and organ functions by recreating native tissue microenvironments. These microphysiological systems achieve enhanced biomimicry by facilitating the integration of multiple cell types and enabling their culture under dynamic perfusion [34, 35]. The co-culture of different cell types inside microfluidic channels emulates tissue–tissue interfaces and allows the investigation of their complex interactions. On the other hand, the dynamic perfusion of the cell medium provides cells with nutrients while removing their waste metabolites, mimicking the role of blood circulation in the native human tissues. Microfluidic channels inside OoC devices are used to manipulate fluids at a microscale scale, substantially reducing the usage of expensive reagents [34, 36]. Additionally, fluid flow at the microscale level is inherently laminar, so that fluid flow regimes can be precisely controlled to establish stable biochemical gradients through which biological processes can be simulated (e.g. angiogenesis, chemotaxis, and differentiation) [37].

Another distinct advantage of OoC devices is their potential to finely tune the magnitude and frequency of various organ-specific mechanical cues, such as cyclic stretching and compression, fluid shear stress, and interstitial fluid flow. In their native milieu, cells are exposed to a unique combination of biomechanical cues, which impact their behavior by modulating cell signaling pathways [38]. Furthermore, OoC platforms benefit from compatibility with sensors for real-time imaging [39]. Incorporating sensors into OoC platforms provides *in situ* information about the cellular responses and cell culture parameters, such as pH, oxygen concentration, and temperature [39, 40].

SoC *in vitro* models enable the culture of skin tissues in a dynamic and controlled environment of a microfluidic chip [41]. By deploying advances in tissue engineering and microfabrication, SoC devices enable the culture of *in vitro* skin tissues possessing an improved barrier function and a multilayered architecture [42–44]. These *in vitro* skin models are well suited for drug screening and toxicity studies [45–47]. In addition, the manipulation of fluids at the microscale favors physiologically relevant substrate transport, making SoC devices powerful tools for drug permeation and transport studies [48, 49]. Furthermore, SoC models can incorporate vasculature, immune components, and skin appendages (e.g. sweat glands and hair follicles), increasing the complexity and biomimicry of produced skin models [43, 50, 51]. The skin models of enhanced biological authenticity can serve as more sensitive predictors of skin irritation and inflammatory responses [51, 52]. Being compatible with real-time imaging, SoC platforms facilitate the quantification of cell migration associated with skin inflammation, infection, and wound healing, providing valuable insights into the dynamics of these physiological processes [53–55]. The physiologically relevant mechanical forces can also be applied to skin tissues in SoC platforms to accelerate their aging and wrinkling [56, 57]. This approach can potentially accelerate the development of *in vitro* models for testing anti-aging drugs [58]. Thus, SoC can be considered a novel means of probing the pathophysiology of skin diseases and screening drugs for their treatment.

3. Factors to be considered in the design of skin models

3.1. Overview of the skin anatomy

In order to be able to recapitulate the skin complexity *in vitro*, it is critical to have a fundamental understanding of the histological structure and key functions of the skin. Therefore, the following paragraphs

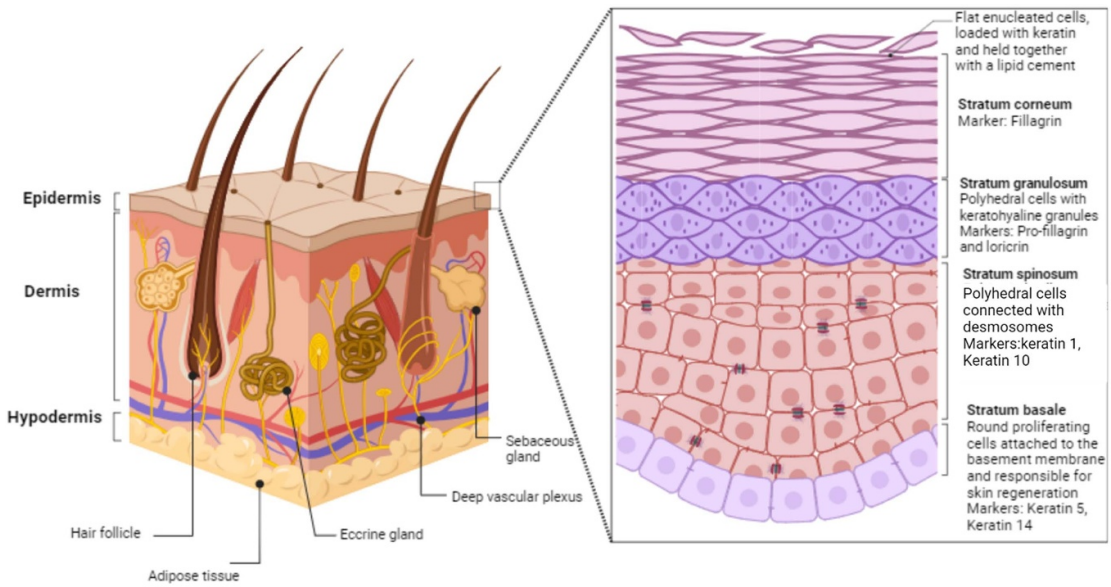


Figure 1. The schematic figure of the skin anatomy. The illustration depicts the complex, vascularized, multilayered structure of human skin, containing the epidermis, dermis, and hypodermis, along with skin appendages, including hair follicles and sweat glands. The diagram further highlights the stratified nature of the outermost epidermal layer, demonstrating its various sub-layers, each playing an important role in skin regeneration and barrier function. Adapted from 'Desmosomal Protein Distribution in the Epidermis', by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates. Created with BioRender.com.

provide a brief overview of the layers, cellular constituents, ECM components, and functions of the human skin.

From a histological perspective, skin is a tissue composed of three distinct layers: epidermis, dermis, and hypodermis (figure 1). The epidermis, the outermost layer of the skin, is made of stratified squamous epithelial tissue containing four different layers, with the stratum basale located at the bottom and the corneal layer lying on the top of the epidermis. The stratum basale, containing a population of stem cells, has a remarkable proliferative capacity and is responsible for renewing the entire epidermis [59]. The dominant cellular components of the skin epidermis are keratinocytes, which own their name to the cytoskeletal filamentous protein, keratin, that they produce [60]. As keratinocytes mature, they migrate from the basal layer to the stratum corneum, losing organelles and upregulating keratin expression through the process known as cornification. As a result, the stratum corneum cells adopt the scale-like flattened morphology and contain only amorphous aggregates of keratin fibers crosslinked with filament-binding proteins, filaggrin, and loricrin. Those keratin-loaded cells are held together by an envelope of highly-ordered and densely-packed lipid layers [61]. The high keratin content and the lipid-rich cement are critical components of the waterproof epidermal barrier, which precludes environmental threats from entering the body and prevents excessive water loss [62]. Tight junctions also contribute to the skin barrier integrity by sealing neighboring keratinocytes together and controlling intercellular transportation between them [63]. As the hydrophobic epidermal barrier regulates the passage of external agents across the skin, efforts have been directed at engineering skin tissues *in vitro* with barrier values close to those of the native human skin [64–66].

The dermis is a supporting layer of connective tissue sparsely interspersed with fibroblast cells [67]. The dermal layer of the skin is mainly composed of fibrous proteins, such as collagen and elastin embedded within a hydrated ground substance of proteoglycans. While the collagen fibers provide the skin with tensile strength, elastin endows its suppleness and resilience [68]. The high water-retaining property of proteoglycans, containing highly negative sulfate groups, ensures skin hydration [69]. Together, the complex network of these proteins establishes the ECM, which serves as a durable and stable scaffold for the dermal fibroblasts, and undergoes a continuous remodeling in response to external cues by relaying biochemical signals to all skin cells [70]. In contrast to the epidermis, an avascular tissue, the dermis contains a rich network of blood vessels, branching from two major plexuses. While the superficial vascular plexus branching into capillary networks is located beneath the epidermis, the deep vascular plexus, made of larger blood vessels, lies at the

dermo-hypodermal junction [71]. Apart from nourishing skin cells, the dermal vasculature also participates in thermoregulation and blood pressure control. Furthermore, the dermal vascular network initiates dermal inflammation by recruiting immune cells, such as neutrophils, T-cells, and macrophages, during the wound healing process or in response to pathogen invasion [59].

The basement membrane, a highly specialized network of proteins, lies at the dermo-epidermal junction and anchors the epidermal layer of the skin to the underlying dermis via hemidesmosomes and other adhesive components. It also regulates keratinocyte and fibroblast interactions and controls nutrient diffusion from the dermal vasculature to the epidermis [72]. The hypodermis, located at the bottom of the skin, is made of adipose tissue. Being a highly vascularized section of the skin, the hypodermis facilitates the swift drug uptake into the bloodstream. In addition, this layer takes part in hormone production and energy storage [59].

3.2. Constructing skin equivalents for SoC studies

The SoC platforms can be grouped into transferred or *in situ* depending on the method of skin tissue integration into the microfluidic system [73]. The first approach involves the off-chip generation of the skin tissue constructs, either in the form of human skin biopsies or reconstructed equivalents, followed by their subsequent on-chip cultivation and exposure to the dynamic perfusion [74]. This approach, utilizing pre-existing skin models, is particularly advantageous for clinical and drug toxicity studies due to its ability to maintain human skin's complexity and facilitate rapid experiment initiation [45, 47, 75]. Conversely, *in situ* SoC platforms, which involve the direct engineering and cultivation of skin tissues within the microfluidic environment, are optimally suited for exploring skin diseases, and understanding skin development and maturation mechanisms, as they allow for precise control over experimental conditions [42, 43, 76].

The skin tissue components of SoC platforms are commonly engineered as dermo-epidermal equivalents by culturing keratinocytes on the top of fibroblast-embedded hydrogel matrices. These reconstructed skin equivalents capture the key hallmarks of the skin, such as layered morphology and cell–cell interactions between keratinocytes and fibroblasts. Furthermore, the biomimetic scaffolds mimicking the dermal ECM provide skin cells with a 3D environment and promote cell-matrix interactions. Regardless of the application of SoCs, all platforms reviewed in this paper utilize a porous synthetic membrane. The membrane is either placed at the dermo-epidermal junction, mimicking the basement membrane [42], or situated between the whole skin tissue and the cell medium perfused below, controlling the diffusion of nutrients and shielding cells against the shear stress of the medium perfusion [66, 77, 78]. The cell medium perfusion below the skin tissue or through its dermal component enables the SoC platform to serve as a continuous bioreactor by replenishing the skin tissue with fresh medium and discharging its cellular wastes. Thus, the continuous media perfusion helps to maintain skin tissues for extended periods of time without compromising their integrity and viability [58, 75]. It is essential to mention that, depending on the application of SoC platforms, a number of studies were directed at introducing additional levels of complexity to the skin constructs, such as perfusable vasculature [76, 79–81], immune cell components [53, 82], and hypodermis [80]. The engineering approaches used in these studies are presented in the following sections.

3.2.1. Selecting cell types for engineering skin equivalents

The cellular source of skin tissue is one of the most critical parameters for SoC design. Skin tissues within SoC platforms were mostly developed from primary cells, cell lines, and iPSCs (induced pluripotent stem cells). Primary cells have been extensively used in SoC studies due to their functional and phenotypic resemblance to the native human skin [45, 52, 78, 83]. Being derived directly from mature dermal tissue, primary cells possess relevant genetic markers, indicating the applicability of primary cells for functional skin studies [84]. However, due to their fully differentiated phenotype, primary skin cells have a finite lifespan, precluding their expansion and utility for large-scale studies [85]. The isolation of primary cells is challenging due to the diverse cellular composition of the native skin, and their maintenance is resource-intensive, reflecting their sensitivity to environmental factors [86].

On the other hand, immortalized skin cell lines offer advantages of continuous and robust growth, unlimited supply, homogenous population, and reproducibility, addressing the current limitations of primary cells [87]. The epidermal compartment of the skin models is mainly established from HaCaT (human keratinocyte cell line) [43, 53, 66, 82, 88, 89] and human telomerase reverse transcriptase (hTERT) [66, 90] immortalized keratinocytes, while the dermal part is mostly produced from primary dermal fibroblasts. Primary skin cells are immortalized through various genetic manipulations, such as ectopic expression of TERT, mutation of cell cycle checkpoints(p53 or pRb), or introduction of oncogenes [91]. Despite providing cells with a limitless lifespan, these genetic alterations may induce pre-malignant characteristics and functions, aberrant from those of the native skin cells. These deviations

can become even more pronounced with extended culture and serial passages [87, 92]. Thus, skin models derived from cell lines may not fully recapitulate essential skin hallmarks, such as epidermal stratification and barrier function [93]. Furthermore, cell lines do not represent genetic variations between patients, limiting their applicability for personalized *in vitro* disease modeling [94].

iPSCs are also leveraged for the production of reproducible skin equivalents with high genetic stability owing to their self-renewal property and capacity to differentiate into all skin cell types [95]. Petrova *et al* reported the stepwise protocol for the derivation of human epidermal equivalents from human embryonic stem cells (hESCs) and iPSCs [65]. hESCs/iPSCs were induced to differentiate into keratinocytes and purified with a physiological method resulting in a high isolation yield. Keratinocytes were subsequently exposed to the air–liquid interface culture to promote the formation of the mature, stratified epidermis with an endoplasmic reticulum Ca^{2+} storage function.

As iPSCs are derived from individual patient cells, they possess all genetic characteristics specific to each individual [96]. This enables the creation of *in vitro* models on a patient-by-patient basis, paving the path for personalized disease modeling and drug screening. Itoh *et al* reported successful generation of 3D skin equivalents from iPSCs derived from patients with recessive dystrophic epidermolysis bullosa (RDEB) disease [97]. The autologous skin model was produced by seeding iPSCs-derived keratinocytes on top of iPSCs-derived fibroblasts dispersed within the collagen matrix. Human iPSCs-derived fibroblasts were shown to secrete collagen type VII, showing a promise for the treatment of the RDED disease.

Despite holding great potential for developing 3D skin *in vitro* models, iPSCs should overcome several obstacles before becoming the primary cellular source for SoC platforms. In contrast to primary cells and cell lines, which are readily available, iPSCs derivation requires sticking to lengthy and complex protocols, which lack robustness and result in meager yields [98]. Furthermore, the derivation and purification of mature skin cells from iPSCs is equally challenging due to the lack of efficient differentiation protocols and cell separation technologies, which compromise the quality of produced *in vitro* skin models [99, 100]. Hence, iPSCs production and differentiation are time-consuming and costly, obstructing their wide adoption by research laboratories and potential commercialization [96].

3.2.2. Selecting biomaterials for engineering skin equivalents

The proper selection of biomaterial types is essential for the construction of 3D dermal scaffolds to promote cell-matrix interactions and facilitate nutrient exchange [101]. In addition, the dermal matrix should possess the mechanical stability to withstand the contractile forces exerted by the fibroblasts as they proliferate. Fibroblasts are known to actively remodel and reorganize the ECM, affecting its composition and mechanical properties [102, 103]. Being the central part of dermal ECM, collagen is an obvious and the most popular material choice when constructing dermal matrices for SoC applications [104]. Despite its biomimicry, pristine collagen suffers from poor mechanical stability, which leads to the rapid degradation and shrinkage of the dermal matrix, as well as its detachment from the underlying membrane [105]. Another widely utilized naturally hydrogel, fibrin, has also been reported to undergo significant contraction due to its intrinsic properties, independent of cell presence [106]. The matrix contraction issue curtails the lifespan of skin equivalents and impedes their standardization, thereby restricting the utility of engineered skin substitutes across various applications [107].

To address the limitations of rapid degradation and contraction, the mechanical properties of natural biomaterials can be significantly improved by using crosslinking agents or a combination of various materials [28, 108]. Although the employment of chemical cross-linkers, including glutaraldehyde and genipin, enhances the physicochemical properties of skin scaffolds, the potential cytotoxic effects of these agents must be carefully evaluated [28]. To circumvent these issues, Lotz *et al* utilized a non-toxic chemical crosslinker, four-arm succinimide glutarate PEG (PEG-SG), to improve the mechanical stability of collagen hydrogels, resulting in scaffolds that retained their initial surface area for three weeks, contrasting standard collagen models that experienced a 50% shrinkage [109]. Other natural hydrogels, including hyaluronic acid and gelatin, can be crosslinked with methacrylic anhydride to produce their photocrosslinkable counterparts, offering a fine control over their mechanical properties [28, 110]. Gong *et al* functionalized hyaluronic acid with methacrylate anhydride and dopamine(DA-MeHA) to design a hydrogel for skin regeneration [111]. The DA-MeHA hydrogel, when loaded with adipose-derived stem cells (ADSCs), significantly improved skin wound healing by securely adhering to the wound site, facilitating its remodeling, and promoting the early-stage angiogenesis.

Cummings *et al* demonstrated the enhanced mechanical stability and reduced matrix contraction of a collagen-fibrin blend at 1:1 ratio [112]. Alternatively, Brougham *et al* developed a reinforced collagen–fibrin–glycosaminoglycan (CFG) gel significantly resisting the cell-mediated contraction [113]. In contrast to pristine fibrin gel, which shrank by 90%, the CFG gel withstood the contraction, demonstrating a 6-fold and 30-fold increase in compressive

and tensile moduli, respectively. Montero *et al* introduced an innovative hydrogel composed of plasma-derived fibrin, reinforced with thiolated-hyaluronic acid, and crosslinked with poly(ethylene glycol) diacrylate (PEGDA), which was found to significantly enhance skin model development [114]. This specialized formulation resulted in organotypic skins that were up to three times thicker than those formed with fibrin controls, evidencing a reduction in contraction. Instead of using an alternative hydrogel, Jeong *et al* attached the collagen matrix to the walls of the culture chamber by applying a crosslinker. The crosslinker firmly bound the collagen scaffold and prevented its shrinkage, enabling a 28 d-long skin tissue culture on a SoC platform [58].

Alternatively, the dermal component of skin substitutes can be produced in a scaffold-free fashion with the self-assembly approach, whereby fibroblasts are coaxed to produce their own ECM in sheets [115]. Subsequently, three or four sheets are stacked and matured to establish a cohesive dermal tissue, upon which keratinocytes are seeded, thus resulting in bilayered skin substitutes [116]. Skin substitutes produced by this method were particularly promising for wound healing studies, where the usage of self-assembled bioactive dressings was shown to promote the healing of chronic wounds, such as ulcers [117, 118].

Enabling spatiotemporal control over the deposition of cell-embedded hydrogels, 3D bioprinting was also leveraged to fabricate the dermal compartment of the reconstituted skin. Several novel bioink formulations were designed to be compatible with 3D bioprinting as alternatives to contractible collagen for skin dermis production. Pourchet *et al* developed a bioink made of a mixture of gelatin, alginate, and fibrinogen to print the dermal layer of the *in vitro* skin model [119]. While the gelatin component facilitated the printing process due to its rheological properties, alginate imparted mechanical stability to the printed dermis. On the other hand, the fibrinogen constituent provided cellular adhesion sites for skin cell attachment and growth. In order to produce organized skin tissues, the bioink embedded with primary fibroblasts was printed as a dermal layer and, upon solidification, was seeded with primary keratinocytes. As a result, well-differentiated skin tissue was established with a basement membrane in the dermo-epidermal junction and collagen fibrils in the dermis. Kim *et al* used skin-derived decellularized ECM (dECM) bioink as a more stable and biomimetic alternative to the contractible collagen [80]. As an alternative to new bioink formulations, Pontiggia *et al* developed an innovative methodology to automate the printing and stabilization of collagen-based skin substitutes [120]. By means of a novel three-unit bioprinting platform, they mixed, extruded and uniformly compressed prevascularized fibroblast-dispersed collagen scaffolds. This uniform compression facilitated fluid removal from hydrogels, significantly enhancing the mechanical strength and minimizing the shrinkage properties of the natural collagen.

The type of materials, their origin, and concentration affect the mechanical and chemical properties of the resulting scaffold. Song *et al* employed SoC platforms with collagen matrices derived from rat tail, porcine skin, and duck feet to demonstrate the differential effect of the collagen source on skin tissue differentiation [121]. While the duck feet collagen resisted the contraction better than others, the rat collagen excelled in promoting skin stratification and expression of differentiation markers. Kwak *et al* examined the impact of varying GelMA concentration on scaffold's mechanical properties and cell attachment [104]. While keratinocytes favored stiffer scaffolds of higher GelMA concentrations, fibroblasts adhered more to less stiff and more porous matrices of lower polymer concentration. These results demonstrated the importance of fine-tuning the mechanical properties of the dermal scaffold to meet the needs of each cell type integrated into the SoC model.

3.3. The role of perfusion in SoC platforms

The microfluidics component of SoC platforms enables the culture of skin tissues under physiologically relevant dynamic conditions [122]. One distinguishing feature of this dynamic microenvironment is perfusion, which refers to the continuous exchange of fresh medium through the microchannels either within or below the skin tissue equivalents [34, 123]. This continuous nutrient supply significantly enhances the lifespan and viability of skin constructs by providing cells with optimal growing conditions for extended periods of time [56, 75]. The incorporation of dynamic perfusion within SoC platforms was shown to establish high glucose concentration in the skin tissue culture chamber [77, 79], sustain long-term fibroblast viability as well as differentiation [77], and maintain the elevated proliferative state of keratinocytes [57].

Apart from supplying skin cells with fresh media, the dynamic perfusion also exerts shear stress on various cell types integrated within the SoC platforms. Shear stress refers to a frictional force applied by a parallel flow of fluid and is known to induce morphological changes and activate signaling cascades in cells exposed to it [124–126]. Thus, shear stress plays a critical role in the organization of collective behaviors of cells, such as alignment and differentiation, while its dysregulation is associated with a range of diseases, including cancer and atherosclerosis [127–129]. Microfluidic platforms enable fine-tuning of fluid flow regimes and shear stress, which generates biomimetic environments suitable for the quantitative characterization of cellular mechanotransduction interactions [129]. For instance, Agarwal *et al* demonstrated that shear stress of 0.06 dyne cm^{-2} inside the

microfluidic channels induced the reorganization of keratinocyte cytoskeleton and enhanced skin barrier function, while higher shear stress of 6 dyne cm^{-2} damaged cell cytoskeleton and disrupted epithelial barrier [130]. Although in most SoC configurations the dermo-epidermal constructs are shielded from direct exposure to the media flow by a porous membrane, culturing cellular monolayers inside perfused microchannels can expose either the dermis or epidermis to the shear stress [42, 82]. In addition, the underlying flow of the medium can still exert the shear stress on the dermal matrix and uppermost epidermal layer through the interstitial flow, bringing about morphological and functional changes within those tissues. The interstitial flow is generated due to the pressure gradient between the air–liquid interface above the epidermis and media flow below the skin tissue [66].

Ramadan and Ting reported a dramatic rise in skin barrier values after culturing the epidermis under dynamic conditions [43]. In another study, instead of perfusing the cell medium, Sriram *et al* ventilated the epidermal layer to promote its differentiation and barrier function [66]. The dynamic perfusion and controlled air ventilation brought about not only a superior epidermal architecture and maturation, but also an enhanced deposition of basement membrane proteins. The dermo-epidermal anchorage (ensured by the intact basement membrane) is critical for epidermal integrity. Therefore, its successful recapitulation enhanced the biomimicry of engineered skin constructs. Apart from improving the maturation of artificial skin tissues, perfusion also aids in the formation of blood vessels with perfusable lumens and enhanced barrier function [57, 80]. This effect can be explained by the mechanoresponsive behavior of endothelial cells, which are reported to align and differentiate under the impact of fluid flow and flow-induced shear stress, respectively [126, 131].

The cell medium within SoC platforms can either be perfused via pumping devices or with the pumpless approach. Typically, perfusion of the cell medium within SoC devices is carried out via peristaltic, syringe, or piezoelectric pumps. The pumping systems offer the advantage of precise programming of perfusion regimes, including the control over fluid flow rates, shear stress, and pressure magnitudes. Nevertheless, their usage necessitates installing external tubing connections, increasing the risk of bacterial contamination and air bubble formation [77]. Alternatively, gravity-assisted rocking platforms and capillary force-based methods (paper-based microfluidic devices) enable the pumpless perfusion of cell culture medium bypassing the need for external connections [77, 82, 132–134]. Those rocking platforms recirculate the medium through periodic fluctuations of the rotating stage, in which tilting angle and rotation frequency determine the ultimate perfusion rate of the medium [45]. However, pumpless platforms lack benefit of the fine control over the flow rate offered by pumping systems. Moreover, gravity-driven recirculation systems face challenges in establishing a unidirectional perfusion, which is critical for the maturation of shear-sensitive tissues, such as the vasculature [135]. This limitation was addressed by an elegant 'UniChip' fluid network design proposed by Wang and Shuler, which converts the reciprocating flow input into backflow-proof unidirectional perfusion by employing a set of supporting channels and valves [135].

3.4. Engineering approaches to mimic vasculature in in vitro skin models

While human blood vessels transport oxygen and nutrients to all organs, they are also involved in the development of various diseases, including vascular disorders, chronic inflammatory diseases, and cancer [136]. Thus, their integration into *in vitro* skin models is pivotal both for engineering functional skin grafts for regenerative purposes and answering fundamental research questions about skin diseases. Undoubtedly, the formation of vascularized skin models would pave a path for a more accurate evaluation of drug absorption, toxicity, and the assessment of skin irritancy and inflammation [81].

Despite substantial advances in improving the biomimicry of *in vitro* SoC platforms, the incorporation of a functional vasculature within reconstructed skin tissues remains a significant engineering challenge. Most studies directed at vascularizing engineered skin tissues involve dispersing endothelial cells within dermal or hypodermal matrices and their subsequent exposure to angiogenic factors, such as VEGF (vascular endothelial growth factor). This strategy results in the formation of randomly distributed microvascular networks. However, the main hurdle behind engineering vasculature lies in the production of robust, mature, and perfusable vessel-like structures that will be sustained even in the absence of growth factors [137]. Several engineering strategies have been employed to generate perfusable tubular structures simulating blood vessels inside *in vitro* skin models. One of the standard techniques involves casting dermal hydrogels around templates with defined channel structures. The subsequent removal of those spacers leaves behind hollow tubular channels that can later be seeded with endothelial cells to emulate blood vessels [138].

Mori *et al* fabricated the dynamic culture system for growing skin tissues with perfusable vascular channels [57, 79]. Anchoring structures of the system and oxygen plasma treatment ensured the firm attachment of skin equivalents to the device walls. Those anchoring connections also served as inlet and outlet points for nylon wires, which were strung in a grid-like pattern. Nylon wires were mechanically withdrawn once the collagen-based fibroblast-laden hydrogel was cast, producing hollow channels

inside the dermal compartment of the skin equivalent. The subsequent coating of tubular structures with HUVEC (human umbilical vein endothelial cells) cells and perfusing them with cell media facilitated the maturation of blood vessels. The feasibility of the vascularized skin tissue as a model for testing vascular drug absorption was confirmed by applying test substances with or without administration of VEGF. The vascular channel permeability was shown to increase in the presence of VEGF, demonstrating the decisive role of the endothelial barrier for drug permeation.

Abaci *et al* utilized a similar yet slightly different approach to incorporate perfusable microvascular networks into human skin equivalents [76]. In this case, 3D printing technology was utilized to build alginate molds of various vasculature patterns, including the one mimicking vascular bifurcations. Instead of mechanical removal, sacrificial alginate microchannels were dissolved by pumping sodium citrate solution, establishing perfusable microchannel networks. Primary and iPSC-derived endothelial cells were seeded onto these channels, producing continuous endothelial layers with the expression of tight junctions. Comparison of diffusion rates of fluorescent molecules across the channels covered with IECs (IPSC-derived endothelial cells) versus the microchannels containing no cells revealed a drastic reduction of 97% in permeability in the former case, confirming the development of a robust barrier in the endothelium. Furthermore, vascularized human skin equivalents demonstrated enhanced integration response upon grafting onto immunodeficient mice by stimulating vascular ingrowth from the host vasculature. Vascularized human skin grafts also resulted in a significantly higher proliferation rate of keratinocytes than tissues containing no blood vessels.

Salameh *et al* engineered a more intricate perfusable vasculature network inside the skin model by combining the molding technique with the capacity of endothelial cells to self-assemble into capillary-like structures (figure 2(B)) [81]. Similar to the molding approach employed by Mori *et al* [57, 79], three perfusable tubular structures were established by casting dermal matrix around nylon wires, which were subsequently removed upon collagen contraction. Afterward, hollow channels were seeded with HUVEC cells and perfused with a cell medium using a peristaltic pump. An additional dispersal of HUVECs within the dermal matrix brought about their spontaneous assembly into microvascular networks with connected branches. Subsequently, angiogenic sprouts (extending from the fluidic channels) connected to the secondary capillary bed within the dermal compartment, forming anastomose-like junctions. Integration of a complex vascular plexus within engineered skin tissues resulted in a more precise prediction of systemic and topical drug responses. Namely, the presence of a perfusable vasculature increased the systemic penetration of an irritant, revealed by its higher concentration within skin layers compared to samples without any vessels or with just vascular channels.

3D bioprinting can also be used to fabricate complex vascular networks within skin tissues due to its ability to accurately deposit multiple hydrogel-embedded cell types [141]. The primary advantage of additive manufacturing for engineering vasculature is its capacity to produce 3D networks spanning multiple skin layers, thus overcoming the limitation of forming vascular channels across only one plane [138]. Kim *et al* utilized a hybrid custom-built 3D printer to fabricate a full-thickness skin model as a part of a transwell system through a one-step bioprinting process (figure 2(A)) [80]. The skin tissue in this work also included the hypodermal layer, fabricated through the deposition of a hypodermal dECM and fibrinogen bioink via the extrusion-based module of the printing system. On the other hand, the epidermal layer was formed through the uniform distribution of keratinocytes via the inkjet module. For the generation of the vasculature, gelatin and thrombin hydrogel mixture were embedded with endothelial cells and bioprinted in a cylindrical shape. After being stored at 37 degrees, the gelatin melted and left behind hollow tubes with attached endothelial cells. The subsequent perfusion of vascular channels led to a twofold decrease in their diffusional permeability coefficient when compared to that of bare vessels, demonstrating the establishment of an intact barrier by endothelial cells. The structural maturation of the skin model was confirmed by evaluating functional markers representative of each skin layer: filaggrin and keratin expression by the epidermis, collagen type 1 deposition by the dermis, and the storage of lipid droplets by the subcutaneous layer of the skin.

Liu *et al* utilized a 3D bioprinting approach to produce a vascularized model of the inflammatory skin disease, atopic dermatitis (AD) [142]. The vascularized dermis was deposited by extruding the bioink composed of a mixture of neonatal fibroblasts, pericytes, and iPSC-derived endothelial cells encapsulated within a fibrin hydrogel. As a result, endothelial cells organized into defined non-perfusable tubular structures establishing vascular sprouting. The biomimicry of vascularized skin models was demonstrated by their capability to capture key clinical features of AD, including hyperplasia and upregulated expression of proinflammatory cytokines. In addition, the bioprinted AD model was exploited as a high-throughput drug-screening platform, recapitulating not only the therapeutic effects of JAK (Janus kinase) inhibitors on restoring the barrier function and the morphology of the skin, but also their side effects on destabilizing the dermal vasculature. Overall, this study indicated the significance of vasculature in enhancing the fidelity of skin disease

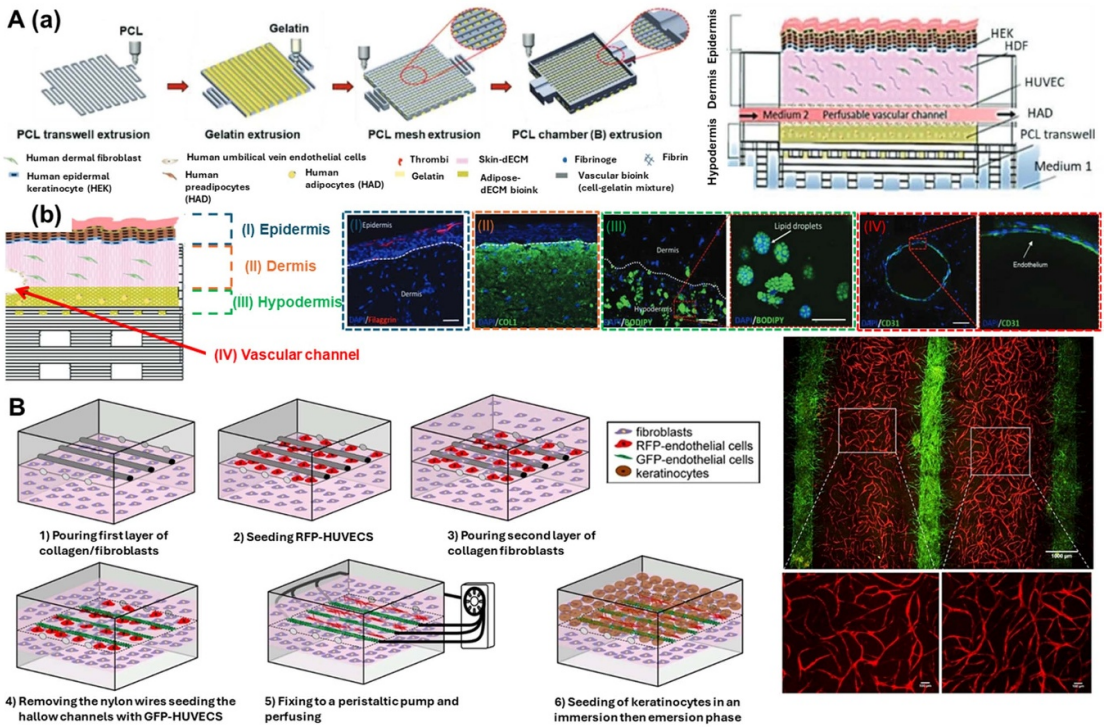


Figure 2. *In vitro* skin models with perfusable vasculature and individual layers of the 3D-printed full-thickness skin. (A (a)) Kim *et al* leveraged 3D printing for engineering a mature vascularized 3D skin model composed of epidermis, dermis, and hypodermis. The schematic diagram illustrates the main steps of the 3D printing process for the fabrication of both the culture chamber and the full-thickness skin tissue. The PCL tissue culture chamber was extruded in parallel with skin layers. The vascular channel was bioprinted on the surface of the hypodermal skin layer and subsequently liquefied to produce a hollow channel between the produced dermal and hypodermal skin compartments. Finally, keratinocytes were overlaid onto the dermal layer, and the produced skin tissue was left to mature. (A (b)) Illustration of each zone of epidermis, dermis, hypodermis, and vascular channel. Subfigures (I), (II) [139], and (IV) refer to individual skin layers. (I) The stratified epidermis was stained with keratin 10 (K10) and filaggrin, demonstrating early differentiation and late differentiation of epidermis, respectively. (II) Fluorescent images of the dermal protein markers of epidermal-dermal junction (Laminin), secreted ECM components (COL1: collagen type I) [139]. Hypodermis stained with BODIPY representing lipid droplets of adipocytes. (IV) Vascular channel walls within the mature 3D human skin equivalent stained with CD31, demonstrating the presence of endothelium (Scale bars: 50 μm).[80]. John Wiley & Sons. [© 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim]. (B) Salameh *et al* combined molding with spontaneous formation of capillary structures by endothelial cells to develop intricate vasculature patterns within skin tissues. On the left: schematic illustration of the stepwise fabrication of vascularized skin models. On the right: fluorescent images of RFP-HUVECs (red) microvasculature formed in-between three GFP-HUVECs [140] perfusable channels. Adapted from [81]. CC BY 4.0.

models for testing new anti-inflammatory skin drug formulations.

3.5. Engineering microfluidic chips for SoC applications

The fabrication of PDMS (Polydimethylsiloxane)-based microfluidic devices for OoC applications widely relies on the soft lithography technique, which involves generating master molds of complex patterns via photolithography, followed by casting elastomeric PDMS to produce an imprint of the master [143]. Soft lithography offers many advantages, including the potential to generate intricate channel patterns with high resolution and the capacity to stack several molded layers to produce 3D multi-chamber devices [144]. The utility and scalability of this method, however, are hampered by the need to fabricate master molds by photolithography, which relies on costly chrome masks and toxic photoresists [145]. In addition, even slight modifications to the chip design require the fabrication of new wafers, making the process relatively inflexible [146]. Therefore, photolithography is expensive, labor-intensive, and rigid. Thus, to overcome challenges associated with photolithography, several other technologies, such as laser cutting and 3D printing, were proposed to fabricate microfluidic devices for culturing skin tissues.

Risueno *et al* leveraged the micromachining technology to produce layered vinyl-based microfluidic chips for SoC applications as an alternative to soft lithography [89]. The cut and assemble approach presented in this study required only an affordable vinyl plotter, adhesive vinyl tapes, and methacrylate sheets. The procedure for fabricating chips was streamlined further by exploiting biocompatible vinyl tapes, which enabled combining multiple layers of different polymers simultaneously, avoiding the usage of complicated plasma bonding. The microfluidic chips possessed a layout of two fluidic chambers, separated by a polycarbonate membrane, where the thickness of each was adjusted by stacking and taping various numbers of vinyl sheets.

In order to bypass the usage of photolithography, Sasaki *et al* utilized a laser cutter to produce acrylic molds for casting microfluidic chips in the form of branched microchannel patterns [88]. This novel microfluidic design enabled a parallel analysis of skin permeation—the intersection of branched microchannel patterns of the top and bottom plates established 24 crossings, permitting 24 parallel permeation experiments of three different analytes. Challenging the keratinocyte monolayer on the porous membrane with a skin irritant compromised its barrier function, demonstrated by increased permeation of fluorescent tracers. Thus, the developed engineering approach facilitated the production of chips amenable for high-throughput studies in a photolithography-free manner.

With the rapid advances in additive manufacturing technology, including higher printing resolution and the development of novel printable materials, there has been an upsurge of interest in using 3D printing for the fabrication of organ transplants, microfluidic, organ-on-chip, and microrobotic devices [94, 147–155]. 3D printing, provides the advantage of producing truly 3D microfluidic devices in one step, with flexibility in rapidly changing intricate microstructure designs in a customizable manner [146, 147]. Recently, many studies have been directed at utilizing 3D-printed molds for PDMS casting, thus avoiding the usage of photolithography for master fabrication [146, 156, 157]. Generated microfluidic platforms were compatible with OoC studies without compromising tissue maturation and viability. Although, to the best of our knowledge, no study reported the usage of 3D printed microfluidic devices for SoC applications, several studies utilized rapid prototyping for producing skin culture platforms [57, 80, 158, 159].

Ding *et al* employed 3D printing to fabricate miniaturized multichannel (MCD) flow-through devices as scaled-down alternatives to conventional Franz diffusion cells for skin permeation studies [158]. In order to optimize fluid flow profiles for efficient drug transportation, three types of MCD of various geometries were printed. 3D printing technology relying on the computer-aided design was amenable for modifications to device design parameters, such as chamber size and geometry. The device was printed in the layout of six parallel diffusion cells enabling drug testing in a high-throughput manner. The administration of two topical formulae onto the skin sample surface in MCDs produced drug permeation profiles similar to those of the conventional static diffusion cells.

Mori *et al* fabricated a platform for cyclical stretching of skin constructs by rapid prototyping technology [57]. The culture devices designed for accommodating and stretching skin tissues were molded by casting flexible silicone material into 3D printed molds. The skin tissue constructs were fixed to the silicon device using 3D printed connections to couple the uniaxial strain of the device with the deformation of skin tissues. The stretching apparatus contained a stepper motor, enabling the control of the stretching frequency via a microcontroller, a device holder accommodating the silicon device, and a crank enabling the adjustment of the strain magnitude. The rapid prototyping of device components facilitated the rapid adjustments of the stretching parameters.

3.6. Integrating biosensors into SoC platforms

To gain a comprehensive understanding of cellular and tissue status in OoC platforms, it is essential to both record the biological responses of cells and monitor cell culture conditions [160, 161]. Conventional analysis methods in OoC systems rely on immunohistochemistry and histological staining, which are predominantly performed by fluorescent microscopy [162]. They require the complete removal of tissue samples from microfluidic chips, resulting in endpoint results that offer limited information on the dynamics of the system. Furthermore, the use of labels in these assays can lead to nonspecific interactions with cells, potentially interfering with cellular metabolism and altering the experimental outcomes [39]. Other analytical techniques, such as enzyme-linked immunosorbent assay, RNA sequencing, and high-performance liquid chromatography (HPLC), are not well-suited for miniaturized OoC platforms, since these methods often require large sample volumes and manual sample collection, which can be labor-intensive and disruptive to the microfluidic system by causing undesirable interference with the biological processes being studied [160].

The integration of OoC platforms with microsensors enables *in situ* and continuous tracking of cellular responses, which can provide a label-free alternative to conventional tissue analysis techniques [39]. Embedding biosensors into OoC systems would also offer an enhanced temporal and spatial resolution in generating meaningful data about living tissues over time without perturbing their behavior [163]. Furthermore, integrating biosensors into OoC platforms would enhance their reproducibility by reducing the need for manual intervention and offering more precise control over cell culture conditions and experimental parameters [164]. OoCs are highly compatible with microsensor embedding, as OoCs are mostly fabricated using the same micromachining or prototyping techniques used to create miniaturized sensors [39]. These sensors can either be completely integrated into the OoC chip or located outside of it, analyzing the fluid at the outlet. While the latter method shields the sensors from fouling or contamination, it introduces an analysis delay. In contrast, integrated biosensors offer direct and fast measurements of changes in analyte levels [39].

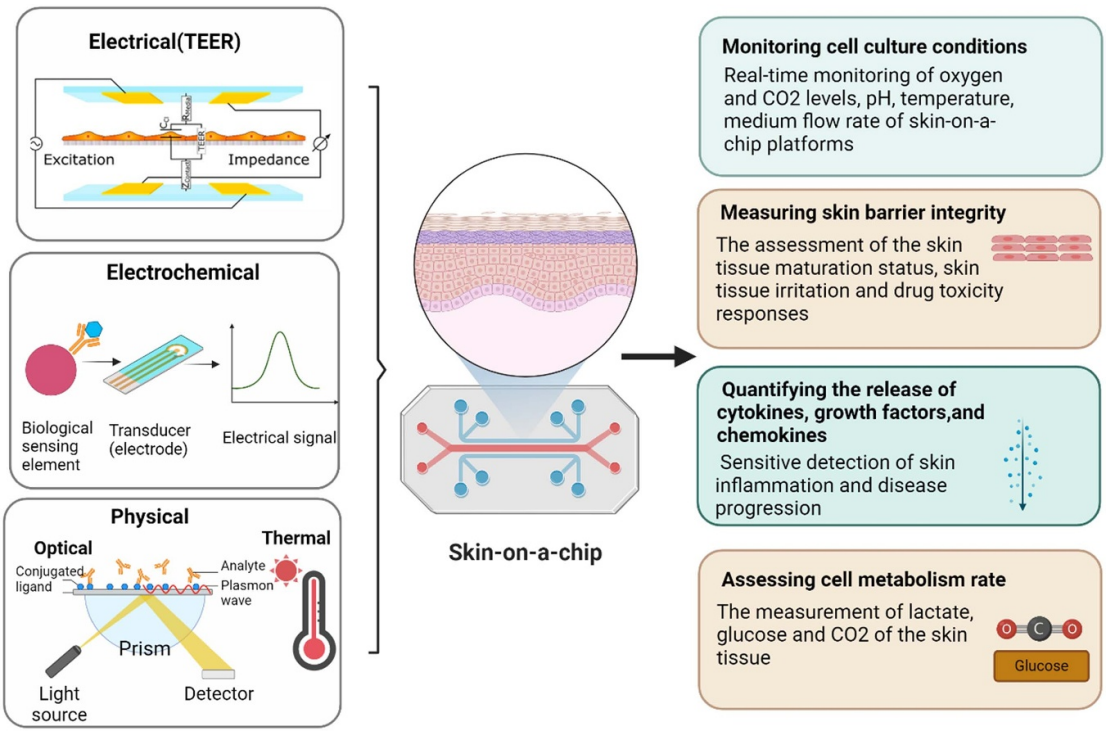


Figure 3. Application areas of various biosensor types for the comprehensive characterization of SoC platforms. The schematic illustration demonstrates how electrical (TEER), electrochemical, physical (optical, thermal) biosensor integration can be exploited to monitor cell culture conditions and to characterize skin tissue inside SoC platforms. The depicted biosensor integration facilitates the real-time assessment of critical skin parameters, including barrier function, cellular differentiation, metabolic activity, and inflammatory or irritative responses, thereby enhancing the predictive accuracy and biological relevance of SoC models. Created with BioRender.com. Created with BioRender.com.

When it comes to skin tissue, integrating biosensors into SoC platforms enables sensitive and real-time monitoring of epidermal differentiation and can provide instant results on drug absorption, metabolism, and efficiency. SoC can be embedded with electrical sensors to measure the barrier function of the skin tissue [44, 165]. In addition, electrochemical and optical sensors can be used to monitor cell culture conditions (such as pH, oxygen concentration, and CO2 level) and detect various biomarkers of skin differentiation, irritation, and inflammation, such as cytokines and growth factors (figure 3) [166]. Furthermore, piezoelectric biosensors can also be utilized to measure changes in the mechanical properties of the skin, which can serve as an inflammation sign [167–169].

Assessing the barrier properties of the skin is critical considering its primary role as the body's largest physical and immune barrier. Monitoring the skin barrier function via sensor integration can provide precise information on skin tissue maturation [43, 44, 64, 170] and some insights into skin irritation, and disease progression [165, 170, 171]. One of the most prevalent ways to assess the barrier integrity of the reconstructed skin tissues is to measure the transepithelial electrical resistance (TEER) of the epidermal monolayer, particularly that of the stratum corneum [44]. TEER recording is a non-invasive and label-free means for evaluating the integrity of the skin barrier. This method is based on measuring electrical impedance changes across skin epithelium associated with the formation of tight junctions between adjacent skin cells [172]. The cell monolayer impedance can be measured using a bipolar or tetrapolar TEER electrode configuration. Tetrapolar electrode configuration involves the use of two electrode pairs, one of which applies the electric current, while the other one measures the voltage drop [39]. Odijk *et al* assessed how the geometrical parameters of the microfluidic chip affect the electrical current distribution over the cell culture membrane and the resulting TEER value in bipolar Ag/AgCl electrode configuration. They emphasized the importance of applying the geometrical correction for various chip geometries since the current density was found to be uneven throughout the cell membrane, leading to overestimation of on-chip TEER measurements, particularly for long and shallow channels and low TEER levels [166]. van der Helm *et al* highlight the advantages of integrating small localized pairs of electrodes to prevent the uneven distribution of electrical potential that can occur with large electrodes [173].

Zhang *et al* proposed a multi-culture microfluidic platform with an integrated de-bubbling unit and TEER electrodes [44] (figure 4(A)). The bubble removal structures ensured the elimination of bubble

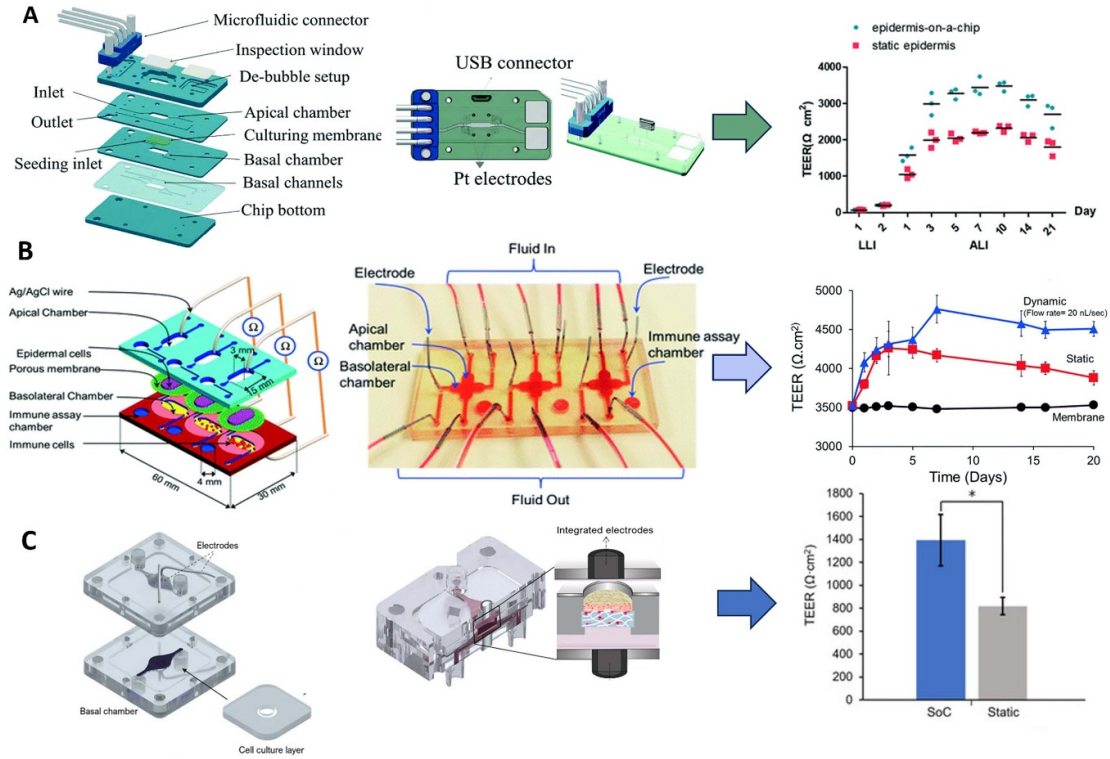


Figure 4. Integration of TEER sensors in SoC platforms for advanced skin barrier function analysis. This figure highlights the utilization of TEER measurements for non-invasive, label-free monitoring of skin barrier integrity across various SoC platforms. (A) Zhang *et al* engineered a platform for a parallel cultivation of 24 SoC microfluidic units and integrated it with four Pt electrodes for the continuous monitoring of the skin barrier function through the epidermal differentiation stages. Adapted from [44] with permission from the Royal Society of Chemistry. (B) Ramadan and Ting introduced a SoC design featuring three parallel cell culture chambers equipped with TEER measuring Ag/AgCl wires, enabling a precise evaluation of skin cell layer integrity against culture duration and exposure to skin irritants. Adapted from [43] with permission from the Royal Society of Chemistry. (C) Zoio *et al* conducted TEER measurements by exploiting concentric Ag/AgCl electrodes at day 11 of air–liquid interface culture. Adapted from [165]. CC BY 4.0. Collectively, these studies underscore the enhanced barrier properties of skin tissues cultivated within dynamic SoC environments compared to traditional static skin models, thereby emphasizing the significant impact of dynamic perfusion on epidermal maturation.

generation, while four integrated Pt TEER electrodes enabled the evaluation of the skin barrier function in a non-invasive and continuous manner. As a result of a 14 d ALI culture, skin tissues exhibited stratification and maturation, indicated by a TEER value of 3 kΩ cm^2, which prevented more than 99% of Cascade Blue-607 Da permeation due to enhanced barrier function. Additionally, the cell culture unit contained 24 sections for parallel cultivation of skin tissues, each made of two polymethyl methacrylate (PMMA) chambers separated by a PET membrane. The suggested device can be exploited for real-time monitoring of skin function in a reproducible and scalable manner.

Ramadan and Ting utilized Ag/AgCl wire electrodes to assess alterations in the epidermal barrier function due to epidermal differentiation, exposure to chemical irritants, and UV irradiation [43]. Consequently, TEER values exhibited a progressive increase throughout a one-week cultivation of the skin tissue at the air–liquid interface, resulting in a measurement of approximately 4500 Ω cm^2, as illustrated in figure 4(B). Notably, across all days of culture, the TEER values for skin tissues cultured within dynamically perfused SoC platforms were significantly elevated compared to those observed in tissues cultivated under static conditions. TEER measurements successfully demonstrated skin irritation and compromised barrier function in response to allergens (nickel and cobalt sulfate).

Similarly, Zoio *et al* developed a barrier-on-a-chip platform incorporating tetrapolar TEER electrodes to evaluate skin differentiation and toxicity response continuously (figure 4(C)) [165]. Two pairs of concentric Ag/AgCl electrodes representing current-carrying and voltage-sensing electrodes were positioned on perfusion chambers both below and above the skin tissue chamber. The 12 d ALI culture of the skin minitissues resulted in a steady increase of their TEER values, reaching a final mean value of 1050 ± 180 Ω cm^2. Conversely, upon exposure of tissues to a benchmark irritant, SDS, for 3 h, the TEER

values witnessed a linear drop, reaching a final value of 53 ± 66 Ω cm^2. In addition, a finite element analysis was carried out to assess the sensitivity of integrated electrodes and compare their performance with that of conventional chopstick electrodes. As a result, chopstick electrodes exhibited lower TEER values with significant variability (37%) versus integrated electrodes with higher TEER results of low variability (5%). These findings demonstrate that the location of chopstick electrodes affects values of resulting TEER measurements, highlighting the importance of immobilizing electrodes directly within chips in close proximity to the tissues being analyzed.

While TEER sensors are important for obtaining information on skin tissue maturation stages and identifying skin tissue irritation and drug toxicity, relying solely on them may not be sufficient for sensitive detection of skin inflammation and disease progression as well as monitoring of cell culture conditions. In addition, even in assessing the overall barrier integrity of the skin tissue, TEER holds certain limitations; namely, TEER measurement cannot accurately quantify tight junction formation if there is a small, uncovered area in the cellular monolayer [174]. To the best of our knowledge, only a limited number of studies are directed at integrating chemical, biochemical, and optical sensors into SoC platforms. However, these integrated biosensors have recently been used in other OoC platforms, examples of which are presented below to illustrate the potential advantages that these biosensors can offer in SoC platforms.

Cognetti *et al* leveraged photonic biosensors in order to quantify the cytokine release profiles of inflamed bronchial epithelial cells in real time [175]. Resonance-based photonic sensors were functionalized with antibodies for three different cytokines and embedded below the cell monolayer membrane in the bottom channel of a bilayered microfluidic chip. Challenging the bronchial epithelium by flowing LPS over the tissue monolayer resulted in a steady increase of the sensor resonance shift for 90–120 min until the cytokine concentrations leveled off. These photonic chips offer the advantage of tracking the secretion of physiologically relevant concentrations of various analytes in real time. Su *et al* offered another innovative approach for the high-throughput longitudinal cytokine profiling of barrier-on-a-chip platforms [176]. Their DigiTACK (Digital tissue-barrier-cytokine-counting-on-a-chip) platform integrated sensitive digital immunosensors in a novel beadless microwell format to perform a rapid 'digital fingerprinting' of analytes with a low limit of detection (LoD) of 100–500 fg ml^{-1}. Importantly, the device employed the reversible 'tacking and fingerprinting' strategy, which involved shifting and exposing different parts of the tissue culture to a fresh set of digital immunosensors at each time interval. This enabled the system to detect rapid real-time concentration fluctuations and establish the secretion rates as well as dynamic ranges of various cytokines. These proposed platforms can also be adapted for the real-time detection of cytokine secretion in SoC studies, providing spatially and temporally resolved information in the context of skin allergy, infection, inflammation, and irritation.

Real-time monitoring of cell culture conditions in SoC platforms would facilitate their standardization and improve the reproducibility of SoC studies. In addition, this capability would permit detailed observation of the dynamic changes in the cell culture environment (e.g. CO2, O2, medium flow rate,pH) and provide timely feedback to make necessary adjustments [177]. Zhang *et al* developed an advanced automated microfluidic platform interfacing with physical biosensors to track cell culture conditions (pH, O2, and temperature), electrochemical immunosensors for detecting soluble biomarkers, and mini microscopes for observing cellular morphologies [178]. Label-free electrochemical sensors were regeneratable, meaning that they could be reused by restoring their sensing elements after each antigen exposure. Optical sensors were selected for the dynamic monitoring of oxygen levels and cell medium pH, as they are well-suited for online monitoring. The miniature microscopes were located at the bottom of tissue chambers and provided *in situ* real-time information on tissue morphologies.

Considering the metabolic importance of the skin in maintaining body homeostasis and breaking down drugs and toxins, gaining real-time information on skin metabolism would be critical for evaluating the functionality of SoC platforms and identifying drug toxicities and side effects [179–181]. Dornhof *et al* introduced a microfluidic cell culture platform with integrated electrochemical microsensors specific to oxygen, lactate, and glucose [182]. The incorporated microsensors demonstrated long-term and precise performance in determining the metabolite consumption and production from matrix-based 3D cultures in a reversible and quantitative manner. This approach can be translated to SoC studies to provide a higher temporal resolution of skin metabolic changes in response to drug exposure and variations in culture conditions.

There are a number of factors that should be considered before deciding to embed biosensors into SoC platforms. One of the most critical factors is the cost associated with sensor manufacturing and its integration into microfluidic platforms. Although integrated biosensors within SoC platforms produce valuable real-time and *in situ* information, their usage may not always be financially justifiable, i.e. most biosensors require intricate microfabrication processes and specialized clean room facilities for the incorporation into microfluidic chips [39, 183]. The second important factor is the long-term performance and stability of biosensors. The stability of biosensors that are in contact with biological fluids (e.g. cell medium within

OoC platforms) can be mainly affected by the non-specific adsorption of proteins, or biofouling [39]. Biofouling causes electrode passivation, deteriorating sensor sensitivity and biasing final measurements. Although not reported for OoC studies so far, the biofouling effect was described for sensors interfacing with biological fluids [184]. For example, the electrochemical properties of gold electrodes were reported to decrease by almost 70% after one hour of exposure to a BSA (bovine serum albumin) solution of 2 mg ml^{-1}. In order to eliminate the biofouling effect on sensor performance, electrodes can be coated with antifouling substances, or their nanostructures can be manipulated [184]. With optical sensors, photodegradation or leaching of sensor dyes can be potential issues affecting their long-term stability. To avoid these problems, sensor dyes with higher photostability and larger sizes can be entrapped within the sensor matrix [39].

4. Application areas

4.1. SoC studies for optimization of on-chip culture parameters and conditions

In an attempt to enhance the utility of SoC platforms for dermatological research and product testing, Lee *et al* engineered and refined the design parameters of an *in situ* SoC model to achieve the long-term culture and differentiation of the skin tissue [77]. A gravity-driven rocking platform facilitated the chip's pumpless operation throughout a 3-week period, effectively reducing risks of bacterial contamination and the formation of air bubbles. Furthermore, parameters such as media volume, perfusion rate, and matrix thickness, were optimized to maintain adequate oxygen and glucose transport rates throughout the skin construct. The matrix thickness was selected as 2 mm to promote optimal fibroblast and keratinocyte viability. The study employed FITC-dextran with time-lapse imaging and COMSOL simulations to establish the nutrient transport models of glucose and oxygen within the SoC model. The modeling revealed a critical role of the optimal flow rate on maintaining adequate glucose concentrations throughout the collagen gel. The medium flow rate of 10 μl min^{-1} enhanced the fibroblast viability when compared to culture conditions without flow. In addition, the skin tissue demonstrated the epidermal stratification and the expression of all key marker proteins of skin differentiation, including Keratin-14, Keratin-10, Involucrin, and Loricrin. Nevertheless, the epidermal skin layer on chip was rough compared to the smooth epidermal layer in a static transwell platform. This finding could be explained by an uneven distribution of nutrients to all skin cells under the dynamic culture conditions, implying a need for further optimization of the microfluidic platform.

In a later study, the same platform was exploited to assess the role of the dynamic on chip culture on collagen hydrogel contraction, skin tissue differentiation and protein expression [78]. The dynamic microfluidic culture mitigated the contraction of collagen-based skin tissue constructs when compared to those grown inside traditional transwell and under static on chip conditions. Histological analyses showed that skin stratification and differentiation were more homogeneous and comparable to the transwell in the dynamic chip condition, highlighting the importance of fluid flow for nutrient delivery and tissue development. Protein expression studies indicated that while the expression of differentiation markers like keratin 10, fibronectin, and collagen IV was slightly slower in the dynamic chip condition, it eventually reached levels comparable to those in the transwell setup, suggesting a delay rather than inhibition of skin differentiation in dynamic conditions.

4.2. SoC platforms for cosmetics testing

In a study by Kim *et al*, a microfluidic platform reconstituting dermal and epidermal layers of the skin was used to demonstrate the restorative effect of Curcuma longa leaf extract (CLLA) treatment. CLLE treatment resulted in a thicker epidermis with an enhanced moisture-retaining and barrier function, demonstrating its anti-aging potential for human skin tissue [185]. Besides, the antiaging properties of coenzyme Q10 were also investigated [83]. Histological analysis of the skin tissue treated with the drug revealed an increase in epidermis thickness and fibroblast cell density. Furthermore, coenzyme Q10 treatment induced the expression of proteins associated with epidermis differentiation and enhanced barrier function. Kim *et al* exploited the pumpless SoC model to demonstrate the anti-aging effects of another substance, α-lipoic acid (ALA), known to be a potent antioxidant [186]. The ALA-treated skin constructs developed a thicker epidermal layer with enhanced expression rates of structural proteins, such as filaggrin, involucrin, and keratin 10, compared to the control groups.

4.3. SoC platforms for drug toxicity, permeability, and absorption studies

Abaci *et al* leveraged a pumpless microfluidic platform for the long-term culture of human skin equivalents and demonstrated its utility for assessing the skin toxicity of doxorubicin (an anti-cancer drug) [45]. The engineered chip was designed in a two-layered configuration with a porous polycarbonate membrane in between. The upper compartment contained a circular housing for skin equivalent placement, whereas the lower one was made up of two media reservoirs connected via five microchannels. The functionality of the proposed platform for drug toxicity studies was supported by the skin's response to doxorubicin treatment, as revealed by immunostaining. Doxorubicin application induced detachment of the epidermal basal layer and stalled the

proliferation of keratinocytes, confirming clinically reported side effects of the chemotherapy treatment on the skin.

A similar gravity-driven pumpless SoC platform was utilized by Jeon *et al* to reproduce clinically-reported skin side effects of the sorafenib anti-cancer drug [187]. Sorafenib destroys cancer cells by preventing the growth of new blood vessels in a tumor. However, it is also known to trigger severe skin side effects, mainly hyperkeratosis or excessive stratification of the skin epidermis. Applying three different doses of sorafenib on an *in vitro* skin model resulted in thickening of the epidermal spinous and basal layers, similar to histological images of the patients with hyperkeratosis. Further analysis through immunohistochemical staining further validated these findings by demonstrating increased expression levels of proteins, involucrin and K-10, associated with hyperkeratosis.

Mah *et al* fabricated a miniaturized flow-through system to reduce the consumption of the candidate drug endoxifen and thus the costs associated with its pre-formulation studies [49]. The platform was made of PDMS-based, concentrically located donor and receptor compartments. The performance of the miniaturized cell for transdermal drug delivery was compared to that of the conventional Franz cell by mounting a rat skin sample between the donor and receptor chambers and applying two model drugs on its surface. As a result, the miniaturized chamber demonstrated drug permeation profiles resembling those obtained from Franz diffusion cells. Finally, the developed platform was used to enhance the permeation of the breast cancer drug, endoxifen, by testing several drug enhancers for achieving its therapeutic plasma concentration.

Lukács *et al* presented a SoC device for conducting drug permeability assays [188]. The microfluidic diffusion chamber was tested for absorbing caffeine, and its performance was compared to that of traditional devices, such as Franz cell and microdialysis device. The design of the chamber resembled that of a Franz diffusion cell. The top donor compartment contacted the test drug, the middle layer contained the skin tissue, and the bottom receptor layer had a channel for continuous collection of the diffused drug sample. As a result, the microfluidic device was suitable for drug penetration analysis, as its absorption rates of caffeine were similar to those obtained through traditional techniques. Yet, there was a 50% difference in drug permeability values obtained from the microfluidic device compared to traditional methods. Different analytic techniques and potential non-specific interactions between the caffeine and PDMS acceptor layer could explain this inconsistency. The same microfluidic device and a similar experimental setup were utilized to demonstrate the absorptive function of permeability glycoprotein (P-gp) transporter in transdermal drug delivery by Bajza *et al* [189]. While inhibition of this efflux protein significantly reduced absorption of tested drugs through excised rat skin samples *in vitro, in vivo* experiments did not show such a pronounced effect. Furthermore, frozen tissue samples were shown to deteriorate P-gp function and increase tissue permeability in a transporter-independent manner. Thus, the authors do not recommend the use of frozen tissues for testing drug permeability, as they do not accurately represent an *in vivo* skin response.

4.4. Body-on-a-chip systems integrating the skin compartment for drug toxicity and efficacy testing

Besides the skin, toxic side effects of medicines can affect other human organs, mainly the liver and the heart. So, in an attempt to enhance the capacity of SoC platforms to predict the systemic toxicity of drugs, some research groups have developed multi-organ micro physiological systems, integrating liver and heart compartments. Apart from improving the physiological relevance of the *in vitro* model in terms of organ complexity, these body-on-a-chip platforms enable crosstalk between miniaturized organ compartments by fluid flow through microfluidic channels, making them particularly promising for drug toxicity assessment [190]. Table 1 summarizes the main features of reported body-on-a-chip systems that integrated the skin compartment for drug-related studies.

Tavares *et al* demonstrated a two-organs-on-a-chip platform consisting of liver and skin compartments that enables a more sensitive assessment of topical and systemic drug toxicities when compared to a static skin model (figure 5(A)) [46]. Integrating the hepatic compartment and the dynamic environment of the microfluidic device recapitulated both skin irritancy and hepatoxicity events upon drug administration. Topical terbinafine administration enhanced the expression of metabolic liver enzymes CYP1A2 and 3A4, indicating the skin penetration of the drug and activation of the liver response. On the other hand, the systemic application of 0.1% terbinafine downregulated liver albumin production and increased metabolic enzyme CYP2C9 activity, serving as a hepatotoxicity model.

Wagner *et al* reported the development of a similar body-on-a-chip platform with crosstalk between a human skin biopsy and liver equivalent [47]. The device consisted of two separate microfluidic circuits, each holding two tissue culture compartments. While the skin chamber was loaded with a human skin biopsy, the hepatic one included 20 liver spheroids. The developed system supported two modes of tissue culture—with the tissues either immersed in the media flow or separated from it by the transwell insert membranes. Under submerged conditions, the co-culture of two tissues established their metabolic equilibrium and steady-state exchange, where albumin produced by the liver was either degraded or

Table 1. Multi-organ body-on-chips systems integrating the skin compartment for drug-related studies.

Included organ compartments	Application area	Representation of the skin compartment	Representation of other organs	Device material	Flow establishment	Ref.
Lung cancer + skin	mAb therapy efficacy and toxicity studies	Commercially available Millicell®, a human full-thickness skin equivalent formed by a dermal layer possessing matrix-grown human fibroblasts and an epidermal layer of human keratinocytes fabricated in standard 24-well inserts	Lung cancer: 15 lung tumor spheroids made of H292 cells	PDMS	Pulsatile flow with a vacuum, on-chip micropump	Hübner *et al* [191]
Liver + skin	Drug toxicity studies	Primary fibroblast-dispersed collagen matrix seeded with primary keratinocytes in the 24-well sized chip	Liver: liver spheroids made of HepaRGs and HSC	PDMS	Pulsatile flow with a vacuum, on-chip micropump	Tavares *et al* [46]
Liver + skin	Drug toxicity studies	Skin biopsies obtained from human juvenile prepuce. A 4-mm skin biopsy was placed in the culture compartment of a multi-organ-chip device, being cultured at an air–liquid interface	Liver: 20 liver microtissues made of HepaRGs and HSC	PDMS	Perfusion with a peristaltic, on-chip micropump	Wagner *et al* [47]
Liver + heart + skin	Drug toxicity sudies	Strat-M membrane that acts as a barrier to mimic skin tissue	Liver: human primary hepatocytes Heart: iPSC- derived cardiomyocytes	PMMA and PDMS	Gravity-driven medium flow with a rocker platform	de Mello *et al* [132]
Liver + intestine + kidney + skin	Drug absorption, distribution, metabolism and excretion studies	Skin biopsies obtained from human juvenile prepuce cultured at an air–liquid interface	Liver: liver spheroids made of HepaRGs and HSC Kidney: human proximal tubule cell line RPTEC/TERT-1 Intestine: human small intestinal epithelial cells on culture inserts	PDMS	Pulsatile flow with a peristaltic on-chip micropump	Maschmeyer *et al* [75]
Heart + liver + bone + skin	Drug PK/PD analysis, cardiotoxicity	Primary fibroblast-dispersed collagen matrix seeded with primary keratinocyte cultured at an air–liquid interface to form and mature stratified epidermal tissues	Liver: hiPSC-derived hepatocytes and fibroblasts within the fibrin matrix Heart: hiPSC-derived cardiomyocytes and fibroblasts within the fibrin matrix Bone: MSC- derived osteoblasts and monocyte-derived osteoclasts seeded on bone matrix	Polysulfone	Perfusion with a peristaltic pump	Ronaldson-Bouchard *et al* [192]

Abbreviations: mAb, monoclonal antibody; FTH, full-thickness human; HepaRGs, hepatoma cell line; human stellate cells (HSC); PMMA, poly(methyl methacrylate); PD, pharmacodynamics; PK, pharmacokinetics.

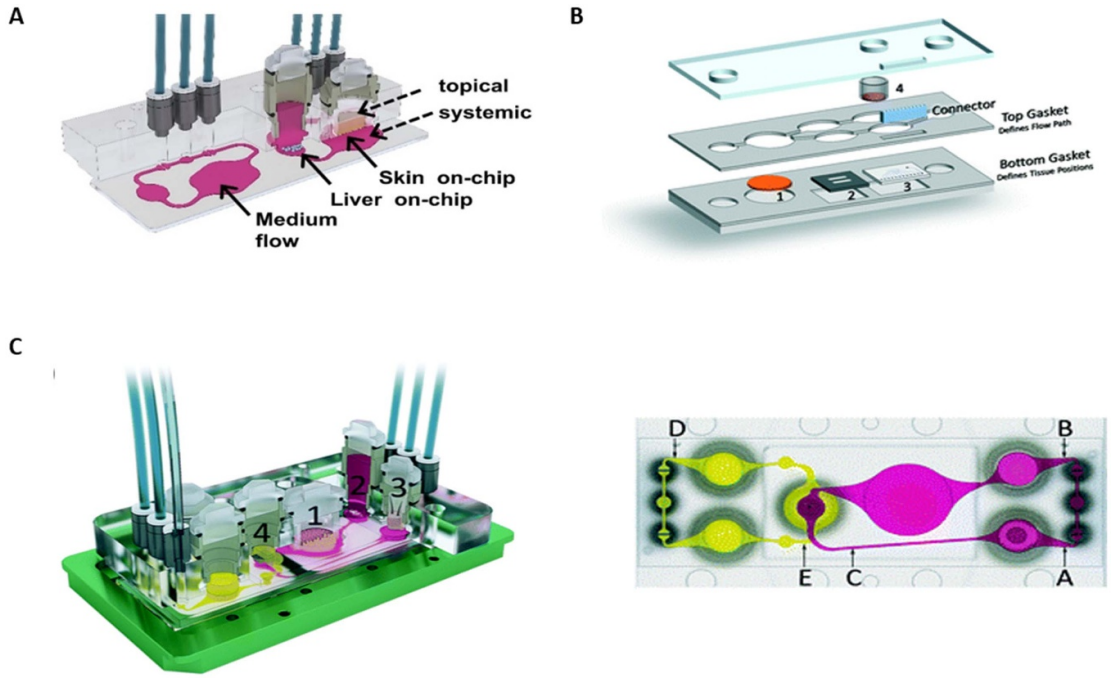


Figure 5. Multiorgan microphysiological systems have been developed to enhance the predictive capacity of SoC platforms for drug toxicity studies. (A) Tavares *et al* connected liver spheroids (cyan, left) with the skin tissue (brown, right) with a microfluidic tube system filled with medium (magenta). Adapted from [46], Copyright (2020), with permission from Elsevier. (B) Mello *et al* integrated the heart compartment into the body-on-a-chip system with a skin module. The platform contained (1) a liver module on a coverslip, (2) cardiomyocytes on bioMEMS cantilevers, (3) cardiomyocytes on a microelectrode array, and (4) a skin module. Adapted from [132] with permission from the Royal Society of Chemistry. (C) Maschmeyer *et al* developed the organ-on-a-chip platform, made of compartments for the (1) intestine, (2) liver, (3) skin and (4) kidney tissues, accommodating a surrogate blood flow circuit (pink) and an excretory flow circuit (yellow). Adapted from [75]. CC BY 3.0.

stored by the skin. In addition, the system showed a dose-dependent response to toxic drug administration, supported by the upregulation of liver enzyme expression.

de Mello *et al* introduced an additional level of complexity to the body-on-a-chip system by integrating the heart compartment [132]. They demonstrated the platform's utility in accurately predicting cardiac and hepatic responses upon systemic or topical administration of four commercially available drugs. The multi-organ microfluidic device containing two PDMS gaskets was perfused by a rocking platform. In contrast to all described studies, the Strat-M, a synthetic and porous membrane without any seeded skin cells, mimicked the skin tissue in this design (figure 5(B)). One innovative feature of the system was the real-time assessment of the electrical activity and force of the cardiac tissue. As a result, the multi-organ system demonstrated differential toxicity responses of various organs depending on whether the drug administration was topical or systemic.

Maschmeyer *et al* established a four-OoC platform composed of skin, liver, intestine, and kidney compartments, which was designed for ADME (absorption, distribution, metabolism and excretion) profiling of drug candidates [75]. The microfluidic device was divided into two circuits: the surrogate blood circuit containing intestinal, liver spheroid, skin biopsy culture compartments, and the excretory fluid circuit (figure 5(C)). While the former circuit mimicked the absorption and primary metabolism of the drug substance, the latter supported the drainage of secondary metabolites. Both circuits overlapped in the filtering kidney compartment, composed of a proximal tubule epithelial cell monolayer covering a polymeric membrane. A 28 d co-culture of four different organs yielded a system with reproducible homeostasis, as demonstrated by metabolic analysis and a steady glucose gradient within a physiological range of 0.65 g l^{-1} and 1.1 g l^{-1} in three different media pools. In addition, all tissues exhibited a high level of viability and maturation during the co-culture period. While the intestinal epithelium established vili-like structures with an apical brush border, skin biopsies formed a multilayered epidermis and the kidney compartment preserved a polarized functional barrier.

In a recent study, Rogerson-Bouchard *et al* reported a body-on-a-chip system, in which heart, liver, bone, and skin tissues resided in separate niches to preserve their tissue-specific phenotypes while communicating in a physiological way through the recirculating vascular flow [192]. Tissue chambers in the chip were designed in a modular fashion enabling flexibility in terms of types, order and number of different tissues to be integrated in the required experimental design. The tissues were produced from iPSCs

of a single donor and matured individually before being transferred to the chip, where they were cultured for additional 4 weeks. A selective vascular barrier-segregated tissue modules from the recirculating flow, which contained exosomes, cytokines and immune cells (CD14+ monocytes). As a result, the multi-organ chip sustained stable structural and functional phenotypes of tissue for the co-culture period: heart muscle maintained the beating rate; liver tissue-maintained albumin secretion; bone tissue exhibited mature osteolytic activity; and skin tissue preserved its thickness. The interlinked tissues faithfully recapitulated the systemic toxicity of the doxorubicin and enabled the identification of miR-1 and miR-1273a, early biomarkers of cardiotoxicity and cardiomyopathy, respectively. The described multi-organ chip presents a promising platform for the establishment of patient-specific models of systemic diseases and identification of early biomarkers of drug toxicity.

4.5. SoC platforms for irritancy,inflammation and immune response testing

Wufuer *et al* established the skin inflammation model by applying tumor necrosis factor (TNF-α) to a three-layered SoC model, containing epidermal, dermal and endothelial chambers [42]. The stratified skin-mimicking model responded to TNF-α (tumor necrosis factor) treatment by upregulating the production of proinflammatory cytokines and exhibiting an elevated endothelial permeability. In addition, the SoC successfully recreated an important skin inflammation hallmark, edema, which was induced by increased fluid transportation from the endothelial layer of the model to the dermal one due to a compromised endothelial barrier. The engineered model responded to an anti-inflammatory drug treatment, as its application reduced the expression of proinflammatory markers and attenuated swelling of the skin and restored its barrier function. The same platform was utilized by Jeon *et al* to evaluate the skin irritation response based on two parameters: the viability of keratinocytes and the expression of tight junctions [52]. The quantification of the endothelial tight junction expression as a sign of edema served to enhance the performance of the SoC in predicting the skin irritation in response to the exposure to 20 test substances. As a result, the dual-parameter model of skin irritation was 30% more specific and 10% more accurate in distinguishing between irritant and nonirritant substances compared to the conventional reconstructed human epidermis model.

Jusoh *et al* proposed a vascularized SoC platform to evaluate the angiogenesis level as a biomarker of skin inflammation [51]. The microfluidic device consisted of two side-by-side channels to promote paracrine interactions between human dermal fibroblasts and dermal keratinocytes (figure 6(B)). HUVEC cells were seeded at the boundary of the dermal channel and established angiogenic sprouts in the direction of the keratinocyte channel. The interrogation of the platform with chemical irritants, being ingredients of common cosmetics, revealed an increase in angiogenic sprouting and a decrease in keratinocyte viability in a dose-dependent manner. Interestingly, the platform displayed enhanced angiogenesis in the absence of keratinocytes death after exposure to a chemical classified as a non-irritant. This result indicated a higher sensitivity of the proposed model for the detection of skin irritation, highlighting its potential as a more predictive alternative to animal testing.

Ramadan and Ting integrated pro-monocytic immune cells into the perfusable *in vitro* skin model to enhance its prediction capability of skin irritation reaction [43]. Challenging the microfluidic organotypic skin model with chemical (Lipopolysaccharides) and physical (UV irradiation) stimuli revealed deterioration of the skin barrier function, which was caused by the disruption of tight junction proteins. Furthermore, the co-culture of keratinocytes with immune cells resulted in an upregulation of inflammatory mediators compared to a keratinocyte monolayer alone.

Ren *et al* developed a 3D, multicellular SoC platform to establish multiple cytokine gradients, simulating skin cutaneous inflammation, for the quantitative assessment of T cell chemotaxis [53]. The device configuration contained a central collagen gel compartment, replicating the skin dermal ECM, with HaCaT and HUVEC cell layers attached from both sides, replicating the skin epithelium and endothelium, respectively (figure 6(A)). In addition, the central channel was separated from side channels by micropillars, which facilitated the establishment of multiple chemical gradients and provided adequate space for T cell migration and tracking at the same time. As a result, the CCL20 chemokine gradient, a hallmark of cutaneous skin inflammation, induced an enhanced T cell transmigration from the endothelium towards the epithelium. The recruitment of T cells to the inflammatory site was blocked by a CCL20 locked dimer (CCL20LD), suggesting its usage as a novel drug to treat chronic inflammatory skin diseases. This study demonstrated the feasibility of using SoC platforms for the investigation of T cell migration in response to complex chemical gradients of skin inflammation and screening of potential immunotherapeutic drug candidates.

Kwak *et al* produced a more physiologically complex *in vitro* model of skin inflammation by integrating leukocytes into the vascularized SoC platform [82]. The treatment of skin constructs with an anticancer drug, doxorubicin, known for its skin cytotoxicity, in the presence of vascular endothelium, caused less severe skin tissue necrosis when compared to the avascular SoC model. The formation of an integral endothelium barrier, preventing drug

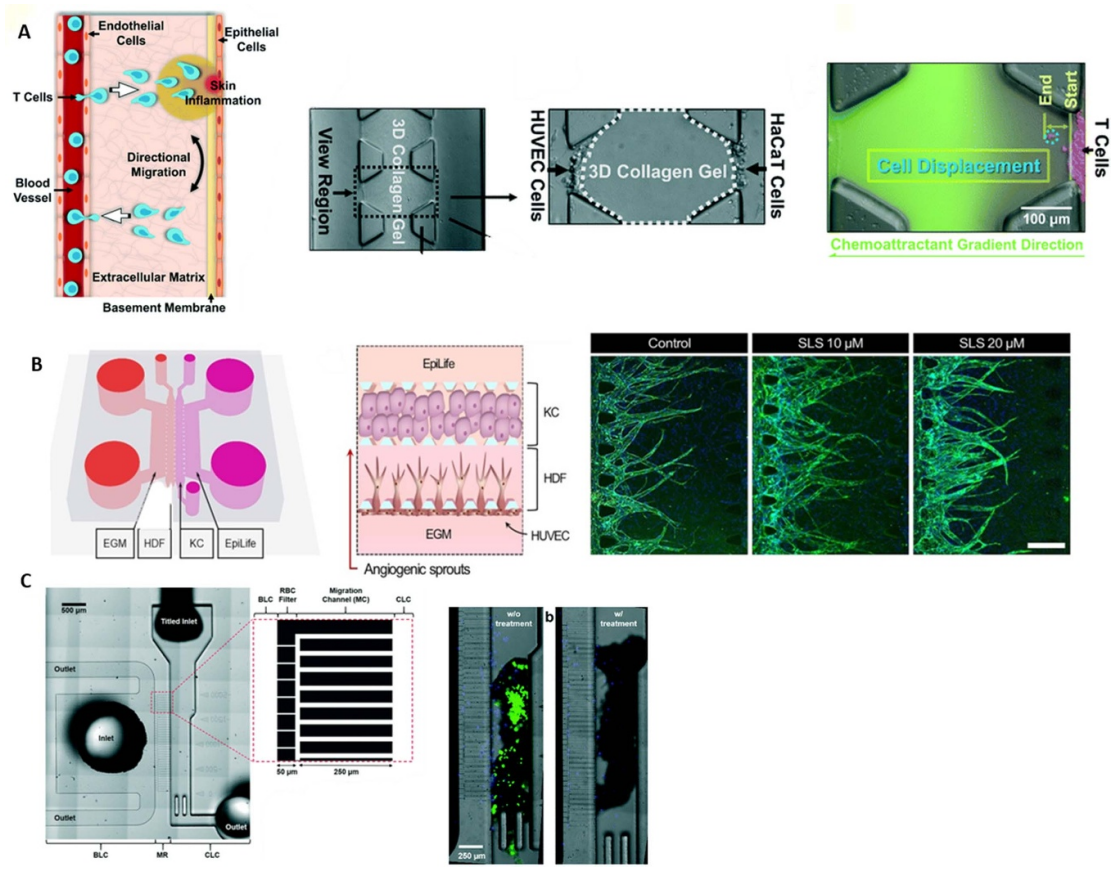


Figure 6. Advanced SoC platforms for simulating skin irritancy, inflammation and immune responses. The figure showcases three innovative SoC models designed to mimic the intricate events associated with skin inflammation. (A) Ren *et al* utilized microfluidics to establish cytokine gradients for the quantitative assessment of T cell transmigration from the endothelial region of the skin to the inflamed epithelium. Adapted from [53] with permission from the Royal Society of Chemistry. (B) Jusoh *et al* proposed a vascularized SoC to evaluate skin irritation by using angiogenesis as an inflammation biomarker. The microfluidic platform contains two layers separated by microposts to fix the hydrogel and enable angiogenic sprouting towards the epidermis (KC) from the dermal matrix (HDF). The immunostaining image shows how the application of SLS (sodium lauryl sulfate), a common skin irritant, enhanced the angiogenic sprouting in a dose-dependent manner. Adapted from [51]. CC BY 4.0. (C) The platform enabling the quantification of neutrophil migration was leveraged by Kim *et al* to study skin bacterial infection. The microfluidic device contained a blood loading channel (BLC), a skin loading channel (CLC), and a migration region (MC). The migration region served to filter all other blood cells enabling the selective migration of neutrophils towards the infected skin. The magnification shows how the antibiotic treatment (on the right) reduced the neutrophil (in green) migration towards the skin tissue, marking the reduction in skin inflammation. Adapted from [54] with permission from the Royal Society of Chemistry.

passage, could explain the mitigated cytotoxicity of the drug to the skin tissue. In the skin irritation model, SDS (Sodium dodecyl sulfate) application resulted in elevated pro-inflammatory IL-6 levels in the presence of endothelium. Together, these findings highlighted the significance of vasculature in producing a physiologically relevant reaction in the context of skin irritation. Moreover, the SoC model irradiation with UV light resulted in the recruitment and subsequent transmigration of leukocytes across the endothelium to the dermal compartment, demonstrating that the developed platform can recapitulate the primitive events involved in the inflammatory cascade of human skin.

In an attempt to assess the bacterial infection of the skin and its response to antibiotic treatment, Kim *et al* engineered a microfluidic platform enabling the quantification of neutrophil migration [54]. The microfluidic device contained three distinct compartments, a loading channel to place skin micro-biopsies, a blood channel for patient blood loading, and a migration region filtering red blood cells and facilitating neutrophil migration (figure 6(C)). The infection of the skin columns with bacteria-induced neutrophil migration was proportional to bacterial concentration, while the subsequent administration of antibiotics attenuated neutrophil migration. This study suggested an SoC platform for the quantification of neutrophil migration as a potential biomarker of skin infection and an indicator of antibiotics' efficiency in a patient-specific manner.

4.6. SoC platforms for simulating wound & wound healing

Li *et al* developed a microfluidic model to explore the impact of bacterial cellulose nanostructure on skin

wound repair response [55]. The PDMS chip made of two parallel channels was used to co-culture skin epithelial cells with fibroblasts and apply bacterial cellulose wound dressing on their top. The evaluation of collective migration rates of two cell types towards the BC patch revealed an enhanced wound healing response in the presence of a more hydrophilic and porous BC film. The anti-inflammatory and therapeutic properties of the bottom BC film were confirmed by *in vivo* studies, where the rat skin wound covered with the bottom side of the BC film recovered at a faster rate compared to the gauze or the top side of the BC film.

Peng *et al* proposed a novel chip design for *in vivo* skin nerve regeneration [193]. Instead of a classical microfluidic platform, this chip was a bioactive patch capable of recruiting mesenchymal stem cells (MSCs) to the wound site and promoting their neuronal differentiation and maturation through a stepwise delivery of relevant chemokines and growth factors. This therapeutic patch was fabricated by 3D coaxial printing in a core-shell configuration. While the outer shell was made of a conductive composite hydrogel, the inner core contained gene-delivery vehicles for stimulating growth factor expression and release by neuronal cells. The gradual diffusion of chemokines caused chemotaxis of MSCs toward the conductive shell layer of the chip. Shell degradation by cellular proteases brought about the cleavage of plasmid vectors from the inner layer and transfection of neurons. The synergetic effect of electrical conduction through the scaffold and expression of growth factors enhanced regeneration of the skin in rat wound models.

4.7. SoC platforms for modeling aged and wrinkled skin through mechanical stress application

OoC systems offer a versatile platform for subjecting various tissues to biomechanical cues that guide cellular activities, thereby facilitating the accurate emulation of critical tissue functions [38]. Beyond the shear stress and interstitial fluid flow discussed in previous sections, SoC platforms enable the application of both tensile and compressive forces, eliciting distinct responses from skin keratinocytes and fibroblasts. This section provides an overview of SoC studies directed at understanding the effect of mechanical stretching on skin tissue responses.

Lim *et al* developed the SoC model of the aged and wrinkled skin by exposing 3D skin equivalents to a uniaxial strain of 10% by using magnetic stretching [56]. While the top compartment of the microfluidic platform comprised the cell culture chamber, flanked by two medium storage chambers, the lower one contained a channel for continuous medium perfusion. The sidewall of the cell culture chamber incorporated a permanent magnet, which stretched the skin equivalent by interacting with an external electromagnet. The application of the tensile force at 0.01 Hz resulted in the formation of grooves resembling wrinkles in the aged skin (figure 7(B)). Furthermore, the stretching at this frequency reduced the skin cell proliferation and production of skin ECM proteins, such as collagen, keratin, and fibronectin.

Instead of stretching the skin equivalents, Jeong *et al* applied a periodic compressive stimulus to skin tissues and induced their accelerated aging. The pumpless microfluidic device was perfused in a gravity-assisted manner for 28 d [58]. All chips were placed in a mechanical stimulus actuating system that applied 5% compressive strain at a frequency of 0.01 Hz. Reduced epidermal layer thickness, contraction rate, and collagen IV production indicated the accelerated aging of skin models under cyclic compressive stress. Elevated levels of aging biomarkers, such as β-galactosidase and reactive oxygen species, further corroborated the aging process in the compressed skin microtissues. These studies can shorten the development process of anti-aging drugs and provide a more comprehensive insight into the aging mechanisms of the human skin.

Mori *et al* developed a dynamic culture system capable of simultaneously perfusing and stretching the skin constructs containing engineered microvasculature [57]. However, in contrast to results obtained by Lim and Jeong, applying the uniaxial stretching at a 10% strain and a frequency of 0.05 Hz brought about an almost two-fold increase in the skin epidermis thickness and the dermal collagen density compared to non-perfused, non-stretchable conditions (figure 7(A)). The potential discrepancy between these studies could be attributed to differences in mechanical stimulus application methods, which elicited distinct cellular responses. The literature also presents conflicting views on the impact of mechanical stimulation on keratinocyte behavior. Keratinocytes, when subjected to mechanical stretching, have been reported to overexpress pro-inflammatory markers and cell death proteins [194, 195]. Conversely, other studies highlighted the beneficial effects of mechanical stretching on keratinocyte proliferation and skin regeneration, attributing these outcomes to the induction of calcium influx and the activation of EGFR and ERK1/2 signaling cascades and epithelial–mesenchymal transition [196]. When it comes to fibroblasts, mechanical stretching was also shown to modulate their behavior, as evidenced by increased fibroblast proliferation, enhanced synthesis of ECM proteins (elastin, collagen I and III), and secretion of growth factors (TGF-β, CTGF) [196]. Together, these findings demonstrate how fine-tuning mechanical forces within SoC platforms promote specific cellular behaviors by offering valuable insights into skin regeneration, aging, and the development of targeted anti-aging therapies.

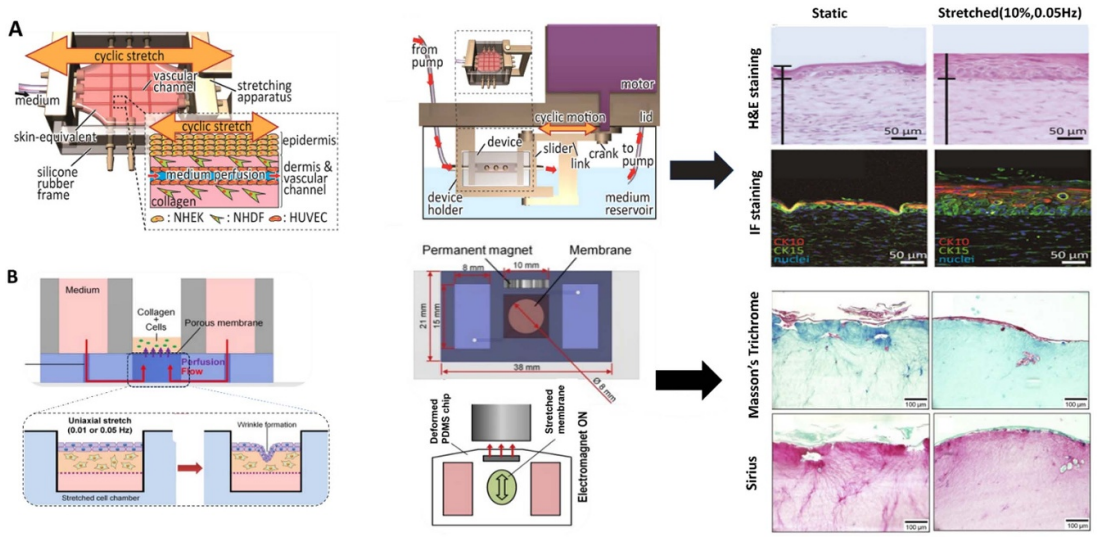


Figure 7. SoC platforms for mechanical stretching of skin tissues. (A) Mori *et al* reported a system for simultaneous stretching and dynamic perfusion of skin equivalents, integrating perfusable vascular channels. Their findings revealed that cyclic mechanical stretching leads to a markedly thicker epidermis with enhanced differentiation, as evidenced by H&E and immunofluorescent staining for CK15 and CK10 markers. Adapted from [57]. © IOP Publishing Ltd. All rights reserved. (B) Lim *et al* developed a microfluidic platform for the uniaxial stretching of the skin equivalents via magnetic forces. They observed the emergence of wrinkles, reduced epidermal thickness, and diminished collagen deposition under stretched conditions, as visualized through Masson's Trichrome and Sirius staining techniques. Adapted from [56], Copyright (2018), with permission from Elsevier.

5. Patent analysis

A review conducted via Google Patents, employing keywords such as 'SoC, skin model, microfluidic skin model, skin equivalents,' revealed that most reconstructed skin equivalent and SoC-related patents are categorized under the classifications of C12N5/0698—Skin Equivalents and C12M23/16-Microfluidic devices; Capillary tubes. Thirty patents under these classifications were systematically reviewed in this section. Among the earliest filings,the patent by Graham and Sabelman presented one of the first engineered skin models [197]. The described human skin model, developed for demonstrating or practicing intradermal injections, contains three layers simulating the human hypodermis, dermis and epidermis. The dermis-simulating layer is made of silicone gel reinforced with nylon fibers to enhance its structure. The epidermis is made of a highly elastic and strong elastomer allowing distension upon fluid injection.

Other reviewed patents highlight the unique contributions and innovations with an emphasis on improving the physiological relevance and enhancing the structural complexity of engineered skin models. For example, Comer *et al* reported the methodology to increase the barrier function of skin equivalents by adding linoleic acid or maintaining the culture at approximately 75% humidity, conditions closely mimicking the natural environment of skin cells [198]. Furthermore, a patent filed by LOreal SA outlined a novel method for producing pigmented dermo-epidermal skin equivalents containing keratinocytes and melanocytes exclusively from hair and matrix cells [199]. The patent emphasis was on creating a more physiologically relevant skin model that can be used for grafting on human patients, particularly for those suffering from third-degree burns. Another patent filed by Retting *et al* focused on a method for creating a full-thickness skin equivalent containing primary keratinocytes and melanocytes through extrusion-based bioprinting [140]. The bioprinted tissues exhibited high survival rates and metabolic activity even after extended periods of cultivation (21 d), indicating their potential for long-term studies and applications.

Some of the reviewed patents focused specifically on developing or enhancing the microfluidic system for SoC applications. Among the earliest ones, the patent filed by Hwan *et al* highlighted the pumpless microfluidic system mimicking the multilayered structure of the skin [200]. The innovative pump-free design of the SoC eliminates the need for external mechanical pumps for perfusing the cell culture medium, reducing the complexity and cost of the skin chip. Another patent by Sung *et al* reported the SoC system with an integrated magnet for the periodic stretching of the skin [201]. The periodic stretching was shown to improve the maturation of skin constructs by supporting the attachment and infiltration of keratinocytes into the dermal collagen layer. Varone *et al* introduced an open-top microfluidic platform for facilitating the monitoring and sampling of the skin tissues [202]. The patent further described the innovative approach for producing patterned gels mimicking the papillae structure of the dermis via a patterned shaping device. The patent by Levner *et al* incorporated structural anchors on the

vertical walls of the open-top microfluidic platform to prevent the gel delamination caused by shrinkage-induced stress [203]. This feature ensured the stability of 3D gel matrices within the device, addressing a common challenge in SoC studies. The patent by Ingber *et al* reported a novel approach to produce lumens within 3D matrices mimicking the vasculature or tissue ducts inside OoC platforms. The 'viscous fingering' method described in the patent relied on the creation of the circular lumens inside 3D hydrogels by passing a low viscosity fluid through the high viscosity matrix [204]. The table 2 provides further details on other SoC patents highlighting their main innovative features (table 2).

In order to provide a systematic analysis of the reviewed patents, they were classified into various categories depending on factors such as the filing date intervals, countries of origin, and specified application categories (figures 8(A)–(C)). As illustrated in figure 8(A), the number of filed patents focusing on skin models demonstrated a consistent rise over the indicated period. This trend emphasizes the growing importance of engineered skin models in modeling and simulating human skin. Distinct from broader OoC technologies, SoC innovations emphasize the criticality of the permeation process, reflective of the skin's primary function as the body's largest barrier. Therefore, as shown in figure 8(C), the number of patents designed for testing drug toxicity and permeation dominates the distribution. The second leading category is cosmetics testing, which is based on the application of the same principle for cosmetic ingredients and anti-aging substances to be tested on the skin. Furthermore, bar charts in figures 8(D)–(F) show the distribution of the culture medium, cell lines, and ECM equivalents used, respectively. Dulbecco's Modified Eagle Medium is the most used cell medium for culturing skin cells, and thus in SoC applications. Fibroblasts are the most common cell line in the connective tissue, therefore they are widely utilized in *in vitro* skin models. Lastly, collagen is the most abundant component of the skin ECM, and therefore the most prevalent material in engineered skin equivalents. Figure 9 clearly states the current focus of the claimed SoC design properties represented with the word cloud that is generated from the collective keywords of the patents. This systematic review and visualized data offer a broad outlook of the current trend of the filed patents in the SoC domain.

6. Market analysis

The market for *in vitro* skin models is experiencing a remarkable growth trajectory, reflecting a transition towards more ethical, precise, and cost-effective alternatives to conventional animal models in drug development and testing. This rise is fueled by a number of factors. The inherent limitations of animal models, notably their lack of standardization and significant interspecies variations, contribute to a 90% discrepancy rate between animal test results and human clinical outcomes [210]. This substantial inconsistency significantly impacts the drug development process, with approximately nine out of ten drug candidates failing to progress past the clinical trial phases to market approval [211]. This, in turn, leads to huge financial losses, estimated at $ 300 billion in the US by 2020 alone [210]. In addition, the increasing prevalence of skin disorders, including psoriasis, acne, AD, as well as the growing patient population, demands the development of more predictive disease models and efficient drug formulations [212]. Furthermore, since March 2013, the use of animals for cosmetic testing has been explicitly banned within the European Union, as per Directive 2003/15/EC of the European Parliament and of the European Council, reflecting a growing legislative and societal push towards human-related research methodologies [213].

The high attrition rates of pharmaceuticals and the ethical concerns associated with the exploitation of animal clinical models have fueled the market expansion of *in vitro* skin models, including reconstructed skin tissue models and SoC platfoms. The global market size of tissue engineered skin substitute, which was $ 326 million in 2022, is expected to reach $ 841 million by 2030, growing by more than 13% Compound Annual Growth Rate (CAGR) over the indicated decade [214]. On the basis of applications, the market of reconstructed skin models is dominated by skin irritation, corrosion and sensitization tests. Based on the end-user analysis, the cosmetical companies segment dominated the market in 2022, while the segment of chemical companies is demonstrating a consistent rise.

Similarly, the significant growth forecast for the global OoC market, from $ 123 million in 2024 to $ 631 million by 2029, with a CAGR of 38.6%, highlights the burgeoning interest and investment in this innovative technology [215]. The escalation in research and development initiatives, marked by rising research funding and a growing number of strategic collaborations between pharmaceutical companies, OoC manufacturers, and regulatory authorities highlight the commitment of the industry to advance this technology. For example, in 2023, the FDA established a collaboration with CN Bio Innovations Ltd, a UK-based company, to improve the estimation of drug bioavailability through the use of multi-organ OoC technology. The FDA Modernization Act 2.0 further encourages the adoption of OoC models in lieu of animal preclinical models, potentially increasing the demand for and investment in this advanced technology [33]. The largest application segments for OoC platforms are the domains of drug discovery and toxicology

Table 2. The comparative overview of SoC patents highlighting their technological focus and innovative features. This table provides a detailed comparison of SoC patents, showcasing their technological focus, structural features, and innovative aspects.

Patent number	Inventor/Asignee	Publication year	Country	Technological Focus	Structural Features	Advantages and Novelty
KR20180040407A	Jong Hwan *et al* [200]/Hongik University Industry-Academic Cooperation Foundation	2018	South Korea	Creating a pumpless microfluidic SoC that simulates human skin tissue with a vasculature	Upper and lower chambers separated with a porous membrane and connected to two cell medium reservoirs via fluidic channels	• Fluid circulation without the need for external mechanical pumps • Integration of both the vascular endothelium and skin dermal and epidermal layers to mimic the multilayered structure of the skin
WO2018030958A1	Bigliardi *et al* [205]	2018	WIPO	Creating a platform with the culturing and testing phases inside a single device	• Dual fluidic compartments separated via a porous membrane, with the top chamber having an access opening that can be covered with a lid • Environmental control system interfacing with the chip and ensuring temperature and humidity control	• High precision in transport parameter estimation, essential for skin permeation studies • Suitable for mass production and high-throughput studies
US20200239857A1	Sung *et al* [78]/Sungkyunkwan University Research and Business Foundation, Industry Academic Cooperation, Foundation of Hallym University	2020	United States	Producing a stretchable SoC that simulates the repetition of relaxation and contraction of the human skin	• Upper and lower layers separated with a porous membrane, where the upper layer contains a skin cell culture chamber and a connector attached to a permanent magnet • The connector is magnetically linked to an external drive, periodically stretching the skin tissue	• Stretching supports the attachment and infiltration of keratinocytes into the dermal collagen layer • Stretching the skin model at a high speed of 5.3 mm s^{-1} for 7 d simulates skin aging
CN111073844A	Yuli *et al* [206]/Guangzhou Huadai Biological Technology Co ltd	2020	China	Developing a method for the long-term cultivation of skin tissues inside the microfluidic chip	Two culture chambers for housing 3D skin model plugins and 2D skin cell cultures connected with microfluidic channels	• Combination of 2D and 3D culture within a single chip • The detailed methodology for simulating topical and systemic skin exposure routes

(Continued.)

Table 2. (Continued.)

US20210003561A1	Ingber *et al* [207]/Harvard College	2021	United States	Developing methods of manufacturing and use for low shear microfluidic devices for different OoC applications, including SoC	• Central channel divided with a porous membrane into mesochannel and microchannel, where the upper mesochannel is significantly taller than the microchannel • The central channel is flanked with two lateral channels that enable the mechanical stretching of the membrane via pneumatic mechanism	• Simultaneous gaseous and liquid fluid flow in the mesochannel and microchannel, simulating the air–liquid interface •The structural configuration of the central channel favors the low shear environment and creation of the stratified 3D skin tissue
KR20210086461A	Jeong and Kim [208]	2021	South Korea	Fabrication method of a multilayered microfluidic platform for enhanced physiological relevance and functionality of SoC models	• PDMS layers separated with a polycarbonate membrane of a predetermined pore size • MEA substrate layers on the outer surfaces of PDMS layers with metal electrodes for TEER measurements • PMMA layers on the outer surface of MEA substrate to protect the chip	• Integrated metal electrode array(MEA) for TEER measurements of the skin barrier function • The detailed methodology for the fabrication of the multilayered SoC
AU2021261826A1	Varone *et al* [202]/Harvard College	2021	Australia	Developing open-top microfluidic devices for topical access and treatment of skin tissues	• Open-top chambers designed for 3D matrix placement and the direct access to the tissue culture area •The top chamber is separated from spiral-shaped microfluidic channels in the bottom layer via a porous membrane	• Open-top design allowing for direct application of substances and sampling of skin tissues •The innovative use of the patterned gels for replicating the topography of the skin dermis
US20210214670A1	Levner *et al* [203]/Emulate Inc	2021	United States	Developing open-top microfluidic devices with structural anchors	• Open-top chambers designed for the direct access to the 3D tissue culture area and separated from the lower fluidic channels via a porous membrane • The open-top cavity contains structural anchors on the vertical walls to attach 3D matrices and ensure their stability	• Structural anchors prevent gel shrinkage for the long-term skin tissue culture and support the uniform stretching of the skin tissue • The patterned gels enhance the physiological relevance of SoC models
US20210229097A1	Kerns *et al* [209]/Emulate Inc	2021	United States	Designing an OoC system that mimics the structure of epithelial tissues, facilitating their interactions with lamina propria-derived cells	• Top chamber for culturing 3D matrix covered by a removable lid and separated from the spiraling fluidic channel via a porous membrane • Lateral vacuum channels flanking the central channel for the uniform stretching of the gel	Versatile co-culture system of various epithelial tissues with lamina propria cells for investigating cellular interactions and inflammatory responses
US20220089989A1	Ingber *et al* [204]/Harvard College	2022	United States	Developing multilayered OoC systems containing permeable matrices with lumens	• First and second chambers separated with a porous membrane and flanked with two lateral channels • The first chamber contains a permeable matrix with plurality of lumens spanning across	Inclusion of lumens lined with cells within the permeable matrices, mimicking vasculature or ducts

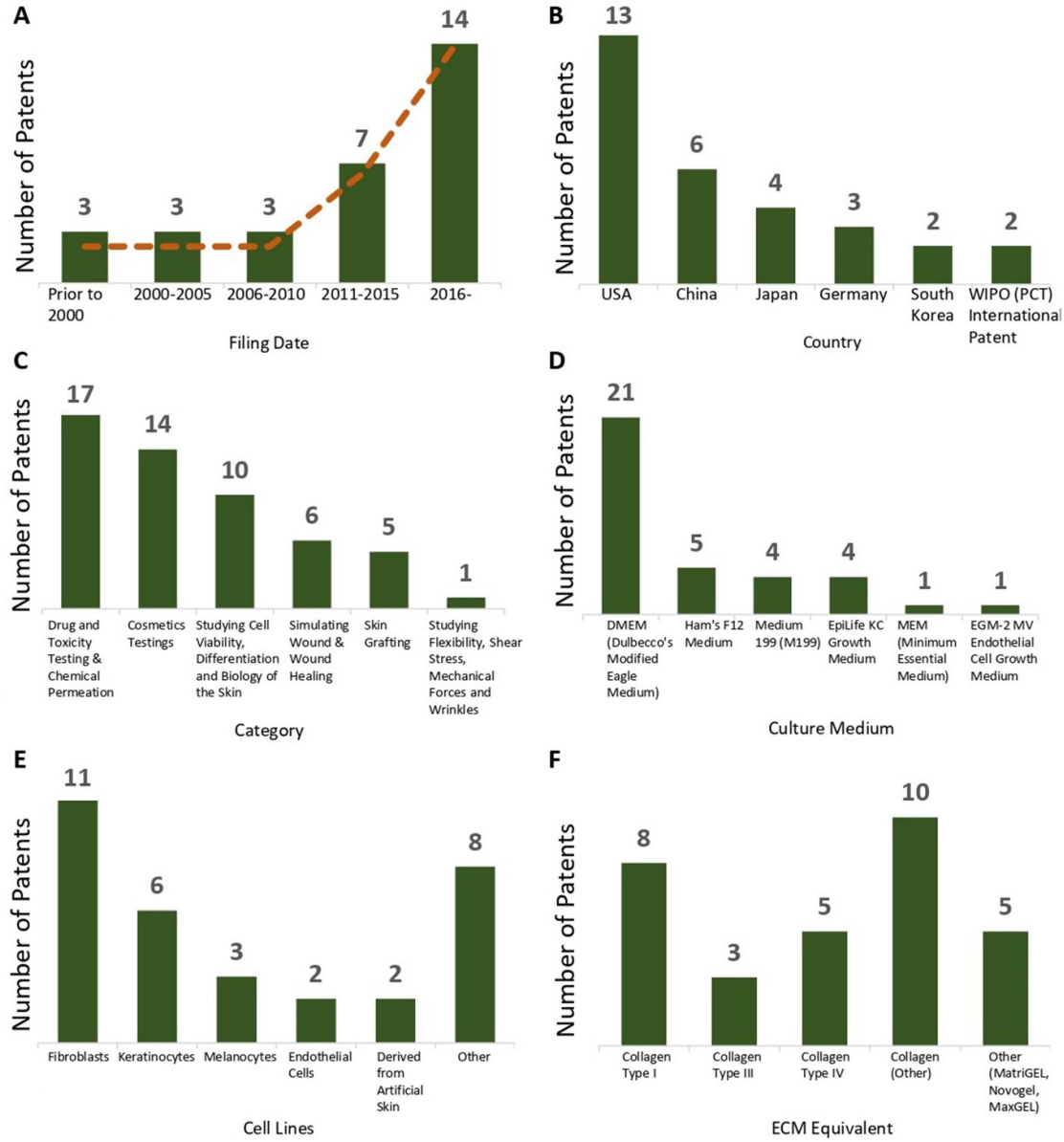


Figure 8. Number of patents investigated in this paper with respect to (A) filing date intervals (prior to 2000, 2000–2005, 2006–2010, 2011–2015, after 2016), (B) countries of origin (United States of America, China, Japan, Germany, South Korea, WIPO (PCT) International Patent), (C) defined categories (Skin Chips for Drug and Toxicity Testing & Chemical Permeation, Skin Chips for Cosmetics Testing, Skin Chips for Studying Cell Viability, Differentiation and Biology of the Skin, Skin Chips for Simulating Wound & Wound Healing, Skin Chips for Skin Grafting, Skin Chips for Studying Flexibility, Shear Stress, Mechanical Forces and Wrinkles), (D) culture medium used (DMEM (Dulbecco's Modified Eagle Medium), Ham's F12 Medium, Medium 199 (M199), EpiLife KC Growth Medium, MEM (Minimum Essential Medium), EGM-2 MV Endothelial Cell Growth Medium), (E) cell line used (fibroblasts, keratinocytes, melanocytes, endothelial cells, derived from artificial skin, other), and (F) extracellular matrix equivalent used (collagen type I, collagen type III, collagen type IV, collagen (other), other (Matrigel, Novogel, MaxGEL).

research, owing to the increasing focus of pharmaceutical companies and research centers on finding more predictive alternatives to animal preclinical models [216, 217]. OoC platforms designed for toxicology are gaining financial support and endorsement from large regulatory bodies such as FDA. For example, in 2017 Wyss Institute received $ 5.6 million from FDA to test the impact of UV irradiation on human physiological responses by exploiting bone marrow, intestine and lung-on-a-chip platforms [217]. While the OoC market is predominantly led by USA, the Asia-Pacific region is experiencing the fastest growth, primarily due to a regulatory framework that supports the OoC approval [216]. All of these factors also contribute to the significant growth of SoC market size, which is predicted to develop revenue and exponential market growth at a high CARG over the next six years [218].

There is a number of companies focused on the development of *in vitro* skin models as well as SoC platforms. One of the prominent companies in the industry is EpiSkin (Lyon, France). EpiSkin currently offers ten different types of skin substitutes: T-Skin™ (Human Full Thickness Model), SkinEthic



Figure 9. Word cloud generated from the trending keywords in SoC patents.

RHE™ (Human Epidermis), SkinEthic RHE ACF™ (Animal Component Free), RHE-LC™ (Human Epidermal Model Langerhans cells), EpiSkin™ (Human Epidermis), SkinEthic RHPE (Pigmented Epidermis), SkinEthic HCE™ (Corneal Ephitelium), SkinEthic HOE™ (Oral Ephitelium), SkinEthic HGE™ (Gingival Ephitelium), and SkinEthic HVE™ (Vaginal Ephitelium). Netzlaff *et al* stated that EpiSkin's main limitation is weak barrier function [219].

Epiderm™ (MatTek Life Sciences) is a reconstructed human epidermis that yields a majority of the barrier function properties of normal human skin and contains all the lipids and ceramides present in vivo. Moreover, the permeability properties of EpiDerm™ were evaluated using a diffusion chamber. According to the evaluation, EpiDerm™'s highly regular structure is considered a possible reason for making it more manufacturable than cadaver skin *[MatTek Life Sciences, 'Epiderm']*. In 2023, MatTek established a strategic partnership with AIM Biotech(Singapore and Boston, MA), integrating AIM Biotech's microfluidic 3D tissue culture platforms and MatTek's advanced engineered skin constructs. This collaboration aims to enhance the physiological relevance of *in vitro* skin models by incorporating features like perfusable vasculature, offering more predictive in vivo outcomes for researchers and the pharmaceutical industry [220].

Phenion (Henkel, Düsseldorf, Germany) offers Phenion FT®, Phenion FT Long-Life®, Phenion FT Aged®, epiCS® and epiCS-M®, which are full thickness models. FT model is reported to be richer in benzoic acid (FT model: 24.8, SkinEthic: 17.8) and caffeine (FT model: 21.6/21.7, SkinEthic: 13.9) than Epiderm, SkinEthic™, EpiSkin™ in terms of apparent permeability coefficient in relation to pig skin. Latency times of FT model are below of the Epiderm, SkinEthic, EPISKIN. Even shorter with Epiderm and SkinEthic reconstructed human epidermis (<0.35 h), formed from keratinocytes grown on a synthetic supporting membrane [221].

CELLnTEC (Bern, Switzerland) offers a variety of skin substitutes, additionally it has the advantage of providing a supplement to increase isolation efficiency. After isolation, the supplement is added to the culture to enhance viability and attachment of primary epithelial cells.

Labskin (Labskin, Cork, Ireland), a commercial 3D *in vitro* skin model, is based on Leeds Model which developed distinctively to study interactions between the skin and the microbiome. Labskin is considered to be a more representative model of the skin ecosystem by being developed with controlled colonization and air-exposed epidermis. Labskin differs from other models by providing relatively dry surface with its highly structured architecture, resulting in making the skin model more convenient for growing skin microorganisms on the surface. This is crucial because colonizing a skin model with proper microorganisms is a complex but necessary process [222].

LabCyte Epi-model (Japan Tissue Engineering Co., Gamagori, Japan) is a multi-layered model which contains a stratum basal, stratum spinosum, stratum granulosum and a stratum corneum as reported by

Katoh *et al* (2009). Its barrier function is tested with cytotoxic substance, sodium lauryl sulfate (SLS, Sigma-Aldrich, St Louis, MO, USA). The SLS concentration required to reduce the viability by 50% is reported as 2.57 ± 0.27 mg ml^{-1}, highly similar value to EpiSkin™ (2.32 mg ml^{-1}) which is validated by ECVAM (EU Reference Laboratory for alternatives to animal testing). Additionally, according to the skin irritation protocol by ECVAM, sensitivity and specificity of LabCyte Epi-model is reported as 66.7% and 80%, respectively [223].

Additionally, other commercially available models are VitroSkin (IMS Inc., Florida, USA), which are mainly designed for SPF/UVA testing and reported to be in correlation with *in-vivo* SPF testing [224], ASTERAND™ (BioIVT, NY, USA) which offers a very wide variety of skin models with diseases such as melanoma, carcinoma, adenocarcinoma, and lentigo maligna in fresh or frozen tissue format, and CreativeBioArray (Creative Bioarray, NY, USA), which offers diseased tissues such as melanoma and carcinoma tissue blocks [225].

While companies mentioned above provide static 3D skin models, there are those specializing in the production of microfluidic systems for various OoC applications, including SoC. ElveFlow (Elvesys, Paris, France) develops various microfluidic instruments designed to improve and optimize microfluidic chip development. Alongside individual instrumentation, ElveFlow offers packages specified for certain applications, including the OoC package containing flow rate sensors, reservoirs and channels with the optional microfluidic distribution valve, bubble trap, pressure sensors, recirculation valve, and additional pumping channels and flow rate sensors [226].

Similarly, Mimetas (Leiden, Netherlands) offers a solution for all *in vitro* tissue cultures, OrganoPlate®. While skin models are currently not offered, the company develops ready-to-use 3D tissue models called OrganoReady®. Mesobiotech (Paris, France), Cherry Biotech (Montreuil, France), Nortis (Washington State, USA), and BiomimX (Milan, Italy) and offer similar instrument packages for OoC applications, while Tissuse (Berlin, Germany) offers microfluidic chip systems that enable integration of up to four different organ models.

7. Future perspective and challenges toward clinical translation

As highlighted in the previous sections, SoC systems are finding a variety of applications, ranging from disease modeling to drug analysis. Nonetheless, their widespread adoption by pharmaceutical companies and clinicians requires overcoming technical and regulatory hurdles.

First and foremost, prior to being integrated into pharmaceutical pipelines or used in clinics, SoCs need to undergo extensive characterization and validation. The validation of SoC models against existing *in vitro* and preclinical models, or actual human skin is essential to ensure their reproducible performance and credibility. In order to be validated, SoC devices have to be meticulously characterized, which requires a comprehensive assessment of the functionality and limitations of each platform, defining its predictive power [227]. The characterization is particularly critical for barrier properties, molecular transport, metabolic and inflammatory responses in SoC devices, which can be facilitated by the integration of sensors (for a more in-depth discussion, please see the Sensor integration section). In addition, leveraging multi-omic approaches, such as transcriptomics, proteomics, and metabolomics, would enable the detailed characterization of cellular responses at the genetic and protein secretion level [228]. However, establishing a universal validation method for SoC platforms may not be feasible, since SoCs are fabricated in different configurations and are utilized for a variety of applications. Therefore, validation methods for SoC devices need to be tailored to the specific application and intended use of the device. For example, for drug absorption studies, the SoC platform may need to be characterized for its physiologically relevant barrier function, thickness, and hydration level. On the other hand, SoC platforms for modeling of inflammatory skin disease may need to be validated for their immune response, such as cytokine release profiles and vascularization level.

Directing efforts towards standardization of SoC systems will facilitate the establishment of validation benchmarks for evaluation of their performance, proving their robustness, reliability, and reproducibility, which ultimately can increase the confidence in this technology among end-users [228]. Standardization of chip material, manufacturing methods, and design will also facilitate the cross-validation of SoC platforms between labs, and facilitate their integration with different on-chip organs/-tissues as well as existing analytical equipment (e.g. microscopes and incubators) [123]. While a universal chip design may limit the flexibility of cellular experiments, specifically for body-on-a-chip platforms, a modular design can help to maintain a certain degree of standardization while also providing versatility. The modular microfluidic design, similar to the one exploited by Rogerson–Bouchard *et al*, allows for different tissue types to be pre-cultured in separate modules under specific conditions prior to being flexibly combined through the plug-and-play assembly [192].

The majority of SoC devices reviewed in this article were fabricated from elastomeric PDMS by utilizing the soft lithography technique. However, PDMS has the propensity to adsorb specific biomolecules and hydrophobic drugs (e.g. sex steroids, quinine, etc), which can be a problem in drug absorption studies. A number of alternative materials, such as PMMA, polystyrene (PS), polyurethane, and other

thermoplastic materials, have been proposed to solve this issue [44, 229, 230]. Despite being suitable for laboratory use, soft lithography is not compatible with the mass production of microfluidic chips, a factor which limits the reproducibility and industrial scaling of SoC platforms. On the other hand, the application of 3D printing technology has the potential to provide a standardized and easy-to-implement solution for the fabrication of microfluidic chips. Yet, the limited range of materials that are both printable and biocompatible remains a significant challenge for the wide adoption of 3D printing as the mainstream manufacturing method of microfluidic chips.

Another significant technical hurdle in the way of SoC standardization and clinical adoption is the fidelity of the cellular source. While primary skin cells exhibit mature and patient-specific phenotypes and can establish multilayered skin architecture, they have a limited lifespan. Despite having an indefinite lifespan, commercially available immortalized cell lines, such as human N/TERT keratinocytes, may not produce biologically relevant results due to being genetically modified. On the other hand, iPSC usage offers a potentially unlimited source of cells, providing a viable solution for the development of personalized models of the skin, which account for genetic variations between patients. However, iPSC derivation and differentiation may be too lengthy and lack reproducibility. Regardless of the cell source, all cell types should be extensively characterized for their phenotype, genetic composition, and ability to demonstrate skin-specific responses relevant to a particular SoC application. Once being validated, skin cell lines can be utilized at the early stages of drug development, where high-throughput studies are the priority. On the other hand, the usage of iPSC would be more advantageous and practical for modeling skin diseases and discovering novel patient-specific drug targets, as iPSC-based SoC systems provide high-content but low-throughput models. Recently, several studies reported the development of skin organoids possessing a high level of differentiation and complexity [231–234]. Skin organoids are 3D static *in vitro* models developed by self-organization of patient-derived iPSCs, thus providing patient-specific insights into skin development and disease phenotypes [235]. Integrating skin organoids into OoC platforms may be a powerful approach to harness the advantages of both technologies and potentially achieve their synergy [236].

The integration of automatic readout systems for the continuous monitoring of skin tissue development and functions is another feature that will accelerate the translation of SoC systems into clinical applications by enhancing their reproducibility and scalability. The future of real-time monitoring is the inclusion of multiplexed sensors that will allow for the simultaneous recording of cell culture conditions, skin maturation, and functions. Creating closed-loop monitoring systems that can automatically adjust cell culture parameters (such as oxygen and glucose concentration, and medium perfusion rate), based on sensor readout values, would enhance the robustness and feasibility of large-scale culture of SoC systems [237]. Furthermore, developing advanced microfluidic sensors with the autonomous decision-making feature (e.g. deciding on the tissue maturity and platform readiness level) would facilitate the development of easy-to-use SoC platforms for end-users with limited clinical expertise in point-of-care [237]. For instance, a sensor-integrated SoC platform can automatically monitor the culture parameters and adjust the medium perfusion rate accordingly to achieve the fastest possible maturation rate of the epidermis.

Another challenge faced by SoC platforms towards clinical translation is the effective and timely analysis of the data generated by sensors. The continuous monitoring of multiple system parameters from several parallel platforms produces a sizeable amount of data. Accurate interpretation of the collected data is a labor-some task that can outpace human experts and is prone to bias (i.e. dissimilar interpretation of similar data by different human experts) [238]. Future studies can focus on the implementation of machine learning [238] algorithms to accelerate the automated collection as well as the interpretation of sensor data [239]. ML, as a subset of artificial intelligence, refers to enabling computers to learn from labeled data and/or past experiences without being explicitly programmed for a specific task with minimum human intervention [240]. In addition, establishing a network of linked SoC platforms to create a universal data server, comprised of SoC sensor data from all around the world (e.g. from different clinics and labs) can provide an open-access source for ML training, which can decrease the needed time and money to develop automated SoC platforms while improving the interpretation capability of ML algorithms using open-source data.

In addition, the operation of SoC devices should be automated to minimize manual handling and, thus, the incidence of human error. Currently, the introduction of cells or cell-dispersed matrices into microfluidic channels or chambers is carried out primarily by pipetting. The manual handling of tissue components prevents reproducible loading, which leads to the formation of air bubbles, reducing the robustness of SoC platforms and questioning the validity of obtained experimental results. Risueno *et al* proposed an alternative method for the *in situ* deposition of fibroblast-dispersed fibrin hydrogel and keratinocytes into microchannels of a chip via the syringe pump, avoiding manual handling [89]. Automatic liquid handling tools, such as robotic pipetting tools, can also be programmed to perform sample collection and reagent dispensing [241]. However, to

fully realize the potential of OoC technology, it is of high importance to automate the entire workflow, from chip preparation to data analysis, reducing the need for skilled personnel and improving reproducibility [242].

Although there are currently no regulatory standards for OoC technology, developing thoroughly characterized, validated, flexible, and biologically relevant platforms will help the establishment of new regulatory standards. Early collaboration with regulatory authorities during the development of microphysiological systems is essential to ensure that these platforms meet regulatory requirements and to create policies that are specific to this technology [228]. Currently, existing standards used by OoC developers are those that are related to medical devices and drug development, such as ISO 9001:2015, FDA 21 CFR Part 58, and FDA FD&C Act Section 507 [228]. However, there is an unmet need for specific guidelines and standards that take into account the unique features of OoC technology, such as the integration of multiple cell types and recapitulation of physiological processes.

Food and Drug Administration [217] has been proactive in understanding the potential of OoC platforms by establishing collaborative agreements with different OoC companies [243]. In addition, FDA launched Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program, which aimed to propose qualification standards that have to be followed by drug development platforms, including OoC, in order to expedite their acceptance by regulatory agencies [227]. One of the key takeaway messages from the recent workshop with the FDA and the Pharmaceutical Industry was that the qualification of OoC systems will depend on their specific context of use. During the workshop, it was also highlighted that ongoing collaboration between the FDA and the industry is crucial for the effective integration of OoC platforms in the drug development process [244].

8. Conclusion

SoC platforms represent the most recent and innovative approach in the field of *in vitro* skin modeling, underscoring the paradigm shift towards more physiologically relevant and reproducible models for dermatological research, disease modeling, and pharmacological testing. Despite being still in its infancy, SoC technology has already made significant advances in enhancing (1) the lifespan and stability of engineered skin equivalents through the integration of dynamic perfusion (2) biological relevance of healthy and diseased skin models via the incorporation of vasculature, immune and nerve components. The versatility of SoC platforms is further highlighted by their miniaturization and adaptability for sensor integration and high-throughput analysis in a reproducible and highly-controlled manner.

However, fully harnessing the potential of SoC technology and ensuring their widespread usage in the biomedical community requires overcoming technological and methodological challenges, specifically related to standardization of SoC platforms. The standardization of cellular source, chip material, manufacturing method and design parameters for different applications would ensure the integration of SoC platforms with the common lab equipment and other on-chip systems. This would create a common framework for these platforms, facilitating the cross-validation across different and accelerating the adoption of SoC technologies in the broader scientific communities. The exploration of alternative materials to PDMS, advances in 3D printing technology for the engineering microfluidic platforms as well as the skin tissues, establishment of characterized skin cell types from cell lines and iPSCs are the avenues for technological innovation that would facilitate this process. The further adoption of SoC platforms by pharmaceutical companies and biotechnology sectors as preclinical models, requires extensive characterization and validation for their predictive accuracy, reproducibility, and biological relevance across various applications. The validation requires the establishment of performance benchmarks tailored to each unique SoC application by regulatory bodies and pharmaceutical entities. Furthemore, such efforts necessitate large-scale evaluation campaigns, requiring the commercial ventures in the design and manufacturing of SoC technologies. In essence, the evolution and eventual clinical translation of SoC technologies relies on the collaborative endeavors of stakeholders across the scientific, regulatory, and commercial domains, requiring a merge of scientific innovation, regulatory foresight and commercial investment.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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