PERSPECTIVE ARTICLE

The future of skin toxicology testing – Three-dimensional bioprinting meets microfluidics

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Abstract: Over the years, the field of toxicology testing has evolved tremendously from the use of animal models to the adaptation of *in vitro* testing models. In this perspective article, we aim to bridge the gap between the regulatory authorities who performed the testing and approval of new chemicals and the scientists who designed and fabricated these *in vitro* testing models. An in-depth discussion of existing toxicology testing guidelines for skin tissue models (definition, testing models, principle, and limitations) is first presented to have a good understanding of the stringent requirements that are necessary during the testing process. Next, the ideal requirements of toxicology testing platform (in terms of fabrication, testing, and screening process) are then discussed. We envisioned that the integration of three-dimensional bioprinting within miniaturized microfluidics platform would bring about a paradigm shift in the field of toxicology testing; providing standardization in the fabrication process, accurate, and rapid deposition of test chemicals, real-time monitoring, and high throughput screening for more efficient skin toxicology testing.

Keywords: Three-dimensional bioprinting; Three-dimensional printing; Additive manufacturing; Skin bioprinting; Microfluidics

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1. Introduction on toxicology testing

Toxicology testing is performed to identify the potential adverse effects a chemical poses to an individual and its surrounding environment[1]. The different types of chemicals include active pharmaceutical ingredients, cosmetics ingredients, household, and industrial chemicals. An estimated number of 2000 new chemicals are produced for various applications; routine toxicology tests are conducted on increasing number of new chemicals on a daily basis to ensure its safety to potential consumers. The global market for *in vitro* toxicology testing market has been estimated to be ~USD 13 billion in 2016, and it is projected to reach USD 20.8 billion by the end of 2021[2]. An ideal study to evaluate the toxicity of a chemical/substance to humans would require an extremely large number of human subjects who are representative of the diversity of humans, which is unrealistic and unethical. As such, the use of animal models provides preliminary safety data to satisfy conservative regulatory requirements. The crucial issue is the extent to which these animal models can predict human responses in an accurate and reliable manner. It is clearly evident that the use of animal models has several caveats: The differences in the absorption or distribution of the chemicals/substances; the way the substances are metabolized and the short duration of animal lifespan (to accurately monitor disease development). As such, the use of animal models remains highly controversial as there are significant discrepancies between adverse effects

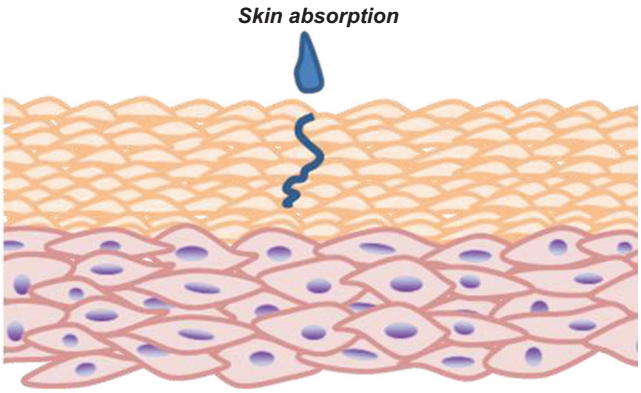
of chemicals in humans and animals[3]. Furthermore, a complete ban on animal testing for cosmetics ingredients in 2013 has necessitated the development of alternative *in vitro* models. A paradigm shift in the testing models has occurred over the past few years; the implementation of non-animal testing strategy has spurred the development of numerous human-based three-dimensional (3D) *in vitro* testing models[4,5].

Notably, the use of 3D bioprinting technology provides a highly-automated and advanced manufacturing platform[6-8] that enables the simultaneous and highly-specific deposition of multiple types of human skin cells and biomaterials with high throughput rates and reproducibility, which is lacking in conventional skin tissue engineering approaches. This facilitates the fabrication of highly-complex human-based 3D skin tissue models with additional types of cells and biomaterials to improve the homology to native skin and enhance the tissue functionalities[9]. Furthermore, the integration of bioprinted skin constructs within microfluidics platform enables the incorporation of more physiologically relevant tissue maturation conditions (controlled medium flow, and supplementation of important growth factors) to achieve more biomimetic 3D skin tissue models[10] and also facilitates real-time monitoring and high throughput screening[11,12]. Here, we provide an overview of different toxicology testing approaches, followed by the ideal requirements of toxicology testing platform.

2. Skin toxicology testing

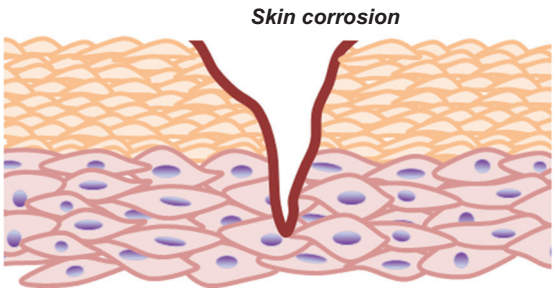
To date, the 3D skin tissue models are one of the most developed and understood *in vitro* engineered constructs[13] and they have been widely utilized as an alternative testing tool by the cosmetics industry to replace the animal models[14]. The native human skin is a large, complex organ containing multiple types of cells that are positioned relative to each other in highly-specific arrangement; it consists of anisotropic distribution of both cellular and extracellular matrix (ECM) components[15,16]. The use of human-relevant skin tissue models enables more reliable and accurate cosmetics testing; different types of skin toxicology tests have been investigated and are well-documented by the test guidelines (TG) under the Organization for Economic Cooperation and Development (OECD) using the united nations (UN) globally harmonized system (GHS) of classification and labeling of chemicals. The four key OECD TG involving *in vitro* skin tissue models include (i) skin absorption (OECD TG 428), (ii) skin corrosion (OECD TG 431), (iii) skin irritation (OECD TG 439), and (iv) skin sensitization (OECD TG 442D) (Table 1).

2.1 Box 1: Skin absorption (OECD TG 428)



Skin absorption can be defined as the absorption of a test chemical by the skin through passive diffusion process when in direct contact to evaluate the systemic exposure and perform risk assessments[17]. Excised human or animal (pig or rat) skin in the range of 200-400 µm thickness is typically used for skin absorption studies. Preparation of the epidermal membranes is performed through heat separation (60°C for 1-2 min for human and pig skin) or chemical separation (2M sodium bromide for rat skin). It is important to perform *in vitro* skin integrity test to ensure an intact stratum corneum which is retained during skin preparation and a test substance, which may be radiolabeled, is applied to the surface of a skin sample separating the two chambers of a diffusion cell. The application should mimic human exposure, normally 1-5 mg/cm^2 of skin for a solid and up to 10 µl/cm^2 for liquids. The chemical remains on the skin for 24 h at a constant temperature of 32±1°C to ensure constant passive diffusion of chemicals before removal by an appropriate cleaning procedure. The receptor fluid is sampled at time points throughout the experiment and analyzed for the test chemicals and/or metabolites. A key limitation is that the skin has been shown to metabolize some chemicals during percutaneous absorption, but the metabolites of test chemicals can still be quantified.

2.2 Box 2: Skin corrosion (OECD TG 431)



Skin corrosion can be defined as a cause of irreversible damage to the skin manifested as visible necrosis through the epidermis and into the dermis, following

Table 1. A comparative analysis of different OECD testing guidelines for skin tissue models.

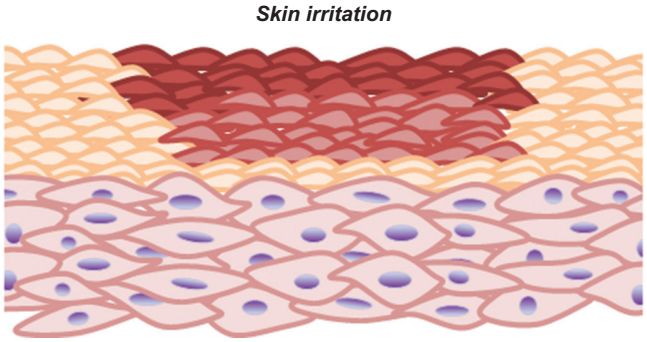
Toxicology testing	Skin absorption	Skin corrosion	Skin irritation	Skin sensitization
Test guidelines	OECD TG 428	OECD TG 431	OECD TG 439	OECD TG 442D
Definition	Absorption of chemical through passive diffusion when in direct contact	Irreversible skin damage following application of a test chemical	Reversible skin damage following application of a test chemical	Allergic response to a chemical following application of a test chemical
Validated *in vitro* testing models	Excised human or animal (pig or rat) skin in the range of 200-400 µm thickness	Epiderm™ EpiSkin™ SkinEthicTM RHE epiCS®	Epiderm™ EpiSkin™ LabCyte EPI-Model SkinEthic™ RHE	KeratinoSens™ (immortalized HaCaT stably transfected with a selectable plasmid)
Principle	A radiolabeled test chemical is applied to the skin sample separating the two chambers of a diffusion cell to check for passive diffusion at different time points throughout the experiment	A corrosive chemical can penetrate the stratum corneum of 3D RHE model by diffusion or corrosion and are toxic to underlying cells	An irritant can penetrate the stratum corneum of 3D RHE model by diffusion and cause the underlying damaged cells to release inflammatory mediators or induce an inflammatory cascade	A sensitizer can upregulate the luciferase activity and allows quantitative measurement of luciferase gene induction
Classification		Class 1A (<50% viability after 3 min exposure) Class 1B and C (≥50% viability after 3 min exposure AND <15% after 60 min of exposure Non-corrosive (≥50% viability after 3 min exposure AND ≥15% after 60 min exposure)	Irritant (UN GHS Category 2) (≤50% viability) Non-irritant (>50% viability)	Sensitizer (≥50% increase in luciferase activity with cell viability >70%) Non-sensitizer (<50% increase in luciferase activity with cell viability >70%)
Limitations	Potential metabolization of test chemicals during percutaneous absorption	Poor categorization of sub-category 1B and 1C Interference in MTT results from light-absorbing test chemicals	Poor classification of UN GHS Category 3 (mild irritants) Interference in MTT results from highly-colored test chemicals	Only addresses the 2nd key event of skin sensitization's AOP
Proposed methods to overcome limitations	Metabolites of test chemicals can be quantified	Use of adapted controls for corrections of interference measurements	Use of adapted controls for corrections of interference measurements	Use of IATA by combining other AOPs

OECD: Organization for Economic Cooperation Development, 3D: Three-dimensional, TG: Test guidelines, GHS: Globally harmonized system, RHE: Reconstructed human epidermis, AOP: Adverse outcome pathway, UN: United Nations, IATA: Integrated approaches to testing and assessment, RHE: Reconstructed human epidermis

the application of a test chemical[18]. The reconstructed human epidermis (RHE) is typically used for skin corrosion studies; the four validated commercially available *in vitro* skin models are Epiderm™, EpiSkin™, SkinEthic™ RHE, and EpiCS®. The principle of the skin corrosion test is based on the hypothesis that corrosive chemicals are able to penetrate the stratum corneum by diffusion or erosion and are cytotoxic to the underlying cell layers. At least two tissue replicates should be used for each test chemical and controls for each exposure time; sufficient amount of test chemical should be applied to uniformly cover the epidermis surface (a minimum of 70 µL/cm^2 or 30 mg/cm^2) should be used. Concurrent negative (NC) and positive controls (PC) should be used in each run to demonstrate that viability with NC, barrier function, and resulting tissue sensitivity with the PC of the tissues are within a defined historical acceptance range. Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from tissues. Corrosive chemicals are identified by their ability to decrease cell viability below-defined threshold levels (<50% viability after 3 min exposure – Class 1A, ≥50% after 3 min exposure AND <15% after 60 min of exposure – Class 1B and 1C), while chemicals that produce cell viabilities above the defined threshold level may be considered non-corrosive (i.e., ≥50% after 3 min exposure AND ≥15% after 60 min exposure). A limitation of existing TG is that it does not allow the discriminating between skin corrosive sub-category 1B and sub-category 1C in accordance with the UN GHS due to the limited set of identified *in vivo* corrosive sub-category 1C chemicals. The TG is currently only applicable to solids, liquids, semi-solids, and waxes; gases and aerosols have not been assessed yet in a validation study. It should also be noted that the test chemicals absorbing light in the same range as MTT formazan and test chemicals able to directly reduce the vital dye MTT may interfere with the tissue viability measurements and need the

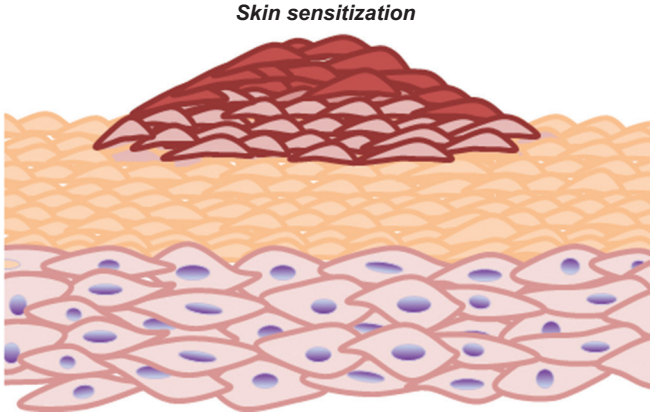
use of adopted controls for corrections. Hence, the use of adapted controls for corrections of interference measurements is critical.

2.3 Box 3: Skin irritation (OECD TG 439)



It can be defined as the cause of reversible damage (an inflammatory reaction that usually disappears after a few days) to the skin following the application of a test chemical[19]. The RHE is typically used for skin irritation studies; the four validated commercially available *in vitro* skin models are Epiderm™, EpiSkin™, LabCyte EPI-Model, and SkinEthic™ RHE. There are two proposed mechanisms that lead to skin irritation, namely, the damage to the barrier function of the stratum corneum and the direct effect of irritants on the skin cells. At least three replicates should be used for each test chemical and for the controls (PC and NC) in each run and sufficient amount of test chemical (26-83 µL/cm^2 or mg/cm^2 should be applied to uniformly cover the epidermis surface. The *in vitro* RHE test system measure the cell/tissue damage using cell viability as readout. Cell viability in RHE models is measured by the enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from tissues. Irritant chemicals are identified by their ability to decrease cell viability below-defined threshold levels (i.e., for UN GHS category 2). Depending on the regulatory framework and applicability of the TG, chemicals that produce cell viabilities above the defined threshold level may be considered non-irritants (i.e., >50%, no category). A limitation of existing TG is that it does not allow the classification of chemicals to the optional UN GHS category 3 (mild irritants). The TG is applicable to solids, liquids, semi-solids, and waxes; gases and aerosols have not been assessed yet in a validation study. It should also be noted that the highly-colored chemicals may interfere with the cell viability measurements and need the use of adopted controls for corrections. Hence, the use of adapted controls for corrections of interference measurements is critical.

2.4 Box 4: Skin sensitization (OECD TG 442D)



It is defined as an allergic response to a chemical following skin contact as defined by UN GHS. The chemical and biological mechanisms associated with skin sensitization have been summarized in the form of an adverse outcome pathway (AOP), going from the molecular initiating event through the intermediate events up to the adverse health effect. Skin sensitization begins from (1) the molecular initiating event through covalent binding of electrophilic substances to nucleophilic centers in skin proteins to, (2) specific cell signaling pathways such as antioxidant/electrophile response element (ARE)-dependent pathways to, (3) activation of dendritic cells, and finally (4) T-cell proliferation[20]. The TG addresses the 2nd key event whereby the dissociated transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) can activate the ARE-dependent genes. The only *in vitro* validated ARE-Nrf2 luciferase test method involves the use of KeratinoSens™ (RHE) which is made up of an immortalized adherent cell line derived from HaCaT human keratinocytes stably transfected with a selectable plasmid and the ARE-Nrf2 luciferase test method allows quantitative measurement of luciferase gene induction following exposure to electrophilic test substances. Three replicates are used for the luciferase activity measurements, and one parallel replicate used for the cell viability assay. Test chemicals are considered positive if they induce a statistically significant induction of luciferase activity above a given threshold (i.e., 50% increase) below a defined concentration which does not significantly affect cell viability (i.e., below 1000 µm and at a concentration at which the cellular viability is above 70%). As this testing guideline only focuses on the 2nd key event of the skin sensitization AOP, the results from the test are unlikely to be sufficient when used on its own. Hence, combining them with other complementary information addressing other key events of skin sensitization AOP would give more accurate predictive outcomes.

Although most of the current validated *in vitro* skin models are RHEs, it has been shown that the presence of fibroblasts increases the resistance of keratinocytes to

toxic chemicals[21]. These findings indicate that the use of full-thickness (FT) skin models would generate more meaningful data for toxicology testing. Furthermore, the use of FT skin models in skin absorption studies has also generated good data reproducibility[22]. Hence, the use of 3D bioprinting technology would enable the fabrication of more complex and biomimetic 3D FT skin models in a standardized manner that can potentially improve the reliability and accuracy of different skin toxicology tests (Figure 1). In the following section, we discuss the ideal requirements of a toxicology testing platform in terms of 3D bioprinting of complex tissue models, automated deposition of test chemicals and use of skin-on-chip microfluidics platform.

3. Ideal requirement for toxicology testing platform

3.1 3D Bioprinting

The use of advanced bioprinting platform not only facilitates the patterning of different living cells, biomaterials and growth-factors to fabricate highly-complex tissue models[24-27] but it also enables automated fabrication of skin tissue models by depositing the bio-inks[28] in a highly scalable and reproducible manner. The main key advantage of 3D bioprinting is the ability to standardize the printing process and facilitate the on-demand manufacturing of standardized skin tissue models anytime, anywhere. Different bioprinting processes have been applied to fabrication of skin tissue models; they include (i) extrusion-based[29-32], (ii) laser-assisted[33-35], and (iii) microvalve-based[36-38] bioprinting.

3.1.1 Extrusion-based bioprinting

Primary human fibroblasts and keratinocytes obtained from skin biopsies of healthy donors were printed directly to fabricate fibrin-based skin constructs using the extrusion-based bioprinting approach. The plasma-derived fibrinogen matrix was first crosslinked with calcium chloride solution to form the 3D fibrin-based dermal constructs with human fibroblasts[30]. The immuno-histochemical analysis indicated that the 3D bioprinted skin constructs exhibited high degree of similarity to the native human skin. Another work has demonstrated the incorporation of PCL mesh to stabilize the collagen-based dermal matrix from severe contraction during the maturation process[39]. A recent work has highlighted the importance of ECM proteins (gelatin, fibrinogen, collagen, elastin, laminin, and entactin) in fabrication of biomimetic human-like skin equivalents[40].

3.1.2 Microvalve-based bioprinting

Multi-layered collagen constructs containing human keratinocyte and fibroblast cell lines were deposited on a non-planar surface through layer-by-layer manufacturing approach[41], and greater cell viability in the 3D constructs with fluidic channels (85% viability) as compared to the ones without any channels (60% viability) was reported[42]. A recent work emulated the native cellular density of different skin cells (HFF-1 fibroblasts and HaCaT keratinocytes) within the 3D bioprinted skin constructs using contactless microvalve print-heads[37], but the use of human skin cell lines resulted in poor stratification and keratinization of the printed keratinocyte layers. As such, recent works on skin bioprinting utilized primary human stem cells and skin cells. The amniotic fluid-derived stem cells (AFSCs at a cell density of 16.6×106 cells/ml) were encapsulated in the fibrinogen/collagen solution at 0°C. Microvalve-based print-heads were used to print the fibrinogen/collagen solution and its cross-linker, thrombin, over the FT skin wounds (2 cm×2 cm) of nu/nu mice[43]. Although the AFSCs only remained transiently in wound sites, the secretion of important growth factors from AFSCs expedited wound closure rates and angiogenesis. Furthermore, the feasibility of *in situ* printing was also explored on full-thickness large wounds (10 cm×10 cm) of nude mice[44]. A single layer of fibrinogen/collagen hydrogel precursor containing fibroblasts (1.0×105 cells/cm^2) was first crosslinked by nebulized thrombin to form fibrin/collagen hydrogel, followed by bioprinting another layer of keratinocyte cells (1.0×107 cells/cm^2) above the fibroblast-populated fibrin/collagen matrix. Although complete re-epithelialization of the large wound was achieved after 8 weeks, it is highly challenging to deposit cells on areas of significant curvature. Notably, a recent study has reported the

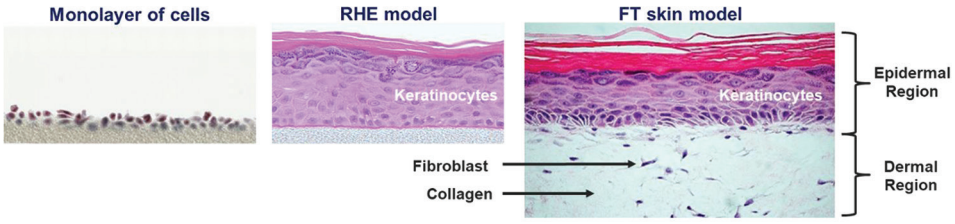


Figure 1. Bioprinting facilitates the deposition of a monolayer of cells with homogeneous cell distribution[23]; the bioprinting technique can be used to fabricate reconstructed human epidermis or full-thickness skin models.

fabrication of pigmented human skin constructs[45]. A two-step bioprinting strategy was implemented; hierarchical porous collagen-fibroblast dermal matrices were first fabricated using macromolecular crowding, followed by pre-defined patterning of the primary human keratinocytes and melanocytes in highly-specific arrangement found in native human skin. The 3D bioprinted human skin constructs were then matured under optimal culture conditions to eventually achieve the 3D pigmented human skin constructs. The 3D printed human skin constructs in most studies showed high resemblance to native skin structure and they can serve as potential *in vitro* tissue models for toxicological testing.

3.1.3 Laser-based bioprinting

A recent *in vitro* study deposited 20 layers of fibroblasts (mouse NIH-3T3) and subsequent 20 layers of keratinocytes (human HaCaT) embedded in collagen gel onto a sheet of Matriderm® (decellularized dermal matrix)[33]. The presence of cadherin and connexin 43 in the epidermis indicated tissue morphogenesis and cohesion. Another study reported good graft-take of printed skin construct with the surrounding tissue and angiogenesis from the wound bed was observed after 11 days of transplantation[34].

3.1.4 Advantages and limitations of 3D bioprinting in skin toxicology testing

The bioprinting approach enables the rapid deposition of cells and biomaterials to form a monolayer of cells with homogeneous cell distribution[23] or fibroblast-laden dermal matrix[41,42] in a highly repeatable manner. Numerous works on skin bioprinting have been conducted over the past decade with the aim of achieving higher degree of complexity and functionality; patterning melanocytes for skin pigmentation[45,46], incorporating immune cells[47], and creating sacrificial channels for perfusable vascularized dermis[48] to create more biomimetic skin constructs that can potentially provide more accurate and reliable readouts for *in vitro* toxicology studies. The bioprinted skin constructs like its conventional manual-cast skin constructs showed some resemblance to the native skin in terms of histological and biochemical properties (Figure 2). The highly-automated fabrication platform provides a higher degree of consistency over conventional manual fabrication approach, which resulted in a less significant batch-to-batch variation that is critical for standardized and reproducible toxicology studies. An important aspect of the bioprinting process is to mitigate the effect of cell sedimentation effect during the printing process[49]; this can be achieved by increasing the polymer concentration[50,51] or the use of surfactants such as polyvinylpyrrolidone[52] to obtain a more consistent cellular output over time.

3.2 Automated deposition of test chemicals

The ability to accurately and reliably titrate different test substances directly onto the skin tissue models would be advantageous for toxicology testing by improving the efficiency and screening throughput for toxicology studies. The different test chemicals are commonly dissolved in dimethyl sulfoxide (DMSO), and compound dilutions are typically performed using manual dispensing approaches that are prone to human-based errors and limited in accuracy (≥0.1 μL). The accurate measurement of chemical concentration responses is critical for achieving reliable and reproducible toxicology testing results. The use of automated equipment can potentially reduce human error through automated compound serial dilution and dispensing and eliminates the aqueous intermediate dilution by allowing accurate low-volume titration of test chemicals directly to assay wells[53]. A study has reported the use of HP D300e digital dispenser to achieve small-volume dispensing (down to 13 pL) that allows concentration-response testing using the direct dilution paradigm[54]. The ultra-low volume dispensing capabilities of the HP D300e Digital Dispenser allows direct dilution of compounds from the DMSO solution in assay plates without the need for an intermediate compound dilution plate that streamlines the experimental process for determination of compound dose response (Figure 3). The results indicated that the HP D300 dispenser generates clean and reproducible results that correlate with those produced with conventional instruments such as the pin tool. The main advantage of direct dilution using automated dispensing machine is (i) elimination of serial dilution step, (ii) reduction of the compound consumption, (iii) prevention of compound cross-contamination due to the contact-free nature of direct dispensing, and (iv) reducing consumables such as tips and plates used in compound dilution. The use of such highly-automated dispensing machine can help to reduce human errors and also increase the accuracy of the measurement of the compound potency in a high-throughput manner.

3.3 Microfluidics: skin-on-a-chip platform

In recent years, there is an increasing trend of organ-on-a-chip applications as they represent a minimally functional unit that can recapitulate specific aspects of human physiology in a direct and controlled manner[55,56]. The first attempt to integrate 3D cell culture with microfluidics was introduced in 2003 and since then it has evolved into today's organ-on-a-chip platform[57]. The use of microfluidics platform enables the evaluation of pharmacological modulation with well-characterized drugs; the integration of automated flow and pressure control using micro-engineered pumps and valves facilitates the high throughput screening

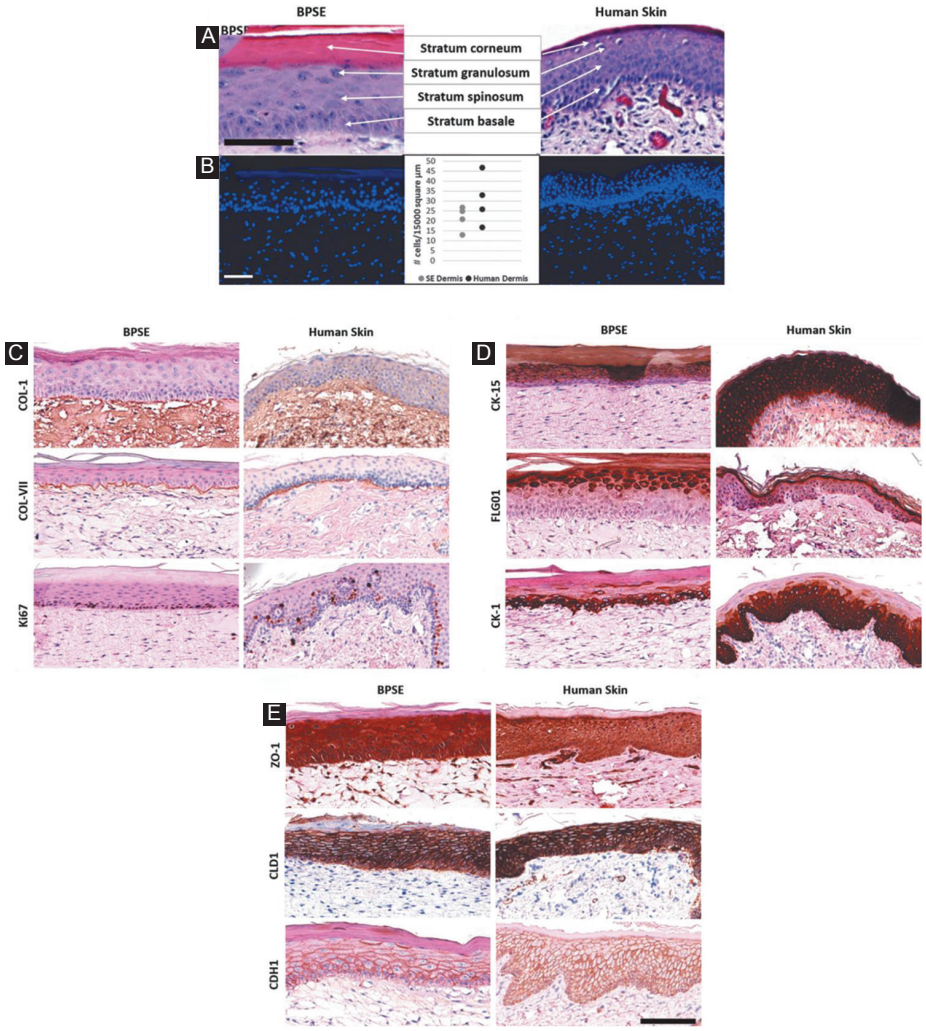


Figure 2. Histological and immunochemistry comparison of protein expression between bioprinted skin tissues and native human skin. (**A**) H and E staining, (**B**) DAPI staining of cell nuclei, (**C**) extracellular matrix proteins: Collagen I, VII and cell proliferation marker Ki67, (**D**) Epidermal differentiation proteins: Cytokeratin 15, filaggrin, cytokeratin 1, (**E**) Tight junction proteins: ZO-1, claudin I, e-cadherin. Scale bar = 100 µm. Reproduced with permission[40].

Figure 3. HP D300e digital dispenser that enables small volume dispensing for high-throughput deposition of test chemicals. Reproduced with permission from HP Inc.

and analysis for toxicology testing[58-60]. A recent study has reported the use of the microfluidic skin-on-a-chip platform for the long-term maintenance of FT human skin equivalent (HSE) comprising primary human skin cells and collagen matrix over PET membranes[61]. The design of the microfluidics chip is carefully constructed to provide a physiologically relevant blood residence times and at the same time enables the establishment of an air-liquid interface for tissue maturation and terminal differentiation of HSEs. The microfluidic skin-on-a-chip platform enables the pump-free, long-term maintenance of HSEs (up to 3 weeks with proliferating keratinocytes) and also significantly reduces the required quantity of both cells and media. The skin barrier function was evaluated by applying a solution of FAM-tagged oligonucleotides on the surface of the skin constructs. The measured concentration in the media showed that the permeability

of oligonucleotides through the HSE was not significantly different in week 1, 2, or 3, indicating the long-term maintenance of skin barrier function for up to 3 weeks. A similar study has highlighted the use of microfluidics platform with dynamic perfusion and facile control of the microenvironment can help to develop HSEs that better recapitulate the structure and functionalities of human skin as compared to conventional static culture systems[62]. The skin-on-a-chip platform not only improves epidermal morphogenesis and differentiation, deposition of basement membrane proteins and skin barrier functions but it also has the capability of performing real-time and high-throughput *in situ* assays without disassembling of the device or disruption of the culture. These studies have highlighted the importance of controlled and dynamic environment for achieving functional and biomimetic 3D skin tissue models using microfluidics platform. Another study has demonstrated the use of microfluidics skin-on-a-chip platform to achieve reproducible and highly-precise skin permeation testing[63]. Furthermore, high-throughput screening can be easily achieved by increasing the number of parallel microfluidic permeation arrays. Notably, there is flexibility of incorporating skin-on-a-chip platform with other functional organ mimetic to create a "human-on-chip" that can potentially provide an improved method to explore different routes of drug delivery (oral, aerosol or dermal) and evaluate the toxicity of different drug formulations in a high-throughput, multi-organ approach[64].

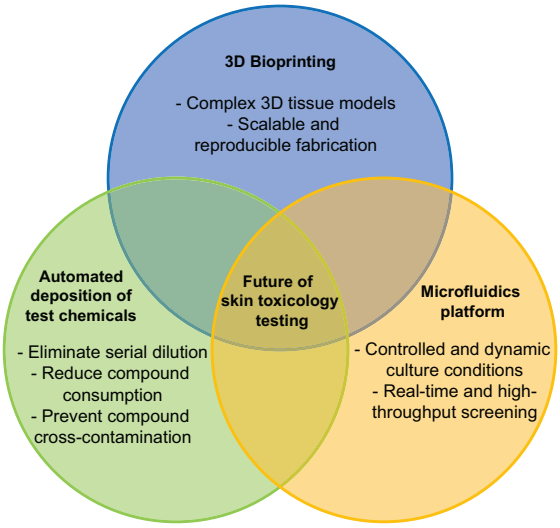


Figure 4. A conceptual figure of important components in skin toxicology testing platform. (i) 3D bioprinting facilitates fabrication of complex 3D tissue models in a scalable and reproducible manner, (ii) automated deposition of test chemicals eliminate serial dilution step, reduce compound consumption and prevent compound cross-contamination, (iii) microfluidics platform provides controlled and dynamic culture conditions for maturation of functional tissue models and facilitates real-time, high-throughput screening through multiple arrays in parallel configuration.

4. Outlook

The use of conventional 2D cell culture is unable to adequately recapitulate important *in vivo* cell-cell and cell-matrix interactions, and numerous cell types have express different phenotypes and genomic profiles in 2D versus 3D cell culture[65,66]. The use of 3D bioprinting technology enables highly-reproducible automated fabrication of 3D skin tissue models in an assay format; the rapid advancement in bio-printers and bio-inks[67-71] enables high precision, accuracy, and scalability of miniaturized skin-on-a-chip platforms that can be used in parallel for high-throughput screening.

Nevertheless, there are still numerous challenges with the integration of 3D skin tissue models for high-throughput screening. Most existing high-throughput screening is predominantly performed using 2D cell culture (monolayer or cell suspension) to yield screening data[72], and it is important to note that the high-throughput screening with 3D bioprinted skin tissue models would require improved and quantifiable outcomes[73]. The use of 3D skin tissue models brings about increased complexity that would require additional measurement of relevant outputs through assays and quantitative measures to ensure the reliability and accuracy of test outcomes. Hence, these outputs have to be modified and adapted for additional components within the 3D cell culture and at the same time evaluate the advantages and limitations of new protocol. Although these new changes may be radical and require significant modifications to existing well-established protocols, they are necessary for utilizing 3D physiological-relevant skin tissue models in more reliable and accurate toxicological testing.

5. Conclusion

The use of advanced technologies such as 3D bioprinting and microfluidics platform can bring about a paradigm shift in the way toxicology testing is performed (Figure 4). The proposed use of such highly-automated systems allows (i) the scalable fabrication of complex 3D skin tissue models, (ii) rapid and small-volume dispensing of test substances, and (iii) real-time monitoring and high-throughput screening for toxicology testing. The use of bioprinting enables the fabrication of miniaturized skin-on-a-chip models with multiple types of skin cells and biomaterials simultaneously with good spatial resolution and reproducibility and also facilitates small-volume dispensing of test substances in a rapid and accurate manner that can reduce human errors and also increase the accuracy of the measurement of the compound potency in a high-throughput manner. Finally, the advancement

in the microfluidics platform enables automated real-time monitoring and high-throughput screening that is advantageous for skin toxicology testing. The integration of these two advanced technologies (3D bioprinting and microfluidics system) would bring about significant improvements in the efficacy of toxicology testing.

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