Vascularized Microfluidic Platform for Skin Toxicity Testing

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Abstract:

In-vitro skin models are highly demanded for drug development and cosmetics testing. As the largest organ, skin serves as an important barrier against exogenous substances penetration and has various critical physiological functions that mostly regulated by capillaries within skin dermis. As the largest organ with epidermis and the dermis, skin serves as an important protective barrier with various physiological functions. Skin is rich with vascular networks that transport nutrients and oxygen to promote cells survival and integration in thick skin tissues. Microfluidic devices have been engineered to mimic tissues and organs to model physiological cellular microenvironment. This organ-on-chip platform has shown great potential as alternatives for replacing animal testing for toxicological applications. Under various physiological and pathological conditions, skin will initiate rapid angiogenesis in generating new blood vessels to restore oxygenation and allow the growth of new tissue. Therefore, in-vitro vascularized skin tissue is highly demanded for toxicity studies. Here we review the development of vascularized microfluidic platform that can be used as alternative platform for further skin toxicity assays in cosmetic and pharmaceutical testing applications.

Keywords: Angiogenesis, Keratinocytes, Microfluidic, Toxicity, Skin

1. Introduction

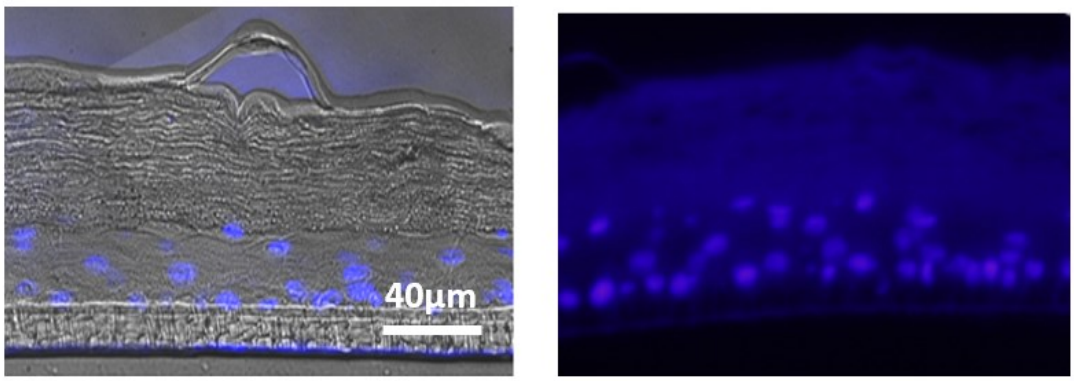
Skin in a complex organ with epidermis and the dermis layers that serves as an important barrier against the penetration of exogenous substances into the body and have various physiological functions such as fluid homeostasis, thermoregulation, immune surveillance, self-healing, as well as sensory detection [1]. Furthermore, dermis is richly supplied by blood vessel, lymphatic channels and sensory nerves. Blood vessel play a critical role on the transportation of the oxygen and nutrients for cell survival and proliferation. Whilst, lymphatic vessels play a major role in tissue fluid homeostasis and immune cell trafficking that respond to interstitial liquid pressure by taking up and removing excess tissue fluid [2]. Besides other physiological functions, skin is also a potential avenue for the transport of active drugs or ingredients into the skin [1]. Therefore, in vitro skin models are beneficial for the systemically acting drugs which can overcome the barrier and transfer the drug into vessels or adipose tissue and also for efficacy testing of drugs at sites of action for disease models [3]. Thus, the human skin equivalents have to fulfill some criteria, such as a suitable barrier function for penetration studies or exhibiting the typical phenotype of a specific disease for drug efficacy testing [3]. Nevertheless, current reconstructed human skin can provide specific skin models for diseases such as vitiligo, melanoma, squamous cell carcinoma, psoriasis, and blistering skin disorders as well as healthy models [1].

The skin rich with vascular networks that transport nutrients and oxygen to promote cells survival and integration in thick skin tissues [4]. Under various physiological and pathological conditions, skin will initiate rapid angiogenesis in generating new blood vessels to restore oxygenation and allow the growth of new tissue. Skin angiogenesis is generated under conditions such aging, wound healing, hair growth, skin neoplasis, vascular tumors, inflammatory dermatoses, bullous diseases and psoriasis, eczema, hemangioma, UV-damage and heat [5]. One of critical growth factor in angiogenesis is VEGF that was expressed from low to moderate amount in normal epidermis. Skin angiogenesis mostly are modelled in-vivo. One of previous in-vitro model is based on reconstructed skin from same split-thickness skin graft and angiogenesis response was observed by the penetration of endothelial cells into the reconstructed skin [6]. However, this model did not clearly demonstrate angiogenesis vessel sprouting in the dermis. Nevertheless, cultured human keratinocytes also were proofed to secrete VEGF [7]. Therefore, understanding skin angiogenesis is important in the development of safe topical products like transdermal drugs and cosmetics, as well as

in wound healing, skin disease and other skin conditions. Besides, a major challenge in toxicity is to define relevant in-vitro systems that accurately predict the effects in human. For the past few years, animal skin models have been banned widely in many countries especially in pharmaceuticals and cosmetics industry that resulted in highly demanded of in-vitro skin models to evaluate the toxicity and efficacy of drug candidates [8]. Therefore, in-vitro vascularized skin tissue is highly demanded for toxicity studies. Here, will review about the development of vascularized skin models and their significant for toxicity studies.

2. In-vitro Skin Model

The motivation to accomplish artificial skin structures is driven by the needs of clinical skin replacements and grafts, models for drug permeability tests and toxicity screening. Numerous skin substitute is commercially available either with or without living cells [1,9-11]. These skins substitute can be used to replace the skin either temporarily or permanently. These artificial skins incorporated human cells which are fibroblast for dermal, keratinocytes for epidermal or combination both cell types for dermal-epidermal skin substitutes. The fabrication of human skin equivalents is generally consisted of multistep process including construction of dermal equivalents by culturing fibroblasts within biological or synthetic matrix, keratinocytes seeding on top of dermal equivalents and culturing the fibroblasts and keratinocytes at the air-liquid interface [12]. Figure 1 and Figure 2 shows the example of constructed skin epidermis by culturing keratinocytes in a Trans-well after 2 weeks of air-liquid interface.



(a) (b)

Fig. 1 Cross-sectional of epidermis from a Trans-well.

Besides, an ideal in-vitro 3D skin model should be mimicking physiological functions by containing vasculature, immune cells, melanocytes and appendages to enable skin disease modelling, substance testing and personalized medicine [8, 13,14]. Therefore, current in-vitro skins models have been expanded from epidermal to full-thickness integrated with others skin components. Developing a functional skin construct with multiple components is challenging since this process rely on precise control over density, structure and arrangement of such components in a 3D microenvironment [8]. However, there were few problems in existing commercially available skin substitutes such as slowed vascularization, scarring, absence of differentiated structures as well as long time cell culturing [9]. Therefore, it has been suggested that the highest complexity of in vitro models for the purpose of drug development can be achieved with multi-organ on chip approaches [3].

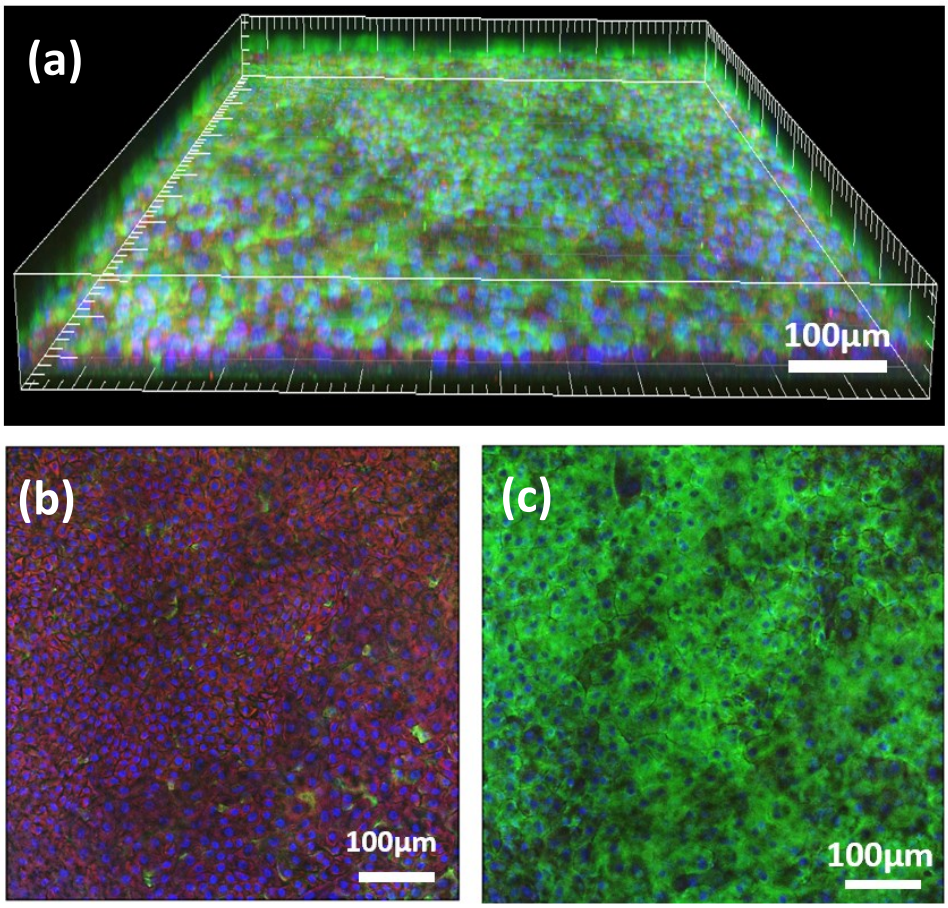


Fig. 2 Confocal images of epidermis constructed after 14 days of air-liquid interface. (a) 3D image of constructed epidermis with (b) basal layer, stained with K14 (red) and (c) keratin layer, stained with K10 (green).

2.1 The Need of Skin Vascularization

Skin vasculature is important to allow perfusion of the dermal component for diffusion of nutrients and signaling molecules that enhances long-term survival and functionality of skin substitutes [8,15]. Thus, one of essential keys for the successful outcome of skin tissue engineering is to provide a vascular network that able to promote survival and integration of cells in thick artificial dermal. Thus, various approaches have been done to develop vascularization in skin tissue engineering including the incorporation of various types of cells from different origin such as bone marrow, fat and blood vessel [16]. However, conventional platforms still have many limitations to meet the skin biologically complex system that is rich with capillaries for tissue metabolism [4,17-18]. The systemic delivery of substances is not being effectively recapitulated due to the lack of an endothelial barrier [8]. Therefore. various approaches are currently used to improve vascularization of skin substitutes before transplantation [2, 19-20] by co-culturing primary endothelial cells and stromal cells such as mesenchymal stem cells or dermal fibroblasts in 3D matrix such collagen and fibrin. Human dermal fibroblasts (HDF) have been co-culture with few types of EC, such as human dermal microvascular endothelial cells (HDMEC), human umbilical vein endothelial cells (HUVEC), or human blood outgrowth endothelial cells (HBOEC) [20]. A tissue-engineered skin composed of fibroblast sheets with endothelial cells and keratinocytes has been constructed and this construct able to develop functional anastomoses with the host's blood vessels and promoted a rapid, complete and optimal vascularization of the implanted tissues [19].

2.2 Engineering of perfusable blood vessel on chip

Organ on chip with relevant physiological cellular microenvironment is predicted to be perfect platform to replace animal testing For the past few years, microscale engineering technologies have been utilized in the development of tissue engineering due to the capability in providing excellent cell culture microenvironments by utilizing the critical tissue–tissue interfaces, spatiotemporal chemical gradients, and dynamic mechanical microenvironments of living organs [21]. Thus, this progress leas to the development of organs-on-chips, which living cells are cultured within microfluidic devices. Microfluidic technologies apply the laminar flow concepts which is the flow and diffusion around the cells can be accurately predicted and mass transport to cells and shear force can be systematically incorporated [22]. Microfluidics devices have been engineered to mimic the living organs tissue arrangements and thus enabling the study of organ physiology as well as to develop specialized in-vitro disease models [21]. Synergy with development of vascularized skin, various effort has been done towards engineering the blood vessel on-chip as well as vascularized organs-on-chips. Figure 3 shows the naturally derived vessel experiments were conducted based on the established vascularization procedure in a microfluidic device [23].

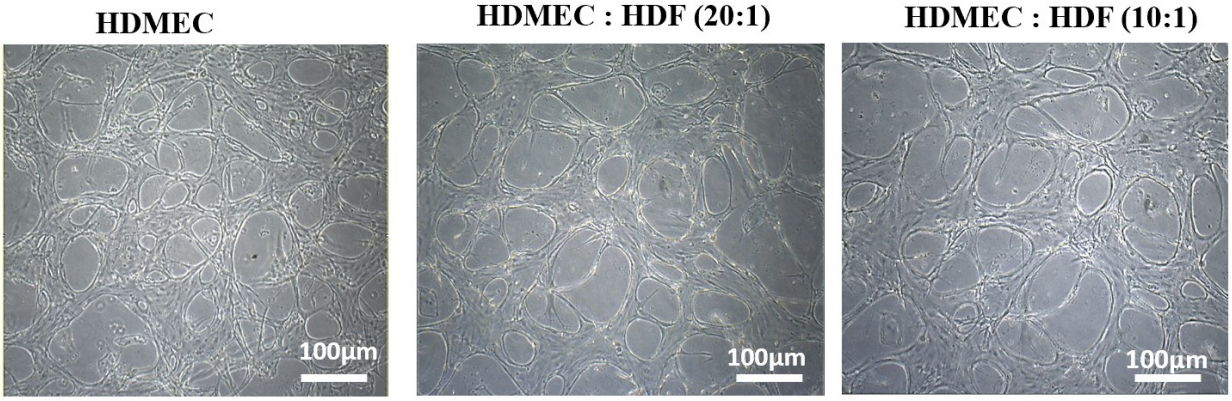


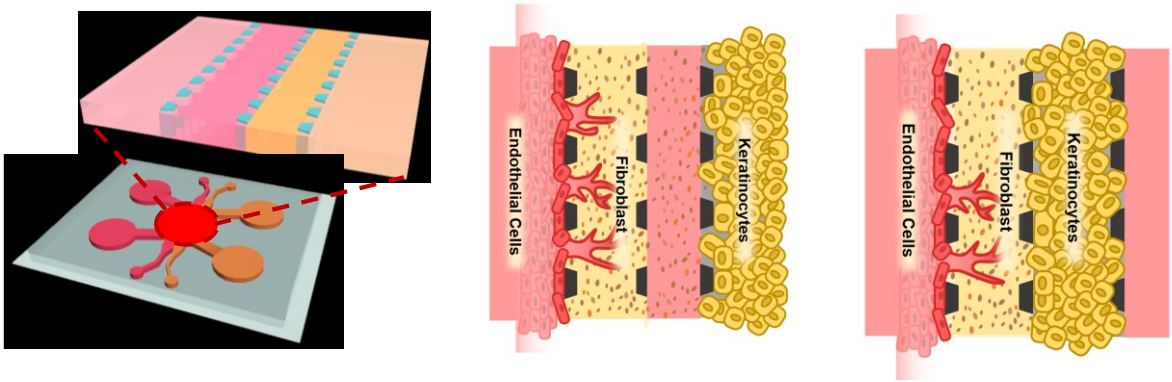
Fig. 3 Vascularization in a microfluidic by incorporating at different ratio of HDMEC and HDF.

The microfluidic design is generally based on single channel perfusable blood vessel device [19]. The microfluidic chips were fabricated by using polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) with channel structures that was patterned by standard photolithography and soft lithography. Functional perfusable microvascular network was engineered with controlled morphology using a multi-culture microfluidic system [23, 24]. Human endothelial cells were seeded in fibrin gels and cultured alongside human lung fibroblasts [23]. By spatially controlled co-culture of endothelial cells with fibroblasts, pericytes or cancer cells, this microfluidic can be a versatile platform in developing vascularized organ-on-a-chip as well as human disease models [23]. Simultaneous tuning of biomechanical and biochemical simulation in a microfluidic device bring important factors for sprout initiation, sprout elongation, sprout navigation as well as the lumen formation [25].

2.3 Development of skin vascularization on chip

Compared to conventional transwell system, skin on chip platform able to provide more efficient transport of nutrients, which important in maintaining the viability of cells within a scaffold matrix to support a longer period of culture time [26]. Advances have been made by numerous researchers in constructing microfluidic skin culture platforms to mimic physiologically relevant blood flow. In most of developed microfluidic platforms, the dermal compartment of skin is not in direct contact with the vasculature and is usually separated from the vasculature by a permeable membrane, which introduces an additional and non-physiological diffusion barrier [26, 27, 28, 29]. Recently, skin-on-chip model based on co-culture of cells monolayers have been developed recently and showed the ability in simulating inflammation and edema [27]. Besides, skin models have been constructed outside the chip and then transferred to the chip that resulting in inconsistency in the immobilization process that lead to variable experiment results of skin equivalents [26, 28]. Recently, skin angiogenesis model in microfluidic device have been developed for toxicity studies

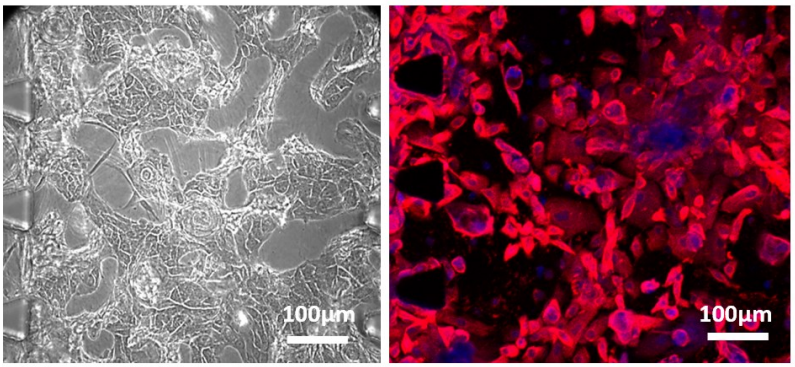
[30]. Figure 4 show the example of proposed model of skin angiogenesis by using a microfluidic. Compare to previous platform, the device was modified with double channels to allow direct contact between human dermal fibroblasts (HDF) and keratinocytes [30].



(a) (b) (c)

Fig. 4 Channel configuration for engineering skin angiogenesis in a microfluidic device, (a) schematic of a microfluidic device, (b) cells configuration for single channel device and (c) cells configuration for double channel device.

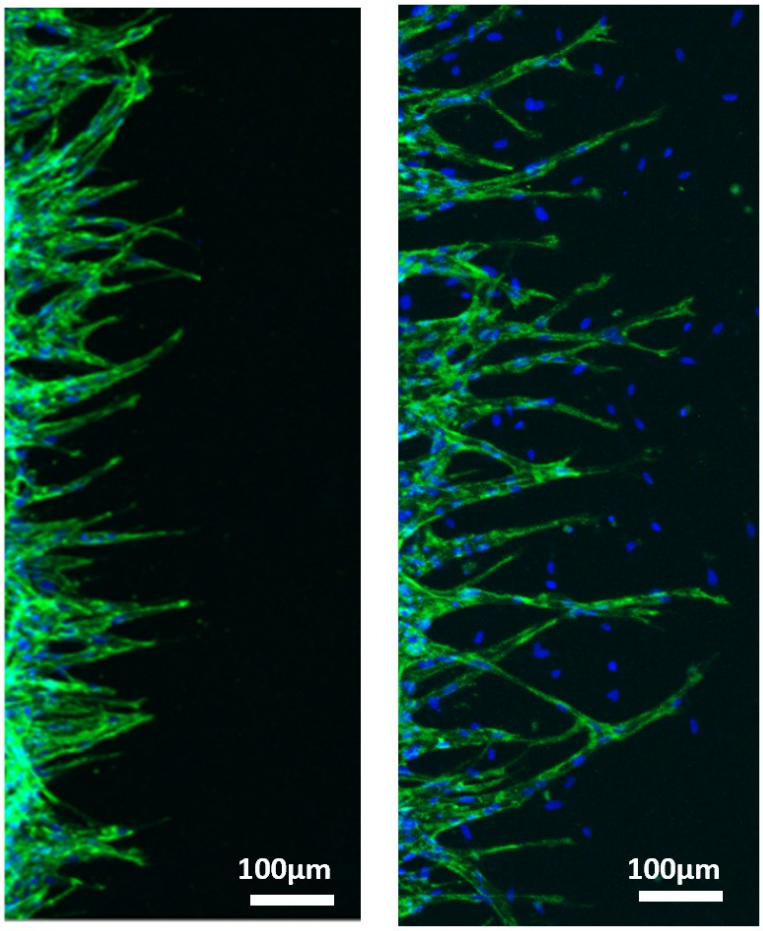
To mimic the angiogenesis in dermis, keratinocytes (KC) were used and cells seeding were done according to designated channels, as shown in Figure 4. The microfluidic channels were mimicking the skin layer that comprise dermis and epidermis with HUVECs were positioned besides fibrin channel to represent the blood capillary of human skin [30]. Human keratinocytes were laid at the opposite channel as the source of VEGF growth factor to enhance formation of functional vessel sprouts [30]. The microfluidic device consists of four parallel channels that were separated by 100µm microposts gap to prevent leakage and to capture the hydrogels in the microfluidic device [23]. The multiple channels enable the supplying of two different mediums in one device which is critical in maintaining multiple cells viability successfully.



(a) (b)

Fig. 5 (a) Keratinocytes in fibrin (brightfield image); (b) Keratinocytes in fibrin (K14, red);

Moreover, the addition of HDF also gave significant effect to the skin environment due to the interaction between keratinocytes and fibroblasts [30]. In-vivo, this epithelial-mesenchymal interactions play a critical role in regulation of epidermal growth and differentiation and thus effect the skin homeostasis [31. This interactions is regulated by autocrine and paracrine activities, cell-matrix interactions and cell-cell contact signaling between these two types of cells [31]. Fibroblasts are believed to promote keratinocytes proliferation and migration and consequently improved epidermal morphology. At the same time, epidermal keratinocytes also induced fibroblasts activities. Figure 7 shows the angiogenesis sprout formation of HDMEC on day 3 with the addition of HDF.



(a) (b)

Fig. 6 (a) Angiogenesis of HDMEC without fibroblast interaction in the main channel at day 3; (b) Angiogenesis of HDMEC with fibroblast interaction in the main channel at day 3. Confocal image with CD31 (Green) and Nuclei (Blue).

Human Dermal Fibroblasts (HDF) were incorporated in the fibrin gel in mimicking skin dermis [30]. The addition of HDF in the fibrin also enhanced the vessel formation in the fibrin channel with the formation of many tip cells. The result shows that the angiogenesis sprouts were sensitively controlled in response of keratinocytes [30]. Other study proofed that VEGF was mostly released extracellularly in cultured keratinocyte. Similar with secreted VEGF, the amount of VEGF intracellular content also was increased with time [7].

3. Skin Toxicity Testing

Reconstructed skin models have been optimized for toxicological screening [3]. Most of in-vitro skin irritation models are based on evaluation of cell viability, morphology changes, gene expression and cytokines analysis for the toxic potential of chemicals [32-35]. However, skin exposure to chemicals, drugs or cosmetics led to a wide variety of skin reactions that involves the randoms changes in epidermis [36]. Keratinocytes that represent the major cells in the epidermis, play an initiator in skin inflammatory and immunological reactions along with Langerhans cells and melanocytes [35]. These random changes in epidermis keratinocytes enhanced the secretion of Vascular Endothelial Growth Factors (VEGF) that led to the formation of skin angiogenesis [30]. VEGF has been suggested as a novel biomarker for keratinocyte damage in skin toxicity testing due to the significant role of VEGF in pathological alteration of keratinocytes [37]. A new skin angiogenesis model in microfluidic device have been developed for toxicity studies by using SLS and SC as the chemical irritants [30]. SLS has been reported to increase significantly VEGF in keratinocytes by affecting the gene transcription of VEGF [38]. VEGF is an endothelium and microvessel hypermeability growth factor that lead to angiogenesis formation VEGF acts as a specific mitogen in-vitro for human dermal microvascular endothelial cells and induces endothelial cells migration towards several extracellular matrices [39, 40]. Thuss, in response to the chemical stimuli, keratinocytes secrete various cytokines and growth factors which promote angiogenesis and vascular permeability.

4. Conclusions

With the huge demands of artificial skin, rapid development of blood vessel of reconstructed skin is crucial towards development of fully functional in-vitro skin model. Various vascularization strategies have been developed with various type of endothelial cells as well as stromal cells. With success of vascularization on chip as well as organ on chip, development of vascularized artificial skin on chip can be a promising strategy. This platform offers new approach as a platform for safety evaluation test in cosmetic industry as well as drug screening in pharmaceutical industry.

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