

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



2D vs. 3D Cell Culture Models for *In Vitro* Topical (Dermatological) Medication Testing

Arezou Teimouri, Pollen Yeung and Remigius Agu

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79868>

Abstract

Due to ethical concerns regarding animal testing, alternative methods have been in development to test the efficacy and safety of pharmaceutical products and medications, specifically topical (dermatological) medications. Two-dimensional (2D) and three-dimensional (3D) skin cell cultures are examples of *in vitro* methods used as an alternative to animal testing. The first skin cells cultured were keratinocytes, a type of cell predominantly in the epidermal layer of the skin. However, with differences in skin characteristics and pathophysiology of different skin conditions, various skin cell cultures and models to better mimic these differences have been developed. These cell cultures include not only keratinocytes but also other skin cell types, such as fibroblasts, which are predominantly in the dermal layer of the skin, and certain immune cells and even melanocytes. To have a better understanding of the type of cell cultures used for testing dermatological products, this chapter aims to outline the differences between 2D and 3D skin cell cultures while considering the advantages and disadvantages of each culture. Different types of cell culture models used for wound healing and for inflammatory skin conditions such as psoriasis will also be discussed.

Keywords: cell culture, 2D cell culture, 3D cell culture, skin cell culture, *in vitro*, topical, dermatological, wound healing, psoriasis

1. Introduction

The growth of cells in an environment outside of an organism's body is referred to as a cell culture [1, 2]. Cells obtained from human tissues are often used for cell cultures to mimic the physiological and metabolic functions of humans for *in vitro* studies [3]. Cell cultures are

maintained in controlled environments and under controlled temperatures for cells to maintain their ability to survive and to grow [3]. For this reason, a medium for the cells to obtain the required nutrients for their growth is essential [2].

Cells obtained directly from living tissues and then cultured are referred to as primary cell cultures [4]. These cells have a short time frame of survival and scientific use [4]. Cells that have been modified to survive indefinitely with continuous cell division are referred to as either continuous cell lines [1] or immortalized cell lines [2]. Cancer cells are an example of the type of cells that may be used to create cell lines due to their genetic mutations and ability to divide continuously [2].

The very first cell cultures developed were of human skin which allowed for a better understanding of the physiological functions of the skin [5]. Cell cultures are available today in two-dimensional (2D) and three-dimensional (3D) forms. Cell cultures that grow in controlled flat environments, such as a Petri dish, are 2D cell cultures [6]. Cell cultures that are 3D, however, combine and shape cells into a 3D form using a surrounding medium or specialized conditions to help maintain a 3D figure [7]. *In vitro* skin cell cultures developed initially were primarily 2D with keratinocytes as the primary cell types [8, 9]. In the epidermal layer, the main cell types are keratinocytes, while fibroblasts are the main cell types in the dermal layer of the skin [10]. It is thus evident that an *in vitro* model consisting of both keratinocytes and fibroblasts is required to better mimic the physiological functions of the human skin, especially with relation to the wound healing properties of the skin [5]. There are therefore ongoing efforts to develop various *in vitro* skin models that more closely resemble the skin's physiological functions and that will allow for testing of dermatological products [8]. To have a better understanding of the type of cell cultures used for testing dermatological products, this chapter aims to outline the differences between 2D and 3D skin cell cultures while considering the advantages and disadvantages of each culture. Different types of cell cultures used for wound healing and for inflammatory skin conditions such as psoriasis will also be discussed.

2. 2D cell cultures

2D cell cultures have been the main type of cell cultures used by scientists in various fields for decades [6]. As explained in the introduction, cell cultures that grow in controlled flat environments, such as a Petri dish, are 2D cell cultures [6]. To maintain cells outside of an organism's body, it is important to have the correct equipment and supplies to ensure the provision of an environment in which cells can survive and divide [2, 3]. Equipment involved typically includes a biosafety hood for maintaining the safety of cultures and of the individuals working with the cultures, a cell incubator for correct storage with a temperature of 37°C and 5% levels of CO₂, a refrigerator for culture media and other supplies, and flasks or Petri dishes for placement of cells, amongst other required equipment [2, 3].

Besides having the correct equipment for adequate handling and storage of cell cultures, the culture medium or environment in which cell culture grow is also important. The cell culture

medium in 2D cell cultures initially consisted of blood plasma; however, over the years, synthetic medium with the right amount of nutrients and even antifungal and antibiotic ingredients have been developed [11]. Cell culture medium is expected to contain amino acids, a buffering system, vitamins, trace elements and more, to maintain cell viability [12]. Eagle's minimum essential medium (EMEM) and Dulbecco's modified Eagle's medium (DMEM) are two examples of cell culture media that have been developed [13]. Different cell types may require different culture media, and thus, the nutrient requirements may vary [13].

2.1. 2D skin cell cultures

The very first cell cultures developed were derived from human skin and were of primarily keratinocytes [5, 8, 9]. As the largest organ in the human body, [14] the skin consists of the epidermal and dermal layers [14, 15]. In the epidermal layer, the main cell types are keratinocytes, while fibroblasts are the main cell types in the dermal layer of the skin [10]. Initially, 2D skin cell cultures consisted primarily of keratinocytes [8, 9]. It was however determined that fibroblasts were essential for the growth of keratinocyte cultures [16] as fibroblasts are the cells responsible for secreting substances of the extracellular matrix such as collagen [17]. The primary fibroblast cell line, cultured by George Todardo and Howard Green, is known as the 3T3 cell line and was named based on its culturing method of transferring cultures every 3 days [16, 18]. The 3T3 cell line is typically used as a feeder layer in keratinocyte cultures [18]. HaCaT cells are a cell line of keratinocytes [19].

Prior to animal testing and clinical trials, traditionally, when testing pharmaceutical ingredients, the first step has been to test *in vitro* using 2D cell cultures [7]. This has however provided limitations with respect to testing as the results obtained from *in vitro* 2D cell culture studies have not translated to *in vivo* studies [7]. For this reason, *in vitro* 3D models have become increasingly popular as they are considered to more closely resemble *in vivo* processes [11, 20].

3. 3D cell cultures

Cell cultures that are 3D involve cells that are combined and shaped into a 3D form using a surrounding medium or specialized condition to help maintain the shape [7]. The equipment used for 2D cell cultures such as a biosafety hood, cell incubator with a temperature of 37°C and 95% O₂, refrigerator for culture medium storage and other supplies [2, 3] may also be used for 3D cell cultures. Supplies used for 3D cultures which differ from those in 2D cell cultures include the matrices, scaffolds, and proprietary plasticware for aggregate formation (e.g., AlgiMatrix, microplates, and multidishes) [21]. Other supplies used in 3D cell cultures may include cell reagents for determining cell health and viability, such as the live/dead Viability/Cytotoxicity Kit and CellTracker Deep Red Dye by ThermoFisher Scientific [21].

The techniques used in creating 3D cell cultures are typically divided into methods that use a scaffold and methods that do not (**Figure 1**) [22]. Methods that do not use a scaffold are typically considered to be a better representation of *in vivo* activity as the cells in this method aggregate on their own [22]. When cells come together and aggregate, they are referred to as

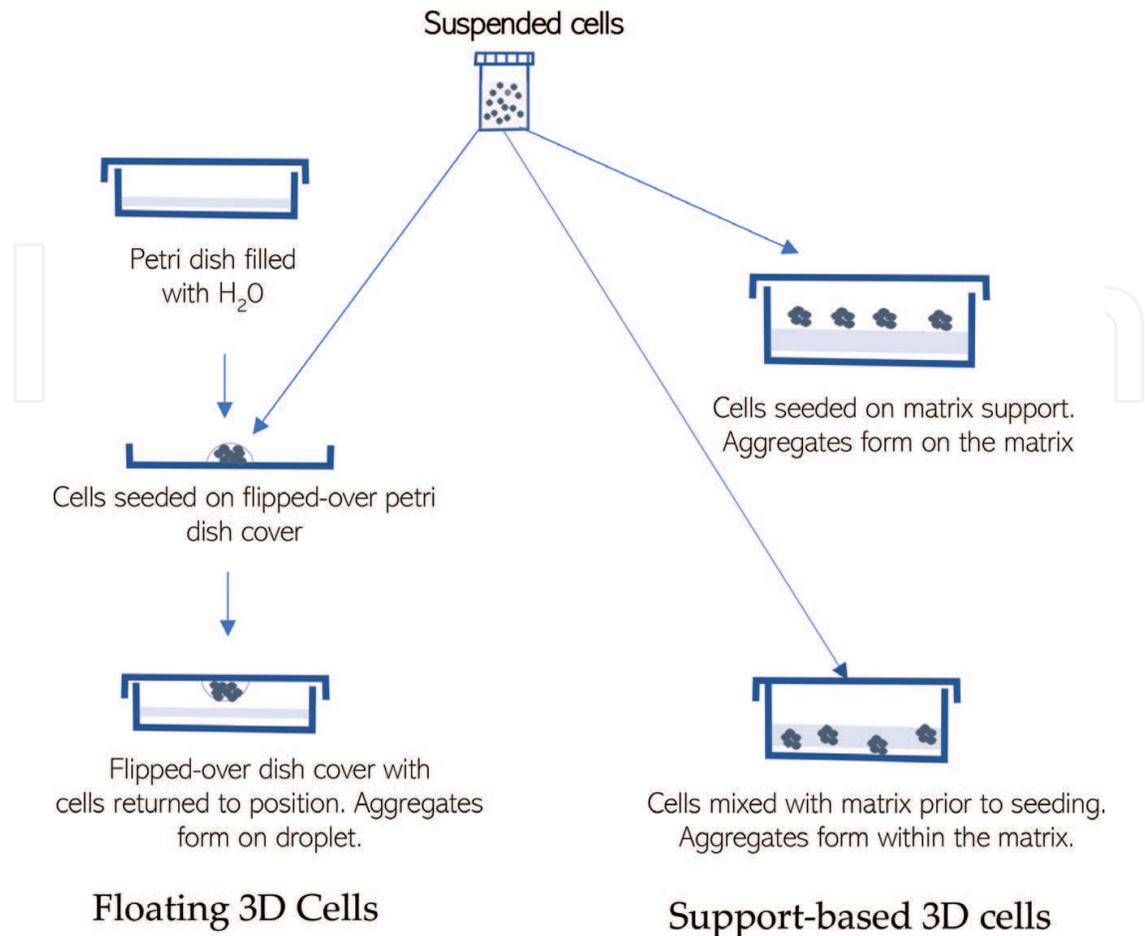


Figure 1. Illustration of general 3D tissue culture methods (floating and matrix-based). Commercially available well-plates and cell-support matrices are often used.

a spheroid in 3D cell cultures [22, 23]. Some of the methods used in 3D cell cultures that will be further explained include the hanging drop method, agitation-based approaches, forced-floating method, and the use of scaffolds [11, 24, 25]. Microfabricated 3D culture systems and bioprinting will also be discussed.

The hanging drop method provides a suspension of cells on a surface which is then inverted, allowing for the cells to hang and form cell-to-cell interactions and aggregate, forming spheroids [24, 25]. Another method is the utilization of continuous mixing methods in agitation-based approaches, which allows cells to aggregate to one another to form a 3D cell culture, as a container that is moving will not allow the cells to stick to the walls of that container [11]. Another method of preventing cells from sticking to the container in which they are placed in is using the forced-floating method which involves the placement of a nonadhesive coating such as poly-2-hydroxyethyl methacrylate (poly-HEMA) or agar, which will in turn force the cells to form interactions and aggregate as opposed to sticking to the surface of the container [11].

The use of scaffolds is another method of forming 3D cell cultures [11]. Scaffolds are materials with pores that allow cells to form aggregates [24]. Scaffolds can be prepared through different methods such as weaving and freeze drying [26] and are typically composed of

gelatin, collagen, agarose, and fibronectin, amongst other materials [27]. The idea of scaffolds and matrices is to provide an imitation of the extracellular matrix, allowing for cells to grow and differentiate using these materials [27]. The extracellular matrix consists of essential substances, such as proteins, and surrounds cells in a fluid form that operates as a natural scaffold for cellular growth and differentiation [28, 29]. As discussed previously, fibroblasts in the body are the cells responsible for secreting extracellular matrix and the substances within it such as collagen [17].

Another type of 3D cell culture method is the microfabricated systems, also referred to as cells on chips, which uses microtechnology to provide cells with the required growth environment that can be easily controlled and uses microfluidics for providing nutrients [30]. Microfluidics is the handling of fluids within the microliters range [31]. Microfabricated systems have the advantage of being highly reproducible and provide a better control of the cell culture environment [30]. Another advantage of microfabricated systems is greater cell-to-extracellular matrix interactions while requiring less cell culture [32].

Bioprinting is another method of developing 3D cell cultures. Bioprinting involves the printing of 3D systems by placing living cells in layers [33]. Bioprinting involves both a pre-processing stage as well as the printing stage [34]. The use of imaging diagnostics such as magnetic resonance imaging (MRI), computed tomography (CT), and X-rays to scan an image of the organ or tissue of interest is the first step involved in bioprinting and is part of the pre-processing stage [33, 34]. Selecting the materials such as biomaterials (i.e., biopolymers) as well as cell selection is also part of the preprocessing stage [33, 34]. The printing stage is then completed using different methods such as inkjet printing [33, 35, 36]. Inkjet printing uses bioink for the process of bioprinting and may include the use of cells as the bioink [37, 38].

3.1. 3D skin cell cultures

As discussed previously, the results obtained from *in vitro* 2D monolayer cell culture models have not translated to *in vivo* successes [7]. Thus, alternative methods for testing dermatological products have been sought out. According to an article by Bergers et al., [39] there are various 3D models that exist for a number of different dermatological conditions such as psoriasis and other autoimmune diseases. Skin cell cultures that are 3D have the advantages of containing a stratum corneum and thus the ability of testing pharmaceutical products on the stratum corneum as a skin barrier [40]. Another advantage to the use of 3D skin cell cultures is the longer cell culture use, which is usually between 10 and 30 days [40]. More recent human skin equivalent (HSE) cultures can even be used for up to 20 weeks [41]. The main disadvantage to the use of 3D skin cell cultures however is the cost associated with developing these cultures [40]. The different types of 3D skin cell cultures that will be discussed in detail are histocultures, human skin equivalents, on-chip skin cultures, and pigmented cell cultures. A discussion on bioprinting of skin constructs is also included.

3.1.1. Histocultures

Histocultures are cultures of intact tissues that were developed to better mimic *in vivo* skin responses [42]. The process of hair growth is an example of the ability of histocultures to

mimic *in vivo* processes, as this process occurs in histocultures of skin cells and allows for the testing of pharmaceutical products aimed at inhibiting or improving hair growth [42]. The process of histocultures involves growing skin tissues on a growth medium on its own or with the support of collagen [42, 43]. Research has shown that both epidermal and dermal cells as well as hair follicles maintain their functions and physiology in skin histocultures [44]. Histocultures have thus successfully been used as *in vitro* models for testing dermatological products, particularly with respect to toxicity screening [42, 44].

3.1.2. Human skin equivalents

In order to test pharmaceutical products on the skin *in vitro* and provide suitable skin replacement options for patients with various skin conditions, such as burn victims, HSEs were created [45]. HSEs are 3D cell culture models created from various human skin cells and materials that mimic the extracellular matrix [45] and are created as either epidermal equivalents, dermal equivalents or skin equivalents consisting of both layers [8, 45]. Skin equivalents that are used as replacement skin for patients, known as skin grafts, are useful in conditions where the skin cannot adequately heal on its own, such as in the case of a burn victim or chronic ulcers/wounds [10].

An example of the method involved in developing full thickness human skin equivalents includes the isolation of keratinocytes and fibroblasts from donor skin which are then cultured in medium at 37°C at 5% CO₂ [46]. Keratinocytes are then incubated for 2 days following their transfer onto a de-epidermized dermal equivalent and then placed at air-liquid interface for the development of full thickness human skin equivalent [46].

Currently available HSEs are primarily epidermal substitutes composed of keratinocytes in 3D cell culture models such as Epiderm™, SkinEthic RHE™, and EpiSkin™, which are used for pharmaceutical and cosmetic testing of skin irritation from topical products [45, 47, 48]. As these skin substitutes are derived of only the epidermal layer and primarily keratinocytes, it limits their use for testing of products related to particular types of skin conditions that involve the immune system, including testing of products for wound healing [47]. Full thickness models consisting of both the epidermal and dermal layers are thus beneficial [8]. One such model is the full thickness model developed by MatTeck Corporation, the EpidermFT™, which includes both normal human keratinocytes and fibroblasts cultured into a 3D model of several layers of the epidermis and dermis to more closely mimic human skin [49].

3.1.3. On-chip skin culture

Microfabricated systems, also referred to as cells on chips, are a type of 3D cell culture that uses microtechnology to provide cells with the required growth environment that can be easily controlled [30]. On-chip culturing also uses microfluidics for providing nutrients [30]. In order to mimic *in vivo* properties of the skin, an on-chip skin culture was created by Lee et al. [50] and is composed of a microfabricated cell culture of keratinocytes (HaCaT cells) and fibroblasts with microfluidic channels to mimic vasculature. This model provides an opportunity for dermatological medication testing on a microfabricated cell system as growth and differentiation of skin cells were made possible through this model [50]. Another on-chip skin

culture model with the ability to mimic vasculature is the model developed by Mori et al. [51], which includes vascular channels attached to an external perfusion system and can be used to test vascular absorption of topical products.

A comparison of on-chip cell cultures to traditional transwell skin cultures was completed by Song et al., [52] in which a static (no flow) chip, dynamic (flow, based on a gravity flow system) chip, and transwell skin equivalents were compared. The comparison revealed that the static chip was not the ideal method as differentiation of skin did not occur and the epidermis was not attached by the end of 1 week [52]. It is thought that this is a result of lack of flow or perfusion in the static chip and thus insufficient flow of nutrients for the growth of the cells [52]. Thus an advantage to using microfabricated skin cell cultures vs. typical 3D cell cultures is the provision of microfluidics which can aid in the supply of essential nutrients for the growth and differentiation of skin cells as well as more closely mimicking *in vivo* processes such as vasculature [51, 52].

3.1.4. Pigmented skin cell cultures

A reconstructed pigmented human skin model was developed by Duval et al. [53]. Using a dermal equivalent with a steel ring on top, seeding of human keratinocytes and melanocytes was completed [53]. The culture developed a monolayer after a period of 1 week in medium after which it was exposed to air for another week to allow differentiation of keratinocytes [53]. This 3D model allows for a better understanding of the interactions between melanocytes, keratinocytes, and fibroblasts [53] and also allows for a pigmented human skin model that could potentially be used for testing of pharmaceutical products on pigmented skin.

3.1.5. Bioprinting of skin constructs

The process of bioprinting explained above is still the same process followed for bioprinting of skin constructs. An important consideration, however, in the pre-processing stage, is that the imaging equipment used ideally should be able to differentiate skin color [34]. Also, with respect to cell selection, keratinocytes are the primary cell types used for bioprinting of skin cells [34]. The advantages to using bioprinting of skin constructs includes greater accuracy in placement of cells and extracellular matrix as well as having the potential of imbedding vasculature in the skin construct as bioprinting of vasculature is also possible [54]. Skin constructs made through bioprinting are also considered to have great plasticity [54]. Skin bioprinting may also be used for developing 3D models for drug testing, such as diseased skin models, and are believed to provide more uniform models compared to manually developed skin models [55]. The main disadvantage of bioprinting for skin constructs is the high cost associated with its use [54].

4. Skin cell cultures for wound healing

Wound healing is a physiological process that consists of four phases: hemostasis, inflammation, proliferation, and remodeling [56]. In the first phase, after a wound injury, hemostasis,

platelets are activated and migrated to the site of injury [57]. The second phase, inflammation, begins about 1 day postinjury and inflammatory mediators such as histamine are released, providing the typical traits of inflammation such as heat and swelling [57]. Proliferation is the phase in which granulation tissue forms at the site of injury and after which re-epithelization occurs [58]. In the last phase, remodeling of the tissue occurs to improve the strength of the skin tissue, which is only ever within 80% of the original tissue's strength [58]. The normal wound healing process could be affected, however, leading to chronic ulcers or excessive wound healing resulting in hypertrophic scars [59]. For this reason, topical agents to improve wound healing or reduce scarring may be of interest and as such *in vitro* testing models for wound healing will be discussed.

Both 2D and 3D skin cell cultures are available as wound healing models [60]. 2D wound healing models involve the creation of a site of injury in a monolayer of skin cells, either through mechanical or chemical means, in which cells then migrate to the site of injury [60, 61]. Cells in 2D monolayer cultures are thought to adhere to the flat environments, such as a Petri dish, in which they are cultured and will therefore migrate to areas of free space within the dish, an activity thought to mimic *in vivo* migration involved in cell differentiation [62]. One method of mechanical introduction of a wound to a 2D cell culture is through the scratch assay that utilizes materials such as pipette tips or needles to introduce a wound or scratch into the monolayer cell culture [63, 64]. Images of the wound are taken within set time frames to assess the migration of cells [63]. Typically, however, it is difficult to ensure wounds that are equal in size using this method [63], and thus for this reason, testing of pharmaceutical products on these types of cultures are not ideal. 2D skin cell cultures also lack essential functions that could mimic *in vivo* processes, such as immune functionality and blood perfusion [65]. 3D wound healing models have thus attempted to more closely mimic *in vivo* wound healing processes and are available as histocultures or HSEs.

Histocultures are cultures of intact tissues, consisting of more than one type of skin cell, such as neutrophils and other cells involved in wound healing, and are thus able to better mimic *in vivo* skin responses of wound healing [42, 43]. HSEs, on the other hand, are 3D cell culture models created from various human skin cells and materials that mimic the extracellular matrix [45] and are created as either epidermal equivalents, dermal equivalents or skin equivalents consisting of both layers [8, 45]. Some examples of 3D wound healing skin cell cultures and HSEs are described below.

A 3D wound healing skin equivalent developed by Herman et al. [66] included capillary endothelial cells which were capable of creating a similar formation to *in vivo* capillaries in the skin. This wound healing model involved several different cells, such as keratinocytes and epithelial cells, and used 3D matrices composed of Matrigel™ and collagen [66]. As angiogenesis is involved in the process of wound healing [66], this model is an excellent example of improved 3D wound healing cell culturing protocols to more closely mimic *in vivo* wound healing processes.

Another example of a 3D skin cell culture model that could be used for wound healing was reported by Sidgwick et al. [67]. This model allows for the immersion of biopsies in Williams E culture media with the epidermal layer of the skin uncovered and uses whole tissue biopsies

in 24-well cell plates with transwell inserts [67]. This model allows application of topical medications to the uncovered epidermal layer and is thus useful for testing of pharmaceutical products, including those used for transdermal drug delivery [67].

The skin substitutes derived of only the epidermal layer and primarily keratinocytes, however, are limited in their use for testing of products related to particular types of skin conditions that involve the immune system, including the testing of products for wound healing [47]. Full thickness models consisting of both the epidermal and dermal layers are thus beneficial [8]. One such model is the full thickness model developed by MatTeck Corporation, the EpidermFT™ which includes both normal human keratinocytes and fibroblasts cultured into a 3D model of several layers of the epidermis and dermis to more closely mimic human skin [49]. This kit can be purchased with a wound healing assay kit [49] and is thus a commercially available 3D model for testing of dermatological products aimed at wound healing.

It is thus evident from the examples provided in this section that 3D wound healing models more closely mimic *in vivo* wound healing processes and thus may be a better choice for testing of pharmaceutical products aimed at wound healing. Wound healing models are also commercially available which may improve the use of *in vitro* models as a replacement for animal testing.

5. Skin cell cultures for psoriasis

As an inflammatory skin condition that involves the immune system, psoriasis has the characteristic appearance of silver scales that arise from increased proliferation of the keratinocytes in the epidermal layer [68, 69]. Psoriasis is typically treated with topical steroid medications or with vitamin D analogue medications such as calcipotriol as well as with moisturizing agents [68]. The treatment of psoriasis also includes systemic medications that suppress the immune system such as methotrexate and cyclosporine as well as biologic drugs [68, 70].

Psoriasis has been linked to various mental health illnesses such as anxiety and depression which are thought to result from having a chronic visible skin condition [71], and therefore the need for developing new pharmaceutical products and testing of these products is evident. As psoriasis is primarily treated with topical medications [70], having *in vitro* cell cultures or models for testing the safety and efficacy of these topical medications is essential.

Skin cell cultures that are 2D for dermatological conditions such as psoriasis and other autoimmune disorders exist [39]. 2D cell cultures of psoriasis initially consisted of psoriatic keratinocytes; however, this proved to be an ineffective model for psoriasis as the cells were not able to grow and lost their psoriatic genes with time [72]. As a result, alternative methods such as adding cytokines to normal human keratinocytes to induce psoriatic features were developed [73]. As stated previously, however, testing of medications on 2D cell culture models does not always translate to *in vivo* responses [7], and thus, 3D models to better mimic *in vivo* responses for testing of topical medications for psoriasis have been developed.

Barker et al. [74] introduce an *in vitro* model of psoriatic human skin, whereby a monolayer cell culture of psoriatic keratinocytes on top of collagen and fibroblasts from the dermis was

created, onto which an epidermal layer was formed approximately 3 weeks later. Similarly, a 3D skin substitute using psoriatic skin cells was developed by Jean et al. [75] with either both psoriatic keratinocytes and fibroblasts or only one type of psoriatic cells (either keratinocytes or fibroblasts were psoriatic). Skin biopsies were obtained from patients with plaque psoriasis, and keratinocytes were extracted and seeded on to mouse fibroblasts and incubated at a temperature of 37°C [75]. For skin substitutes, ascorbic acid was used to culture the fibroblasts which formed dermal sheets that were altered to create a dermal layer onto which keratinocytes were then seeded to form an epidermal layer [75]. This method is the self-assembly method as the fibroblasts release their own extracellular matrix to maintain their growth [75]. A serum-free 3D psoriatic skin cell culture was similarly developed by Duque-Fernandez et al. [66]. This method is similar to the one presented by Jean et al. [75] and may be due to the fact that some authors are the same in both articles. The model by Duque-Fernandez et al. [76] was even used for testing percutaneous permeation of benzoic acid, caffeine, and hydrocortisone, using a Franz-diffusion cell method. This was compared to healthy skin substitutes and revealed that the psoriatic skin substitute had a greater permeability response to the three compounds [76].

For safety and efficacy testing of medications for psoriasis, commercially available 3D *in vitro* models also exist. Two examples of such models are the Psoriasis Skin Model by Creative Bioarray [77] and the model by MatTek Corporation [78]. According to Creative Bioarray [77], their skin model more closely mimics *in vivo* psoriatic skin vs. typical cell cultures as it allows for cell differentiation and can be used for testing of pharmaceutical products. The psoriasis model by MatTek Corporation can also be used for testing of dermatological products as it is a 3D model composed of psoriatic fibroblasts while using normal human epidermal keratinocytes and is capable of mimicking *in vivo* psoriatic responses such as basal cell proliferation [78]. These examples were presented for a better understanding of the methods used in developing psoriatic human skin equivalents as well as the commercially available models for testing the safety and efficacy of dermatological products used for psoriasis.

6. 2D vs. 3D cell cultures: morphology and other characteristics

Cell cultures that are 2D and 3D clearly differ with respect to their cell morphology as well as other characteristics. Cell cultures that grow in controlled flat environments, such as a Petri dish, are 2D cell cultures [6], and as such have cells that are flat in morphology [7]. Cell cultures that are 3D involve cells that are combined and shaped into a 3D spheroids using surrounding milieu or specialized conditions [7]. Cell viability also differs between 2D and 3D cell cultures. For example, 2D breast cancer cell cultures showed greater cell viability than 3D breast cancer cells [79]. Drug efficacy may also differ in 2D and 3D cell cultures [79].

7. 2D vs. 3D cell cultures: advantages and disadvantages

Monolayer 2D cell cultures do not always provide an accurate representation of *in vivo* processes [7]. The use of a single cell type [80], such as the use of keratinocytes only in skin cell

cultures, [8] or the lack of interactions in 2D cell cultures of cell-to-cell or cell-to-extracellular matrix may be possible causes [5, 80]. However, advantages to using a 2D cell culture include ease of use, cost, and abundant scientific literature surrounding its use [81, 82].

3D cell cultures, on the other hand, provide a better imitation of *in vivo* processes as they allow for better understanding of the interactions that take place between cell-to-cell and cell-to-extracellular matrix [7]. Cell cultures that are 3D have also revealed to possess gene expression abilities, thus mimicking *in vivo* processes [83]. However, regardless of their improvements vs. the 2D cell cultures, typically 3D cell cultures still lack in their ability to provide key functions such as cellular waste removal [7]. Microfabricated cell cultures or on-chip cell cultures are advantageous in this instance as they provide microfluidic channels that enable the flow of nutrients and waste removal as well as the ability to mimic vasculature [51, 52]. Thus microfabricated 3D cell cultures are arguably the *in vitro* method of cell cultures that most closely mimic *in vivo* processes [50].

With respect to skin cell cultures, those that are 3D have the advantages of containing a stratum corneum and thus the ability of testing pharmaceutical products on the stratum corneum

	Advantages	Disadvantages
2D cell cultures	<ul style="list-style-type: none"> Ease of use, cost and abundant scientific literature surrounding use [81, 82] 	<ul style="list-style-type: none"> Do not always provide an accurate representation of <i>in vivo</i> processes [7] Use of a single cell type, [80] for example with skin cell cultures, the use of keratinocytes only [8] No cell-to-cell or cell-to-extracellular matrix interactions [5, 80]
3D cell cultures	<ul style="list-style-type: none"> Better imitation of <i>in vivo</i> processes [7] Better understanding of the interactions that take place between cell-to-cell and cell-to-extracellular matrix [7] Possess gene expression abilities, thus mimicking <i>in vivo</i> processes [83] Microfabricated cell cultures or on-chip cell cultures provide microfluidic channels that enable the flow of nutrients and waste removal as well as the ability to mimic vasculature [51, 52] 3D skin cultures have a stratum corneum and thus the ability of testing pharmaceutical products on the stratum corneum as a skin barrier [40] Longer skin cell culture use between 10 and 30 days [40]; more recent human skin equivalent (HSE) cultures can even be used for up to 20 weeks [41] 3D bioprinting provides greater accuracy in placement of cells and extracellular matrix, potential of imbedding vasculature in the skin construct and have great plasticity [54] 3D bioprinted skin models provide more uniform models vs. manually developed skin models [55] 	<ul style="list-style-type: none"> Typically lack in their ability to provide key functions such as cellular waste removal [7] Cost associated with developing cultures [40, 54]

Table 1. Summary of the advantages and disadvantages of 2D vs. 3D cell cultures.

as a skin barrier [40]. Other advantages to the use of 3D skin cell cultures include longer cell culture use between 10 and 30 days [40]. More recent human skin equivalent (HSE) cultures can even be used for up to 20 weeks [41]. Skin substitutes that are derived from only the epidermal layer and primarily keratinocytes are limited in their use for testing of products related to particular types of skin conditions that involve the immune system, including testing of products for wound healing [47]. Full thickness models consisting of both the epidermal and dermal layers are thus beneficial [8]. 3D bioprinting of skin constructs are also advantageous as they provide a greater accuracy in placement of cells and extracellular matrices as well as having the potential of imbedding vasculature in the skin construct as bioprinting of vasculature is also possible [54]. Skin constructs made through bioprinting are also considered to have high plasticity [54]. Skin bioprinting may also be used for developing 3D models for drug testing, such as diseased skin models, and are considered to provide more uniform models vs. manually developed skin models [55]. The primary disadvantage to the use of 3D skin cell cultures, however, is the cost associated with developing these cultures [40, 54]. See **Table 1** for a summary of the information presented in this section.

8. Conclusion

Testing the efficacy and safety of dermatological products and medications using *in vitro* methods such as cell cultures can be considered as a replacement to animal testing. Cell cultures currently exist in both 2D and 3D forms. The very first cell cultures developed were of human skin which allowed for a better understanding of the physiological functions of the skin [5]. *In vitro* skin cell cultures developed initially were primarily 2D with keratinocytes as the primary cell types used [8, 9]. However, it became evident that an *in vitro* model consisting of both keratinocytes and fibroblasts is required to better mimic the physiological functions of the human skin, especially with relation to the wound healing properties of the skin [5]. For this reason, 3D cell cultures that allowed greater cell-to-cell and cell-to-extracellular matrix interactions were developed [7].

It is evident from the information presented in this chapter that 3D skin cell cultures can more closely mimic *in vivo* processes vs. 2D skin cell cultures for both wound healing and psoriasis. In addition, full thickness 3D skin equivalents that consist of both an epidermal and dermal layer are a better representation of the human skin [8]. Microfabricated cell systems, however, provide arguably an even better model for mimicking *in vivo* skin processes including vasculature and thus allowing for the testing of absorption of pharmaceutical products [50–52]. Additionally, 3D bioprinting provides greater accuracy in placement of cells and extracellular matrices along with the potential of imbedding vasculature in the skin construct as well as having great plasticity [54]. 3D bioprinted skin models also provide more uniform models vs. manually developed skin models [55].

Conflict of interest

No conflicts of interest.

Author details

Arezou Teimouri, Pollen Yeung and Remigius Agu*

*Address all correspondence to: remigius.agu@dal.ca

Dalhousie University, Halifax, Canada

References

- [1] ThermoFisher Scientific. Introduction to Cell Culture. Available from: <https://www.thermofisher.com/ca/en/home/references/gibco-cell-culture-basics/introduction-to-cell-culture.html> [Accessed: 25-04-2018]
- [2] Carter M, Shieh JC. Chapter 13—Cell culture techniques. In: Carter M, Shieh JC, editors. *Guide to Research Techniques in Neuroscience*. New York: Academic Press; 2010. pp. 281-296
- [3] Philippeos C, Hughes RD, Dhawan A, Mitry RR. Introduction to cell culture. *Methods in Molecular Biology*. 2012;**806**:1
- [4] MicroscopeMaster. Cell Culture—Basics, Techniques and Media. Available from: <https://www.microscopemaster.com/cell-culture.html> [Accessed: 25-04-2018]
- [5] Coulomb B, Dubertret L. Skin cell culture and wound healing. *Wound Repair and Regeneration*. 2002;**10**(2):109-112
- [6] Duval K, Grover H, Han LH, Mou Y, Pegoraro AF, Fredberg J, et al. Modeling physiological events in 2D vs. 3D cell culture. *Physiology (Bethesda)*. 2017;**32**(4):266-277
- [7] Edmondson R, Broglie J, Adcock A, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay and Drug Development Technologies*. 2014;**12**(4):207-218
- [8] MacNeil S. Progress and opportunities for tissue-engineered skin. *Nature*. 2007;**445**(7130):874-880
- [9] Rheinwald JG, Green H. Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. *Nature*. 1977;**265**(5593):421
- [10] Nicholas MN, Jeschke MG, Amini-Nik S. Methodologies in creating skin substitutes. *Cellular and Molecular Life Sciences*. 2016;**73**(18):3453-3472
- [11] Breslin S, O'Driscoll L. Three-dimensional cell culture: The missing link in drug discovery. *Drug Discovery Today*. 2013;**18**(5):240-249
- [12] Davis J. *Animal Cell Culture: Essential Methods*. Chichester, West Sussex: Wiley; 2011
- [13] Celis JE. *Cell Biology, Four-Volume Set: A Laboratory Handbook*. Amsterdam: Academic Press; 2006

- [14] Casey G. Physiology of the skin. *Nursing Standard* (through 2013). 2002;**16**(34):47-51; quiz 53, 55
- [15] Gaboriau HP, Murakami CS. Skin anatomy and flap physiology. *Otolaryngologic Clinics of North America*. 2001;**34**(3):555-569
- [16] Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell*. 1975;**6**:331-344
- [17] PromoCell. Fibroblasts. 2015. Available from: <https://www.promocell.com/products/human-primary-cells/fibroblasts/#> [Accessed: 10-05-2018]
- [18] Molecular Devices. At the Cell Counter: IH3T3 Cells. Available from: <https://www.moleculardevices.com/cell-counter-nih3t3-cells> [Accessed: 10-05-2018]
- [19] Seo M, Kang TJ, Lee CH, Lee A, Noh M. HaCaT keratinocytes and primary epidermal keratinocytes have different transcriptional profiles of cornified envelope-associated genes to T helper cell cytokines. *Biomolecules & Therapeutics*. 2012;**20**(2):171-176
- [20] Tung J. Welcome to the 3rd Dimension. 2017. Available from: <https://sciencellonline.com/blog/welcome-to-the-3rd-dimension/> [Accessed: 26-04-2018]
- [21] ThermoFisher Scientific. 3D Cell Culture Product Selection Guide for Organoids and Spheroids. 2018. Available from: <http://assets.thermofisher.com/TFS-Assets/BID/brochures/3D-Organoid-Spheroid-Product-Selection-Guide-Global.pdf> [Accessed: 20-06-2018]
- [22] MilliporeSigma. 3D-Cell culture: An Overview of Advanced Tools and Techniques. Available from: <https://www.sigmaaldrich.com/technical-documents/articles/biology/3d-cell-culture-technology.html> [Accessed: 26-04-2018]
- [23] Pampaloni F, Reynaud EG, Stelzer EHK. The third dimension bridges the gap between cell culture and live tissue. *Nature Reviews Molecular Cell Biology*. 2007;**8**(10):839-845
- [24] Lin R, Chang H. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnology Journal*. 2008;**3**(9):1285-1285
- [25] Fennema E, Rivron N, Rouwkema J, van Blitterswijk C, de Boer J. Spheroid culture as a tool for creating 3D complex tissues. *Trends in Biotechnology*. 2013;**31**(2):108-115
- [26] Moroni L, de Wijn JR, van Blitterswijk CA. Integrating novel technologies to fabricate smart scaffolds. *Journal of Biomaterials Science. Polymer Edition*. 2008;**19**(5):543-572
- [27] Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FDP. 3D cell culture systems: Advantages and applications. *Journal of Cellular Physiology*. 2015;**230**(1):16-26
- [28] Guruswamy M. The extracellular matrix. *Young Scientists Journal*. 2014;**16**:35
- [29] Brown BN, Badylak SF. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. *Translational Research*. 2014;**163**(4):268-285
- [30] El-Ali J, Sorger PK, Jensen KF. Cells on chips. *Nature*. 2006;**442**(7101):403

- [31] Whitesides GM. The origins and the future of microfluidics. *Nature*. 2006;**442**(7101):368-373
- [32] Wu M, Huang S, Lee G. Microfluidic cell culture systems for drug research. *Lab on a Chip*. 2010;**10**(8):939
- [33] Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nature Biotechnology*. 2014;**32**(8):773-785
- [34] Vijayavenkataraman S, Lu WF, Fuh JYH. 3D bioprinting of skin: a state-of-the-art review on modelling, materials, and processes. *Biofabrication*. 2016;**8**(3):032001
- [35] Nakamura M, Kobayashi A, Takagi F, Watanabe A, Hiruma Y, Ohuchi K, et al. Bio-compatible inkjet printing technique for designed seeding of individual living cells. *Tissue Engineering*. 2005;**11**(11-12):1658
- [36] Xu T, Jin J, Gregory C, Hickman JJ, Boland T. Inkjet printing of viable mammalian cells. *Biomaterials*. 2005;**26**(1):93-99
- [37] Wüst S, Müller R, Hofmann S. Controlled positioning of cells in biomaterials-approaches towards 3D tissue printing. *Journal of Functional Biomaterials*. 2011;**2**(3):119-154
- [38] Guifang G, Ying H, Schilling AF, Karen H, Xiaofeng C. Organ bioprinting: Are we there yet? *Advanced Healthcare Materials*. 2018;**7**(1):1701018
- [39] Bergers LIJC, Reijnders CMA, van den Broek LJ, Spiekstra SW, de Gruijl TD, Weijers EM, et al. Immune-competent human skin disease models. *Drug Discovery Today*. 2016;**21**(9):1479-1488
- [40] Roguet R. Use of skin cell cultures for *in vitro* assessment of corrosion and cutaneous irritancy. *Cell Biology and Toxicology*. 1999;**15**(1):63-75
- [41] El Ghalbzouri A, Commandeur S, Rietveld MH, Mulder AA, Willemze R. Replacement of animal-derived collagen matrix by human fibroblast-derived dermal matrix for human skin equivalent products. *Biomaterials*. 2009;**30**(1):71-78
- [42] Hoffman RM. Histocultures and their use. In: *Encyclopedia of Life Sciences (ELS)*. Chichester: John Wiley & Sons Ltd; October 2010. DOI: 10.1002/9780470015902.a0002573.pub2
- [43] Ali N, Hosseini M, Vainio S, Taïeb A, Cario-André M, Rezvani HR. Skin equivalents: Skin from reconstructions as models to study skin development and diseases. *The British Journal of Dermatology*. 2015;**173**(2):391-403
- [44] Li LN, Margolis LB, Hoffman RM. Skin toxicity determined *in vitro* by three-dimensional, native-state histoculture. In: *Proceedings of the National Academy of Sciences of the United States of America*. 1991;**88**(5):1908-1912
- [45] Zhang Z, Michniak-Kohn B. Tissue engineered human skin equivalents. *Pharmaceutics*. 2012;**4**(1):26-41
- [46] Xie Y, Rizzi S, Dawson R, Lynam E, Richards S, Leavesley D, et al. Development of a three-dimensional human skin equivalent wound model for investigating novel wound healing therapies. *Tissue Engineering Part C: Methods*. 2010;**16**(5):1111-1123

- [47] Akagi T, Nagura M, Hiura A, Kojima H, Akashi M. Construction of three-dimensional dermo-epidermal skin equivalents using cell coating technology and their utilization as alternative skin for permeation studies and skin irritation tests. *Tissue Engineering. Part A*. 2017;**23**(11-12):481
- [48] Matsusaki M, Fujimoto K, Shirakata Y, Hirakawa S, Hashimoto K, Akashi M. Development of full-thickness human skin equivalents with blood and lymph-like capillary networks by cell coating technology. *Journal of Biomedical Materials Research Part A*. 2015;**103**(10):3386-3396
- [49] MatTek Corporation. EpidermFT™. Available from: <https://www.mattek.com/products/epidermft/> [Accessed: 10-05-2018]
- [50] Lee S, Jin S, Kim Y, Sung G, Chung J, Sung J. Construction of 3D multicellular microfluidic chip for an *in vitro* skin model. *Biomedical Microdevices*. 2017;**19**(2):1-14
- [51] Mori N, Morimoto Y, Takeuchi S. Skin integrated with perfusable vascular channels on a chip. *Biomaterials*. 2017;**116**:48-56
- [52] Song HJ, Lim HY, Chun W, Choi KC, Lee T, Sung JH, et al. Development of 3D skin-equivalent in a pump-less microfluidic chip. *Journal of Industrial and Engineering Chemistry*. 2018;**60**: 355-359
- [53] Duval C, Chagnoleau C, Pouradier F, Sextius P, Condom E, Bernerd F. Human skin model containing melanocytes: Essential role of keratinocyte growth factor for constitutive pigmentation—functional response to alpha-melanocyte stimulating hormone and forskolin. *Tissue Engineering Part C: Methods*. 2012;**18**(12):947-957
- [54] He P, Zhao J, Zhang J, Li B, Gou Z, Gou M, et al. Bioprinting of skin constructs for wound healing. *Burns & Trauma*. 2018;**6**(1):5. <http://doi.org/10.1186/s41038-017-0104-x>
- [55] Vultur A, Schanstra T, Herlyn M. The promise of 3D skin and melanoma cell bioprinting. *Melanoma Research*. 2016;**26**(2):205-206
- [56] Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M. Biology and biomarkers for wound healing. *Plastic and Reconstructive Surgery*. 2016;**138**(3 Suppl):18S-28S
- [57] Chin GA, Diegmann RF, Schultz GS. 3 Cellular and molecular regulation of wound healing. In: Falabella A, Kirsner R, editors. *Wound Healing*. Baton Rouge: Chapman and Hall/CRC; 2005. pp. 17-37
- [58] Adams SB, Sabesan VJ, Easley ME. Wound healing agents. *Critical Care Nursing Clinics of North America*. 2012;**24**(2):255-260
- [59] Wang P, Huang B, Horng H, Yeh C, Chen Y. Wound healing. *Journal of the Chinese Medical Association*. 2018;**81**(2):94-101
- [60] Stamm A, Reimers K, Strauß S, Vogt P, Scheper T, Pepelanova I. *In vitro* wound healing assays—state of the art. *BioNanoMaterials*. 2016;**17**(1-2):79-87

- [61] Jonkman JEN, Cathcart JA, Xu F, Bartolini ME, Amon JE, Stevens KM, et al. An introduction to the wound healing assay using live-cell microscopy. *Cell Adhesion & Migration*. 2014;**8**(5):440-451
- [62] Keese C, Wegener J, Walker S, Giaever I. Electrical wound-healing assay for cells *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;**101**(6):1554-1559
- [63] Liang C-C, Ann YP, Guan J-L. *In vitro* scratch assay: A convenient and inexpensive method for analysis of cell migration *in vitro*. *Nature Protocols*. 2007;**2**(2):329
- [64] Belete H, Godin L, Stroetz R, Hubmayr R. Experimental models to study cell wounding and repair. *Cellular Physiology and Biochemistry*. 2010;**25**(1):71-80
- [65] Klicks J, von Molitor E, Ertongur-Fauth T, Rudolf R, Hafner M. *In vitro* skin three-dimensional models and their applications. *Journal of Cellular Biotechnology*. 2017;**3**(1): 21-39
- [66] Herman IM, Leung A. Creation of human skin equivalents for the *in vitro* study of angiogenesis in wound healing. In: Murray C, Martin S, editors. *Angiogenesis Protocols: Second Edition*. Totowa, NJ: Humana Press; 2009. pp. 241-248
- [67] Sidgwick G, McGeorge D, Bayat A. Functional testing of topical skin formulations using an optimised ex vivo skin organ culture model. *Archives of Dermatological Research*. 2016;**308**(5):297-308
- [68] Clarke P. Psoriasis. *Australian Family Physician*. 2011;**40**(7):468-473
- [69] Menter A. Psoriasis. London: Manson Pub; 2011
- [70] Brassinne DL, Failla V, Nikkels A. Psoriasis: State of the art 2013 part I: Clinical, historical, epidemiological and genetic aspects, Co-morbidities and pathogenesis. *Acta Clinica Belgica*. 2013;**68**(6):427-432
- [71] Ferreira B, Abreu JLPDC, Reis JPGD, Figueiredo AMDC. Psoriasis and associated psychiatric disorders: A systematic review on etiopathogenesis and clinical correlation. *The Journal of Clinical and Aesthetic Dermatology*. 2016;**9**(6):36-43
- [72] Jeong I, Lee HJ. Psoriasis skin models as promising tools in psoriasis research. *Biomedical Journal of Scientific & Technical Research*. 2018;**2**(3):1-4
- [73] Bracke S, Desmet E, Guerrero-Aspizua S, Tjabringa SG, Schalkwijk J, Van Gele M, et al. Identifying targets for topical RNAi therapeutics in psoriasis: Assessment of a new *in vitro* psoriasis model. *Archives of Dermatological Research*. 2013;**305**(6):501-512
- [74] Barker CL, McHale MT, Gillies AK, Waller J, Pearce DM, Osborne J, et al. The development and characterization of an *in vitro* model of psoriasis. *Journal of Investigative Dermatology*. 2004;**123**(5):892-901
- [75] Jean J, Lapointe M, Soucy J, Pouliot R, et al. *Journal of Dermatological Science*. 2009; **53**(1):19-25

- [76] Duque-Fernandez A, Gauthier L, Simard M, Jean J, Gendreau I, Morin A, et al. A 3D-psoriatic skin model for dermatological testing: The impact of culture conditions. *Biochemistry and Biophysics Reports*. 2016;**8**:268-276
- [77] Creative Bioarray. Psoriasis Skin Model. Available from: <https://www.creative-bioarray.com/psoriasis-skin-model.htm> [Accessed: 18-05-2018]
- [78] MatTek Corporation. Psoriasis. 2018. Available from: <https://www.mattek.com/products/psoriasis/> [Accessed: 18-05-2018]
- [79] Breslin S, O'Driscoll L. The relevance of using 3D cell cultures, in addition to 2D monolayer cultures, when evaluating breast cancer drug sensitivity and resistance. *Oncotarget*. 2016;**7**(29):45745
- [80] Haycock JW. 3D cell culture: A review of current approaches and techniques. *Methods in Molecular Biology*. 2011;**695**:1
- [81] Gargotti M, Lopez-Gonzalez U, Byrne H, Casey A. Comparative studies of cellular viability levels on 2D and 3D *in vitro* culture matrices. *Cytotechnology*. 2018;**70**(1):261-273
- [82] Mimetas. 2D Versus 3D Cell Cultures. Available from: <https://mimetas.com/article/2d-versus-3d-cell-cultures> [Accessed: 27-04-2018]
- [83] Abbott A. Cell culture: Biology's new dimension. *Nature*. 2003;**424**(6951):870