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Bioengineering in cosmetology – the overview of present state and future prospects

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ABSTRACT

Biological engineering, also referred to as bioengineering, is a branch of biotechnology focused on solving problems of life sciences with the use of the most modern technology, analytical and synthetic methodologies, including possibly lowest cost of the conducted analyses and procedures. Nowadays, bioengineering is in the lead in the scientific world. Unlike traditional engineering techniques, which apply mathematical and physical sciences to analysis processes and structures of biological systems, the biological engineering is capable of molecular analysis due to developing knowledge of molecular biology and progress in technology.

***Keywords*:** engineering, techniques, innovation, molecular biology, technology

1. INTRODUCTION

Bioengineering is a branch of knowledge, which allows execution of studies providing information about molecular structure of biological formation, decent determination of individual organism, population or species, provides information about metabolic, energetic and genetic status of an organism, also provides the possibility of recreating the native tissue or organ, which can be later on implanted in to the body of the patient. Furthermore bioengineering enables laboratory analysis, in which the examined tissue, organism or structure can be replaced with a synthetic model.

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2. DISCUSSION

According to Polish law every cosmetic has to be tested in regard to its safety for human health and life. Furthermore, European Union law forbids testing cosmetics and household products on animals. The law is founded on three the most important rules, known as Three Rs. Its definition applies to three terms: Replacement, Reduction and Refinement. The rule of replacement is defined as replacement of living higher animals by insentient material, such as cell and tissue culture, tissue slices etc. The reduction applies to minimizing the amount of animals used in experiment, but still statistically relatable. At the same time, the amount of animals cannot be too low due to higher risk of increased suffering. The last rule of refinement applies to the personnel, laboratory and to the living conditions of animals. According to this rule people should always educate them; take care of animals in most humane way in order to decrease the discomfort of animals.[1]

This determines a new role of modern techniques of biological engineering and biotechnology in medical, scientific and cosmetic field of knowledge. The alternative non-animal methods of assessment are widely accessible, which makes them equivalent. For various kinds of substances different types of assays are available. With progress of technology and biological knowledge these techniques are constantly developed and improved, which in consequence allows to obtain results faster and at a reduced cost of the procedure.

The most recent knowledge and technology provide possibility of replacing the animal tissue, for example skin, by the use of *in vitro* cultures. Cell breeding *in vitro* is one of the most basic and most demanding techniques, which provides tissue models for study. This method makes use of the individual cells sampled from an organism (e. g. human, mouse, chicken), which are bred in special conditions: specific temperature and humidity, concentration of carbon dioxide, concentration of nutrients, sometimes fortified with specific chemicals or native tissue products. Achieved cell culture creates single layer of living cells, which are going through next stages of growth. These cells can be later on used as a tester for new cosmetics and substances. They are living cells of one kind, their regenerative ability, structure and function are comparable to native cells. The effects of the tested product observed on the cell culture provide the information about predicted reaction of human tissue to this cosmetic.

Organ breeding is a separate type of culture. This process is more complex in comparison with cell culture or even tissue breeding. It offers an opportunity to obtain fully developed organ or its part. The culture is made from sampled native organ. The fragment is the basis of the new culture. Its cells migrate from the sample to the culture medium, where they begin to grow and differentiate into specific types of tissue, which are typical for this organ. This kind of culture has the advantage of providing the integrity of the structure with conserved cell activity and also biochemical, histological and metabolic diversity. Those characteristics make the organ culture one of the most faithful model of the tissue *in vivo*. Their main disadvantage is limited growth range, which is dictated by different access of the oxygen to the distinct areas of the organ. Further, the same issue affects the nutrition absorption and availability as well as metabolic products disposal. Organ culture can be the

[1] U.S. Food and Drug Administration, "Import Alert 66-38," http://www.accessdata.fda.gov/cms ia/importalert 188.html Availability 23.08.2017

source of skin tissue, which can successfully replace traditionally used animal skin for cosmetics testing.[2]

The most recent knowledge and technology provide possibility of replacing the native tissue, for example skin, by the use of a 3-D printer. The machine forms layers of skin cells, which mimics the structure of native skin. Because the cell's location in tissue is more natural it provides better functioning for a tissue as a whole. Moreover, a 3-D model is more comparable to the natural skin, than one layer cell culture. This feature assures more reliable test results, because the structure of the model is comparable to the human skin. This technique can also be used in creation of other kinds of tissue, e.g. bone, epithelium etc. The technique of 3-D printing is becoming more and more popular and accessible, enhancing its meaning in scientific fields and cosmetic assessment.

Biological engineering is focused on implementation of beneficial properties of chemical compounds and substances in the biological systems. This field of knowledge is to a large extent dedicated to performing the experiments and assays, which provide the valuable and crucial information. The scientists are able to not only prepare the test material, but also design the experiment and relate its result to the human organism.

Not long ago, every safety test for cosmetics was performed on living animals. For many years, the alternative non-animal methods of assessment were modified and improved, due to growing interest in animal protection. Nowadays, most of these methods are widely available, but still some of the older methods cannot be replaced.

One of the modern methods of assessment is the alternative test for eye products or products used in the eye area. The traditional test was founded on product application to conjunctival sac of the rabbit eye. The other eye of the animal was used as the control. During the experiment the condition of the eye was observed. The whole procedure could last up to 72 hours and the experiment – up to 21 days. The alternative method is called the HET-CAM test, which stands for Hen's Egg Test Chorio-Allantoic-Membran. This method requires usage of the fertilised and incubated hen eggs. They should be used for experiments between 6th and 11th Day after fertilisation, as the development of the hen embryos nervous system is not complete, which protects it from pain. The shell of an egg is drilled, which makes the accessible way of treatment. Then the tested product is applied. The experiment takes up to a few minutes. The changes on the surface of the egg give information about membrane reaction to the substance. The most important are changes connected with inflammation, irritation of the tissue, narcosis or bleeding. The assessment includes the presence of negative effects, their severity and the time of their arrival. These characteristics give information about the safety of the product for human health and the predicted reaction of the human eye.[3]

Another assay similar to HET-CAM is CAMVA (chorioallantoic membrane vascular assay). The material used in the assessment of the product is the same – fertilised hen eggs. In this case part of egg white is removed and the tested product is inserted into egg. The egg is incubated for the next 6 days. The test helps in assessment of eye irritation potential. These changes are linked to the congestion of hen's egg vascularised membrane. CAMVA is the most effective in prediction of samples of mild or moderate irritation potential. The difficulty

[2] S. Raj, S. Jose, U. S. Sumod, and M. Sabitha, "Nanotechnology in cosmetics: opportunities and challenges," Journal of Pharmacy and Bioallied Sciences, vol. 4, no. 3, pp. 186–193, 2012.

[3] J.A.B. Paul and P.F.S. Roel, "Toxicological characterization of engineered nanoparticles," in Nanoparticle Technology for Drug Delivery, R. B. Gupta and U. B. Kompella, Eds., pp. 161–170, Taylor & Francis, New York, NY, USA, 2006.

with assessment of low irritation scale is connected with variability of the vascularised membrane of hen egg. The same situation is noticeable in assay of products with high irritation potential. This method of assessment is most effective for a specific group of substances.

The alternative for test with the use of hen egg is the BCOP method. The cattle eye cornea is used as the model for assessment of its opacity and permeability after induction of the cosmetic on cornea surface. The material is sampled from the cattle shortly after slaughter. The assessed substance is applied onto the epithelium. Each group in the experiment consists of three eyes at least. Groups are made of study group, negative control (without assessed product) , positive control (with control material, other than cattle eye cornea). Permeability is observed after use of specific dye containing fluorescein, which allows the observation of the material with fluorescent microscope and observation of the changes made. The opacity of the cornea is assessed by cornea's light permeability. Any changes in the permeability of the cornea are connected with injuries. On the other hand, the opacity reflects damage or loss of epithelium. These parameters can be relatable for product's effects made on the human eye.

ICE test is a further one founded on animals' eye model. In this case the hen's eye is used for the experiment. Eyes are excised after the slaughter. The substance is applied on the surface of the cornea. The changes are observed at regular intervals: after 30, 75, 120, 180 and 240 minutes after product application. Changes are presented on the cornea as swelling, change in opacity of the eye, changes in permeability and morphologic changes in the structure. Another variant of this test is one with rabbit eyes used as a model.

The main disadvantage of alternative tests for assessment of irritation eye potential is variety of the animal eye model, even within the confines of one species. This makes the unification and comparison of the results very difficult. Another disadvantage of these models is their lack of regenerative potential. Human eye regenerates regularly, old cells and damaged cells are replaced with the new ones. Because models are posthumously obtained, they are exposed to natural damage, implied from decay processes. This last one characteristic makes them very difficult in conservation and very sensitive to every external factor.[4]

The alternative methods of assessment are also focused on other models, not only those based on animal tissue. Modern engineering gives the opportunity of producing models *in vitro*. These tissues are bred in laboratories, made of cells with high division and life potential. As mentioned above, the synthetic tissues are supposed to replace the living animal and provide the valuable results from experimentations. The modern scientific background provides a very effective and sterile method of breeding. The obtained material is as uniform as possible. This trait guarantees statistical reliability.

One of these tissues is human epithelium model, made of keratinocytes. In native tissue they create the outermost layer of the skin and are responsible for protecting it from bacteria, pathogens and mechanical injuries. Model's structure is similar to the native human epithelium tissue. It is usually used in test for assessment of vitality, permeability. The model can be used as a tester for different kinds of chemicals.

Furthermore, human tissue models are composed of or based on different types of cells. The type used in laboratory for breeding new tissue depends on the purpose of prepared model. Cell types vary in structure, vitality, metabolism, kind of created products, size, resistance etc. All of these characteristics are very important in selection of native cell type

[4] D.M. Bagley, D. Cerven, J. Harbell, Assessment of the chorioallantoic membrane vascular assay (CAMVA) in the COLIPA in vitro eye irritation validation study, 1999.

used in laboratory and the purpose of the final product. The end result has to be as reliable to the native tissue as possible.

In modern world skin is one of the most important subjects in regard to beauty standards. Many women and more and more men pay attention to their skin condition. Its condition should suggest good health and youth. Scars, imperfections like acne, cellulite etc. are unwanted features. This implies the reason for the use of skin products, which will protect and care for it. Products like skin cream, serum, lotions, oils etc. are already very popular. They remain on the skin surface for many hours. That means they need to fulfil strictly defined safety requirements.

Traditional cosmetic tests used, for example, little rodents like rats, mice or guinea pigs. Nowadays, they have been replaced by synthetic tissue cultures, but also synthetic materials, like special membranes. They limit the chemical detector, which is poured into a glass vial. This system has to imitate the corrosive effect similar to the one occurring on the skin. If the membrane gets damaged the detection system will change its colour, which is linked to the possibly made injury on the surface of human skin. The test results are compatible with those induced on rabbit skin. This method allows us to qualify certain substances and products as corrosive or non-corrosive – safe for the skin.

Some chemicals show specific effects not only on the surface of the skin, but also may affect the immunological system. These reactions can occur as allergy, inflammation or necrosis. The skin cells' receptors are able to connect with chemical particles and are responsible for the modulation and administration of immunological system. Every substance identified as foreign or with high allergic potential can cause immediate and aggressive response of the system.

Pyrogenic substances are responsible for sudden activation of the immunological system. They determine occurrence of high temperature by disturbing of neurological system. It may cause overheat of the organism, metabolism disorder, which may result in internal injuries and lasting damage. The alternative non-animal testing methods for this kind of substances are safe and effective. In these tests, known as LAL tests, the marker of pyrogens is limulus blood. It is collected from wild animals, which are later on set free. Their blood characterizes with specific reaction to pyrogenic toxins produced by one kind of bacteria. The change in its structure is the result of the reaction, which stops toxins from spreading into the system. This method is animal-friendly, where rabbits are replaced by small, but sufficient blood sample taken from a wild animal. The test is fast, reliable and easy to perform. Moreover, it allows estimation of actual toxin volume. LAL assay is not a perfect method – it only detects specific kind of toxins, released by one bacteria group.

Pyrogenic substances can also be detected with the usage of human monocytes. Monocytes are blood cells responsible for defending the organism from pathogens. The assay is founded on the reaction between the monocytes and pyrogenic molecules. As the effect of stimulation, the monocytes release substances responsible for stimulation of the immunological system.

Very important aspect in product safety depends on its non-toxic potential. Cytotoxicity describes dangerous, usually lethal effects on cells caused by certain chemicals. Using such compounds on living cells may cause them to undergo necrosis (accidental, sudden, very chaotic cell death) or apoptosis (initialization of natural cell death). Main unit of cytotoxicity is IC50, which stands for inhibitory concentration 50, that describes cell growth and division inhibition potential. In other words, cytotoxic particles can inhibit cells from regeneration and

maturing to a various degree. Additionally, cytotoxic character of certain substance is usually specific for one type of cells, e.g. for liver cells, heart cells or neurons. The main focus in cytotoxicity test is put on testing the cell integrity, cell enzymes activity, cell ability to divide and grow, concentration of genetic material and proteins, protein localisation and changes in genetic material. These traits are investigated with the use of analytic tests, which are based on activity and interaction of different markers. Specific marker dyes allow the effects of the substance on cell to be visible via microscope.[5]

Another kind of toxicity is fototoxicity. Some substances can provide negative skin reaction to the UV light. The molecules of the substance penetrate the skin cells and modify its DNA, making them prone to aggressive reaction to the sunlight. The effects occur after next exposure to the light. The reaction manifests itself by itching, burning sensation, but also necrosis. The safety of the substance is tested on special human cell culture. The cells are exposed to the UV light for a specific period of time after substance application. The cell growth and survivability are the main parameters of fototoxicity.

In the modern world one of the biggest issues are genetic mutations, which manifest as malignant tumours and cancer. Many studies confirm existence of the correlation between increasing longevity and frequency of cancerous diseases. With aging the quality of the genetic material deteriorates which fosters the genetic alternations. These circumstances define need for safety examinations of human health. The ability of some chemicals to damage the genetic material is called genotoxicity. Each ingredient used in cosmetics production has to be tested in regard of its genetic safety. Some substances are extremely dangerous, but only in certain dosage. Their minimal amount, which can be used in cosmetics production is assessed in tests using certain species of bacteria. These organisms are modified accordingly for those experiments. Their genetic material already contains a mutation. During the test the bacteria are exposed to the tested chemical. If the assessed substance is toxic for genetic material, the existing mutation is altered on non-mutated material – the correct version. This test, called Ames Assay, is a fast and convenient method of compound assessment. Its main advantage is the short time and relatively low costs of performing, in comparison to traditional tests on rats and mice. Of course, this assay is not perfect and false results are possible, but this method is very reliable and one of the most popular methods of assessment.

The methods of assessment play as an important role in cosmetic production as branches of biotechnology and bioengineering responsible for production of ingredients. Modern knowledge about physical phenomena and chemical reaction of certain particles gives the opportunity to create completely new ingredients, properties of which give positive results in regular or daily usage.

One of the latest fields of study is nano biotechnology, often used in combination with the bioengineering. Nanoparticles are understood as extremely minimized chemical particles. Their properties are extremely different from the original element. This includes existence of new biological interactions between nanoparticle and biological system or its cells. Usually this affects the reaction of pathogens in the system, the process of regeneration and recovery, activation of immunological system in the process of body defence, neutralization of bacteria. For example nanoparticles of silver are known for antimicrobial and protective activity. These

[5] F. Pflücker, V. Wendel, H. Hohenberg et al., "The human stratum corneum layer: an effective barrier against dermal uptake of different forms of topically applied micronised titanium dioxide," Skin Pharmacology and Applied Skin Physiology, vol. 14, no. 1, pp. 92–97, 2001.

properties make it a very effective alternative preservative, which can successfully protect a cosmetic from external factors. At the same time, the nanoparticles of silver are highly safe for tissues and natural microbial flora. They can be found in products such as oral hygiene products, intimate hygiene products, shampoos and soaps, antibacterial tissues and cosmetics dedicated for skin prone to acne. Other nanoparticles are obtained from gold, platinum and copper. These structures are responsible for protecting skin from UV light and ageing. Furthermore, they are responsible for enhancing the skin barrier and stimulation of natural regenerative processes. These nanoparticles are responsible for improving the skin's appearance and positive impact on cell metabolism and activity. The production of collagen is activated, which results in improving the skin's structure.

The skin protecting products, like sunscreens, increasingly contain nanoparticles of titanium dioxide and zinc oxide. They effectively protect skin from UV light without negative effects on health and human safety. In addition, the size of those particles play important role in efficiency of the final product.

Apart from particles, other kinds of molecules can also be manufactured in nanoscale. The vesicles called liposomes or micelles are built from two layers of natural or synthetic lipids, known as phospholipids. They enclose aqueous volume, where the active product can be introduced. Their structure is similar to the structure of cellular walls. The lipid bilayer makes it possible to fuse the liposomes with cellular membrane. The content of the liposomes is then released to the cell, which allows the molecules to interact. This makes the micelles very effective cosmetics delivery application system. The preparation of the vesicles is easy to perform; also cost of production is relatively low. In addition, their bilayer can be enriched with specific molecules, which are characteristic for specific types of cells. Such action is used in production of cosmetics, which contain the particles especially effective for one kind of tissue. The liposomes can precisely provide vitamins, nanoparticles and nutrients.[6]

The nanoscale can also be applied to emulsions, capsules and lipids. The nanoemulsion is a dispersion of nanodroplets of one kind of liquid within another. Their structure can be manipulated by the use of different production methods. The nanosize makes them more effective containers with higher stability. They also prolong the product's suitability for use. The nanocapsules are vesicles whose oily or aqueous core is surrounded by polymeric capsule. As for nanocapsules, their size provides higher stability and higher effectiveness in ingredient transportation.

The production of nanoparticles is a new field of industry. Some methods have been known for many years, other are relatively new. There is a defined group of metals, which are most popular as material for nanoscale products. Since their physical and chemical properties are extremely different from their original source the safety assessment procedures for human health and environment are very important. The nanoparticles of gold, platinum and silver are the most verified and tested ingredients used in cosmetic products. At the same time new particles are being discovered, many of which have not yet been tested in the nanoscale.

The safety of nanoparticles plays the main role in their future in cosmetics industry. Because nanoparticles are offering many positive aspects of their use indicated by their specific size, they are also potentially dangerous for our biological system. They can easily penetrate the biological membrane, which indicates the concern for their probable entrance to the bloodstream and spreading through the organism. The uncontrolled transportation of

[6] T. Butz, "Dermal penetration of nanoparticles: what we know and what we don't. Cosmetic. Science Conference Proceedings, Munich," SÖFW Journal, vol. 135, no. 4, pp. 8–10, 2009.

nanoparticles may cause extreme internal damage. All these possibilities define the need for specific tests, which allow specifying the safe dosage for each kind of nanoparticle used in skin products. The safety of nanoparticles can be studied using cell and tissue cultures. This allows making the observation of positive, but also negative results of nanoparticles' activity. Of course, the danger appears in the situation when particles used as an ingredient do not rest on the surface, but they penetrate deeply into the tissue or the body. The experiments performed by many scientific teams proved that small concentration of nanoparticles is beneficial for health. At the same time the safest dosage has to be established and imposed on every cosmetic producer.

Even today many chemicals, molecules and substances are being identified and studied. Modern technology grants us access to new compounds. The traditional drugs or cosmetic ingredients are sometimes the starting point allowing to constantly innovate their biological activity for the improvement of human life quality. The devices, such as microscopes, give the possibility of the structure analysis. Application of different light frequencies can enable studying certain substances in distinct aspects. An additional use of specific detectors, cameras and other devices can provide information about specific chemical composition of the tested material, for example of the plant extract used in cosmetics production. The knowledge about its structure, chemical and biological activity forms the basis for innovative experiments. They are very important in case when effective ingredient has the positive effect on scar healing, but at the same time it is very expensive and difficult to obtain. The information about object of interest provides the opportunity to find or synthetically produce new material with properties corresponding to the properties of the source. In bioengineering the important aspect is the implementation of knowledge about chemical and biological activity to a new environment, like human body, which will allow to improve the growth and health. The production of new cosmetics, with effective but relatively affordable ingredients is highly beneficial for cosmetic producers. At the same time cosmetic products are becoming more effective as a means of self-care and improvement of self-image.[7]

It is clearly noticeable that bioengineering and technology are already very important elements of cosmetology and cosmetic industry. The significant proportion of alternative methods of assessment will be growing due to general public opinion about animal testing and protection of animal life. Of course, these techniques are still not perfect in many aspects. Even though they contribute to animal protection, they are still not perfectly relatable to human biology and physiology. As in the case of traditional tests, where animals are completely different from human, in alternative non-animal assays the comparison of effects on bacteria or cell culture to human organism is sometimes very complicated. The differences include not only the size or anatomy. The most important are the connections between metabolically and physiological activities. The assay performed on cell culture does not provide the information about general body reaction to a certain kind of product. The non-animal models have their specific disadvantages. The *in vitro* experiments are very specific in their performance. In *in vitro* tests the examined substance is significantly slower absorbed by the testing material than it takes place in a biological system. Moreover, in *in vitro* tests the liver metabolism is completely eliminated due to its absence in synthetic culture. In a living organism the distribution of substance is not uniform, as it takes place during the assay. The alternative methods of assessment need continuous modifications. However, their meaning in

[7] "Nanomaterials and the EU Cosmetics Regulation: Implications for Your Company," http://www.gcimagazine.com/business/management/regulation/143553126.html Availability 25.08.2017

modern world is still growing due to an increasing interest in ecology and consciousness about environment and animals.

5. CONCLUSIONS

The meaning of bioengineering in cosmetology is still growing. This field offers very effective and useful tools for projecting, studying, producing and also improving on new cosmetic products. The combination of biological knowledge and technology enables cosmetic companies to manufacture modern cosmetics. The current concept of beauty defines society's need for certain types of products. Every day, people buy many cosmetic products. Each of them has to be tested in regard of its safety for human body. Many substances' negative impact is still unknown, because they were not tested in a specific environment. Each substance has to have an assigned dosage, which is either neutral or beneficial for human body. It applies not only to the active ingredients, but also to the preservatives, fragrances, substances having allergic potential. The true overview of every of these aspects can be verified thanks to the accessible technology.

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