

Tissue Culture Technology: A Boon for Cosmeticology



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Introduction

Cosmeticology has been regarded as the science of cosmetic products that mainly focuses on enhancing the beauty or appearance of bodily features by direct application of cosmetic formulations. Nowadays, cosmetic industry is not only a booming business but also a consistently developing and expanding field. Most of the cosmetic products are derived from plants as they are equipped with many active ingredients along with their clinical significance. But their use has been limited to some extent due to various reasons (1). On the other hand, animals are widely used for cosmetic testing to check the safety and hypoallergenic properties of cosmetic products prior further use by humans. Due to the animal use, various ethical issues have also been raised. Somehow, all this may have a negative impact on the market of cosmetic industry. However, tissue culture technology is an emerging interdisciplinary field that deals with development of biological substitutes for restoring, maintaining, or improving tissue functions (2). This article describes the application of tissue culture technology of both plants and animals to overcome drawbacks involved in cosmeticology field.

Plants: Bio-factories for Cosmeceuticals

Today, various cosmetic industries rely on plants as majority of the cosmetic products contain higher percentage of plant-derived natural components. Plants contain enormous amount of different phyto-ingredients like essential oils, saponins, steroids, flavonoids, triterpenes, carotenoids, polysaccharides, peptides, fatty acids, etc. In 1961, to briefly describe active and science-based cosmetics, a member of US Society of Cosmetic Chemist collectively referred them as 'cosmeceuticals' (3). Therefore, plants have been regarded as bio-factories for cosmeceuticals. The following table depicts the extracts derived from plants and used in marketed cosmetic products.

Table 1. Plants used in Cosmetics

Sr. No.	Plant	Scientific Name	Active component	Properties	Reference
1)	Turmeric	Curcuma longa L.	Curcumin	Antibacterial, anti-inflammatory	(4)
2)	Aloe vera	Aloe barbadensis	Mucopolysaccharides	Antioxidant, moisturizer	(5)
3)	Rosemary	Rosmarinus officinalis	Caffeic acid	Antioxidant	(6)
4)	Olive	Olea europaea	Triglycerides, tocopherols, squalene, sterols, carotenoids, polyphenols	Anti-inflammatory and active oxygen scavenging effects	(7)
5)	Green tea		Catechins	Anti-inflammatory and anticarcinogenic	(8)

Though plants have wide significance in cosmetic industries, their use has been restricted due to slow growth, seasonal harvest, existence of toxic metabolites and variation of active concentration from plant to plant and harvest to harvest. Besides this, quality of the extracts ranges widely and depends on climate, soil, latitude, seasonal factors, and time of harvest (1). As a consequence of this, cosmetic industries fail to fulfil the ever-increasing desire of consumers for sustainable and natural products. However, plant cell culture technology has made its own way in the field of cosmeticology with great innovations and development. Therefore, industry market has been elevated drastically over past 10 years.

Plant Tissue Culture Technology

Being the part of modern biotechnology, plant tissue culture uses several techniques for growth and multiplication of tissues using nutrient solution under aseptic environment. It mimics the in vivo system inside the laboratory. It allows propagation of undifferentiated plant cells either to generate single cells or to produce a whole plant for further metabolite production (9). From the very beginning, it was established and extensively used in pharmaceutical industries. But since past few years it has proved its significance in cosmetic field too. The flowchart in figure 1 depicts the fundamental steps in production of culture-based cosmeceuticals.

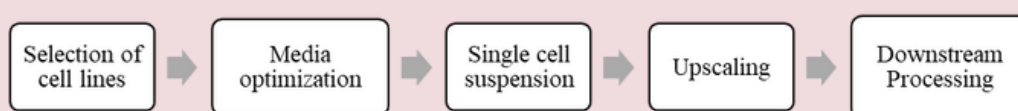


Figure 1. Basic steps of plant tissue culture to get cosmeceuticals

General steps required to obtain cosmeceuticals from plant tissue culture are mentioned below:

1. Selection of cell line based on highest biomass production and shortest doubling time is an essential step to give early production of extracts as most of the secondary metabolites are produced during stationary phase (10).
2. Although standard media such as Gamborg B5 (11), Linsmaier-Skoog (12), Schenk & Hildebrandt (13), Murashige & Skoog (14) medium provide adequate nutrient supply for optimum growth and maintenance of plant cell lines, media modification can increase the yield of extracts.
3. Callus culture can lead to formation of small clusters of cells or a fast-growing suspension culture of single cells (15).
4. The cells grown in liquid media can further be upscaled to bigger volumes by transferring into bioreactors of capacity 75,000 L (16).
5. When cells reach at stationary phase of the growth, they are harvested and allowed to undergo downstream processing for extraction of intracellular products. The obtained extract is then ready to use as active cosmeceutical.

Plant Stem Cells in Cosmetics

Plant stem cells (PSCs) have begun to garner significant attention of researchers due to their unique properties such as:

1. Totipotency
2. Ability to regenerate into whole plant
3. Self-renewal ability
4. Differentiation into specialized type of cells
5. Repair of damaged tissues.

PSCs are found in meristematic tissues, mainly apical and lateral meristem. A new wave of cosmetic ingredients containing PSCs and their extracts has made its way into the industry. This has become possible only because of new technologies provided by plant tissue engineering (1). PSCs and their extracts have wide application in the formulation of antiaging products. They are abundant in various sugars, polyphenols, carotenoids, fatty acids, phenolic acids, and triterpenes which are involved in the anti-aging process. They mainly act on fibroblasts by extending their life and stimulating regeneration. Currently, in the market, XtemCell's patented Stem Cell Technology manufactures a product that contains high concentrations of amino acids, proteins, lipids, and phytoalexins. They get easily absorbed into the outermost cells of the epidermis and stimulate the skin cell renewal and protect the skin from sun and aging by increasing the filaggrin protein level inside the skin which has skin barrier function (17,18).

Marketed products of plant cells and their extracts

Due to advanced technologies, many plant cell-based cosmetic products have been developed with greater commercial values in the market. Some of the marketed products of plant cells and their extracts are tabulated in table 2. Further, the various advantages of plant tissue culture in cosmetics are depicted in figure 2.

Table 2. Marketed products of plant cells and their extracts

Sr. No.	Brand Name	Product Name	Active ingredient	Function	Reference
1)	Juice Beauty	STEM CELLULAR™ Anti-wrinkle moisturizer	Fruit Stem cells	Moisturizes the skin	(19)
2)	Luzern	Serum Absolut Firming Collagen Booster	Orange stem cells	Stimulates collagen fibroblast and connective tissues.	(20)
3)	Eminence	Organic Skin care Monoi Age Corrective Night cream	Argan Stem cells and Nutmeg Seed extract.	Reduces wrinkles depth and improves elasticity and firmness	(21)
4)	Naolys	Unwind Scared Lotus	Nelumbo nucifera leaf extract	Radiance, anti-redness, antioxidant	(22)
5)	Naolys	High tech Natural Face care	Naolys plant cells	Relaxing, anti-wrinkle, skin repair	(23)
6)	Naolys	High tech Natural Cornflower + Vit C	Centaurea cyanus callus lysate extract and ascorbic acid	Anti-aging, anti-wrinkle, antioxidant, Regeneration	(24)

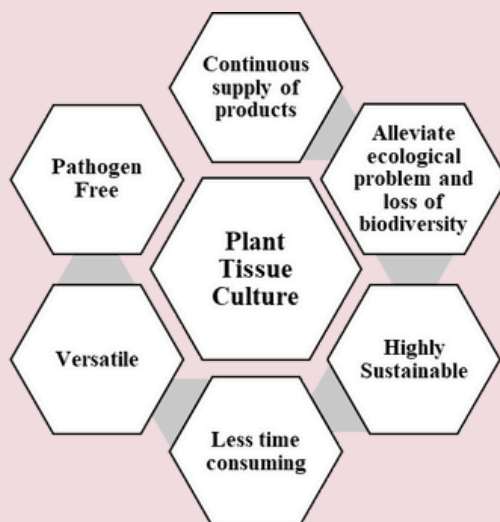


Figure 2. Advantages of plant tissue culture in cosmetics

Animal Testing of Cosmetic Products

Animals have been used as experimental models since many years as it was traditionally assumed that they share physiological similarities with humans. Like pharmaceutical products, cosmetic formulations must undergo some tests to assess whether they are safe for human use. And hence many animals are used for this purpose. There are different types of tests being performed on animals as represented in figure 3. Animal testing has various limitations for e.g.,

one new ingredient in any cosmetic product used in these tests leads to death of at least 1,400 animals (25). Sometimes, tests give unreliable data due to large differences in physiology between humans and animals, also their way of responding to chemicals.

Due to increased concern about the animals and to protect them from unnecessary pain and injuries many countries have passed some rules and regulations. However, cosmetics are considered to be luxury products and hence they are not the part of essential commodity. European Union was the first one who banned animal tested cosmetic products in March 2013. Gradually, other countries like Israel, Norway, Brazil, South Korea banned the sales of these products. India prohibited the animal testing for cosmetic purpose in 2014 (26). Due to this, efforts were made to find alternative solution for cosmetic testing.

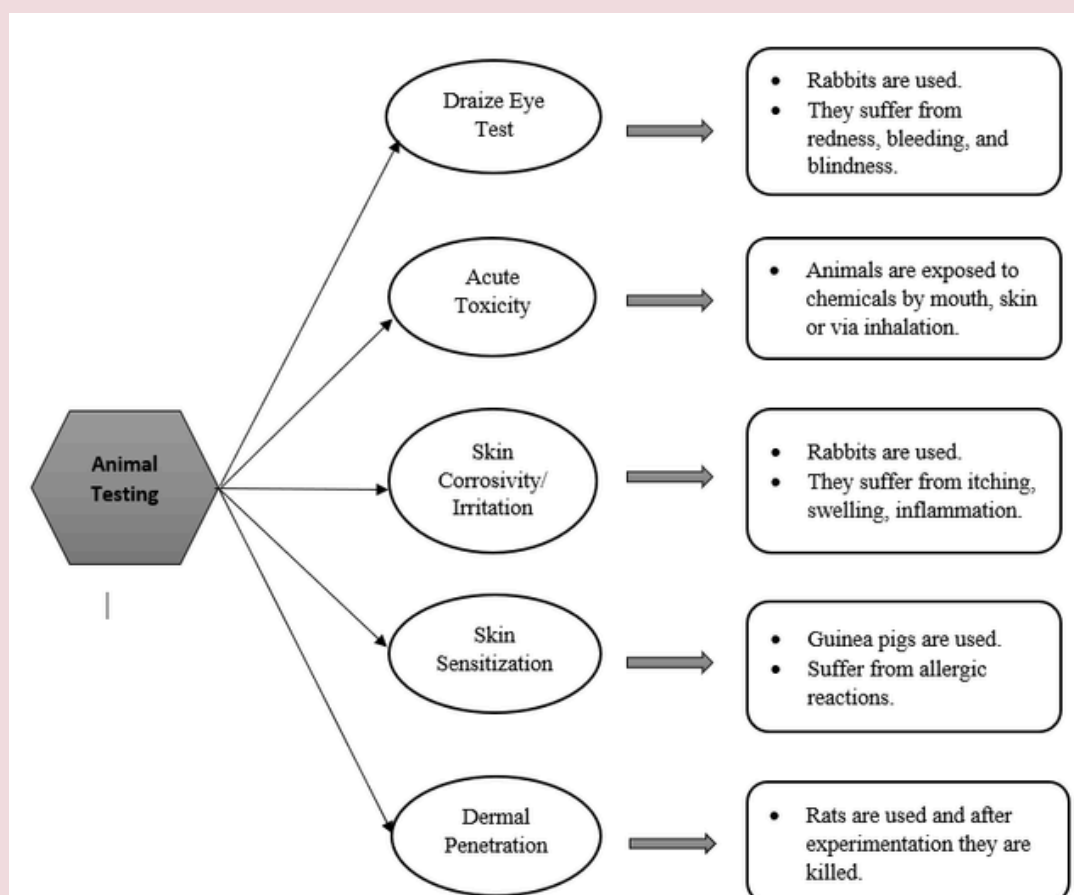


Figure 3. Various tests performed on animals for cosmetic testing (27)

Animal Tissue Culture

Animal tissue culture technology in today’s scenario has become indispensable in the field of life sciences, which provides a basis to study regulation, proliferation, and differentiation and to perform genetic manipulation. It is one of the major tools used in the life science research that have a potential for economic value and commercialization. Animal tissue culture can be combined with Recombinant DNA (r-DNA) technology for production of desired biologicals such as therapeutic proteins, anticancer agents, enzymes, monoclonal antibodies, interleukins, and hormones (28). In addition to this, it is an emerging field for studying toxicity of various novel compounds used in pharmaceutical and cosmetic formulations. Animal cell culture includes following steps:

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1. Cell isolation from organ
2. Media optimization
3. Primary cell culture
4. Passaging
5. Secondary cell culture
6. Cell lines which can be further cultured into monolayers or three-dimensional spheroids or organoids.

There are two types of animal cell culture:

- A. Differentiated culture: Culture of specialized type of tissues
- B. Undifferentiated Culture: Involves differentiation of stem cells into any specialized type of tissues.

Replacement of Animal testing with *In Vitro* models

In vitro systems are the ones that recapitulate the biological systems with more safety and no ethical concerns. Animal cell culture is highly desirable as it offers systems ready for direct access and evaluation of tissues to study the toxicity mechanisms.”. Hence, these techniques can be applied for reconstruction of cell based In vitro models for assessing toxicity studies of various cosmetic ingredients or finished products.

Human stem cells and their application:

Stem cells are undifferentiated or partially differentiated cells that can differentiate into various types of cells and proliferate indefinitely to produce more of the same stem cell. They are the earliest type of cells in a cell lineage (29). They are found in both embryonic and adult organisms, but they have slightly different properties in each. They are usually distinguished from progenitor cells, which cannot divide indefinitely, and precursor or blast cells, which are usually committed to differentiating into one cell type. There are different types of stem cells.

- A. Embryonic Stem Cells
- B. Adult Stem Cells
- C. Induced Pluripotent Stem Cells
- D. Mesenchymal stem cells

Researchers have attempted to construct in vitro models using human stem cells. Chaudhari et al. (2018), studied cardiotoxicity of few cosmetic compounds using human-induced pluripotent stem cells derived cardiomyocytes (hiPSCs-CMs). It has been observed that cosmetic ingredients reach to the blood upon absorption through skin, GI tract, inhalation and cause lethal effect to vital organs like heart.

In the study, cytotoxicity, cell viability, beating rate, beating pattern and total intracellular ATP content were evaluated (30). Aberdam et al. (2017) derived limbal epithelial cells from human induced pluripotent stem cells (LiPSCs) and used them as a cellular model alternative for in vitro ocular toxicity testing of both drug and cosmetic products. Various assays including flow cytometry, RT-qPCR, and zymography demonstrate that LiPSCs share morphological and molecular similarities with adult stem cells. The final data strongly supports that LiPSC model can be a potent alternative cellular model for cosmetic products (31). Currently, two types of commercialized In vitro models are available for testing cosmetics:

1. Skin Models
2. Eye Models

Marketed In vitro Skin Models

Reconstructed human skin models can mimic human skin. These 3D models are based on keratinocytes cultures which can grow at the air-liquid interface on a variety of substrates, making possible the topical application test materials. Following in vitro skin irritation tests are now officially validated in the OECD guidelines (32):

1. EpiSkin™ (L'Oreal, France)

EpiSkin™ was developed by L'Oreal (France) and commercially supplied by SkinEthics Laboratories (France). This model has been validated and recognized as a standalone method for screening and replacement by ECVAM in 2010. In this model, a dermal substrate is generated on type I bovine collagen matrix (representing the dermis), with a film of type IV human collagen, upon which a stratified differentiated epidermis derived from human keratinocytes is laid after 13 days in culture (33).

2. EpiDerm™ (MatTek Corporation, Massachusetts, USA)

EpiDerm system is a leading in vitro testing technology patented by MatTek Corporation for dermal toxicologists and formulation scientists. With multiple ECVAM validations and OECD accepted test guidelines, EpiDerm is a proven in vitro model system for chemical, pharmaceutical and skin care product testing. It shows excellent in vivo-in vitro correlation. The model exhibits human epidermal tissue structure and cellular morphology with greater uniformity and reproducibility. It's 3D structure consisting of organized and proliferative basal cells, spinous and granular layers, and cornified epidermal layers are mitotically and metabolically active. In this model, human-derived epidermal keratinocytes (NHEK) are cultured on specially prepared tissue culture inserts. Cultured at the air-liquid interface (ALI), EpiDerm allows for the evaluation of topically applied compounds, chemicals, cosmetic/personal care product ingredients and final formulations (34).

3. epiCS® (CellSystems, Germany)

epiCSTM (CellSystems, Germany), formerly known under the name EST-1000, is available since 2004, consists of normal human epidermal keratinocytes obtained from a neonatal donor (33). The model is supplied in 24-well polycarbonate membranes and consist of keratinocytes culture seeded over an artificial stratum corneum emerged at air liquid interphase.

4. SkinEthics™ (SkinEthics, France)

SkinEthic™ RHE is an in vitro reconstructed human epidermis from normal human keratinocytes cultured on an inert polycarbonate filter at the air-liquid interface, in a chemically defined medium. This model exists at different stages of maturity. This model is histologically similar to in vivo human epidermis. The epidermis and stratum corneum consisted of, respectively, 5-9 cell layers (23–59 µm) and 14–24 layers (15–32 µm) (35). The major differentiation markers expressed in RHETM, are namely, transglutaminase I and keratin 10 in 11 supra basal cell layers, involucrin and filaggrin in granular cell layers, and loricrin in upper granular cell layers. The basement membrane markers are laminin I and laminin V, type IV collagen, integrin beta 4, integrin alpha a 6 and antigen BP. Free fatty acids and ceramides are detected in the lipid profile (36).

5. 3D Full Thickness Skin Model (Human Skin Equivalent, FTSK, HSE)

3D Full Thickness Skin Model is developed by Creative Bioarray. Reconstructed FTSK (HSE) are composed of a dermal compartment containing human skin fibroblasts embedded in a collagen matrix and human keratinocytes seeded on top to form the epidermis. Most widely presented specific markers are Filaggrin, Involucrin, Loricrin, Tropoelastin, Keratin-10, and Keratin-5. Different epidermal classes of lipids comprising ceramides Collagen, Laminin V, Alpha6, Beta4-integrin, and BP antigen are also present. Creative Bioarray FTSK (HSE) can be utilized for the evaluation of toxicity and efficacy of skin therapeutics and cosmetics (37).

Marketed In vitro Eye Models

Cornea being the outermost part of the eye, its exposure to any chemical upon topical administration is higher compared to other parts of eye including sclera, conjunctiva, and retina. Therefore, currently in vitro human corneal models have been reconstructed as eye models to check the toxicity of any cosmetic compound. Some commercial in vitro corneal models are as follows:

1. EpiOcular™

EpiOcular™ was developed by MatTek to create an in vitro (non-animal) alternative to the Draize Rabbit Eye Irritation Test used in the cosmetics, personal care, household products, chemical, and pharmaceuticals. EpiOcular™ has been validated as a Draize Replacement by ECVAM (OECD TG 492 Reconstructed human Cornea-like Epithelium (RhCE) test method) and since its introduction in 1985, EpiOcular™ has been used to determine the ocular irritancy of their products without using animals by many cosmetic, personal care and household product companies. EpiOcular™ tissues are metabolically and mitotically active and release cytokines that occur in irritation and inflammation in vivo (IL-6, IL-8, TNFα). This in vitro ocular irritation test is based upon the assessment cytotoxicity following exposure to a test article, at three time points (additional time points may be added). Cytotoxicity is expressed as a decrease in mitochondrial conversion of MTT [(3-4,5-dimethyl thiazole 2-yl) 2,5-diphenyltetrazoliumbromide, into a blue formazan salt that is quantitatively measured after extraction from the 3D EpiOcular™ tissues (38).

2. *SkinEthic HCE™*

SkinEthic™ HCE model is composed of transformed human corneal keratinocytes cultivated on an inert polycarbonate filter at the air liquid interface in a chemically defined medium. The reconstructed tissue forms a stratified and well-organized epithelium which is structurally, morphologically, and functionally similar to the human cornea with presence of basal, wing and mucus production cells. Expressed markers are Keratin, CD 44, and Hémidosomes (39).

3. *LabCyte CORNEA-MODEL*

LabCyte CORNEA-MODEL is a 3D human cultured cornea epithelial tissue produced from normal human cornea epithelial cells. LabCyte CORNEA-MODEL was developed by applying cell culture techniques to differentiate and stratify cornea epithelial cells to form a tissue structure like that of the normal human cornea. The measurement of viability of the tissues, after topical exposure to a chemical entity, is commonly used to identify its potential toxicity, carried out by enzymatic conversion of formazan salts (MTT assay) by the viable cells of the tissue into coloured formazan salt, which is quantitatively measured after the extraction from tissues (40).

4. *MCTT HCE™*

The MCTT HCE™ model, was manufactured in MCTT, Seoul, Korea. It is based on primary human limbal epithelial cells and they are cultured to form a multi-layered, differentiated corneal epithelium model. The transepithelial electrical resistance (TEER) detects the expression of corneal cell markers as well as barrier function and hence the model faithfully represents human corneal epithelium. It shows accuracy of 88%, sensitivity of 100% and specificity of 77% (41).

Future perspective

Due to seasonal harvest, slow growth and change in the quality and concentration of phyto-ingredients, it was difficult to fulfil the ever-increasing demands for cosmetic products. But plant tissue culture technology has been successful to overcome all the pitfalls faced by cosmetic industries. For high quality and high yield products, this technology can be combined with techniques of genetic engineering such as r-DNA technology wherein tissues are engineered in such a way to get desired bioproducts. This synergy is expected to push forward the global cosmetic market, since it provides scientific innovation and new opportunities for product development. On the other hand, because of the ethical concerns and less accurate data obtained from the animal tests, use of animals for cosmetic testing is still a matter of debate. Researchers are taking enormous efforts to find the alternative solutions for animal testing. For the moment, techniques of animal tissue culture technology are providing reliable in vitro models to deal with the current issues, but they do not fully reproduce the biological systems and this may result in less accurate results. However, using advanced techniques, co-culturing of cells can be performed to induce cell-cell interaction and hence recapitulating the in vivo system. Besides this, Organ-on-a-chip (OOC) technology where in cells are cultured in microfluidic environment offers a particularly exciting avenue for further research and it is assumed to abolish the need for animals in cosmetic testing.

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