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Review

ANALAIZE OF THE LAST YEARS POSSIBLE DIRECTIONS FOR CULTIVATION AND APPLICATION OF HUMAN KERATINOCYTES

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ABSTRACT

The author have a short view on the cell culture method development in some last years. The goal of the current study is to determine the present condition of the method and to investigate the modern directions, achievements, trends and applications for it. Study is based on a literature available in the specialized magazines and journals, and the most original and helpful for the completion of our goal were selected. Particular attention is paid to the preparation of media for keratinocytes cultivation, the conditions for successful cultivation and the latest developments in this method.

Key words: cell cultures, keratinocytes cultivation, wound treatment, tissue cultures.

INTRODUCTION

Cell and tissue cultivation begins with the experiments of Vilhelm Ru in 1885 (1, 2), who first defines the basic principles of this method by cultivation of chicken embryonic bone marrow in warm saline. During the next 120 years the method has been developed and improved, and the produced cell and tissue cultures used in scientific and practical applications – new drugs tests, production of viral material for vaccines production, usage of epithelial monolayer for skin burns treatment. For the treatment of extensive skin wounds such as in severe burns, autologous skin for transplantation is often not available in sufficient amounts.

Reconstructions in the oral cavity or the vaginal mucosae, as required after tumor resections or cleft palate repair, are often complicated by similar problems. Techniques have been developed for the culture of epithelial grafts, dermal substitutes, and the combination of these two to a 'functional' skin or mucosa equivalent (3, 4).

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GOALS

The goal of the current study is to determine the present condition of the methods for cultivation of epithelial cells and to investigate the modern directions, achievements, trends and applications for it.

For the completion of this goal we formulated the following two tasks:

1. To examine the available literature on the development of the method for epithelial cell cultivation and their application.
2. To point out new and promising directions in the cell cultivation and application of epithelial cells.

MATERIAL AND METHODS

This study is based on a literature available in the specialized magazines and journals, and the most original and helpful for the completion of our goal were selected.

RESULTS AND DISCUSSION

We had found hundreds of studies related to the topic of cell cultivation. Some of them are only theoretical, while others have practical application. From the start of the method the efforts of researchers are directed to the practical use of cultured cells - in poultry, virology, vaccine production, medical treatment, cosmetic and regenerative medicine and others. Since its foundation till nowadays

the method is constantly evolving and improving as the goals and objectives that have set the researchers and their capabilities in relation to current scientific knowledge, concerning the cell culturing. In recent years, paying particular attention to the composition of media on which cultivate epithelial cells and bring new alternative media.

Acevedo et al. (2010) used fibrin in cell encapsulation because it has important biological properties (5). Keratinocyte encapsulation in fibrin is a widely used technique in skin tissue engineering. The production of growth factors (EGF, TGF-beta1 and PDGF-BB) was evaluated when keratinocytes are encapsulated in fibrin. Secretions of TGF-beta1 and PDGF-BB increased more than five times compared to monolayer cultures. Encapsulated cells secreted about 80% active form of TGF-beta1 (monolayer cells only secreted inactive form). An enhanced secretion of TGF-beta1 and PDGF-BB was found in encapsulated cells, showing that fibrin capsules are favourable for the production of these growth factors.
The same author (2010) creates a rolling profile of cell cultures of human skin at various old-age diseases (6).

Cushing&Anseth (2007) propose a modification of the composition of the cell culture medium for a higher cell growth (7).
Caldelari&Müller (2010) in contrast to earlier large-scale, non-specific screening of factors described a simple method to isolate and cultivate epidermal keratinocytes from embryonic or neonatal skin on uncoated plastic. They used a medium specifically designed to retain epidermal keratinocyte progenitors in an undifferentiated state for improved isolation and proliferation and an alternative medium to support terminal differentiation (8).

Campbell et al. (2010) demonstrated that keratinocytes grown directly on adipose tissue have greater proliferation rates than keratinocytes grown alone. Adipose-conditioned medium (ACM) supplementation provides an additive proliferation benefit when combined with a feeder layer producing mature grafts in approximately half the time as keratinocytes alone by accelerating proliferation and increasing keratinization (9).

Egles et al. (2010) describe the fabrication of Human skin equivalents (HSEs) from human keratinocytes and fibroblasts and how HSEs can be modified to characterize the response of the human epithelium during wound repair. The protocols outlined first describe techniques for the generation of human tissues that closely approximate the architectural features, differentiation and growth of human skin (10).

McMullen et al. (2010) did an image analysis to quantify histological and immunofluorescent staining of ex vivo skin and skin cell cultures. They carry out investigation of either skin cell cultures, such as normal human keratinocytes (NHK) or fibroblasts, or ex vivo skin sections. Examples of the analyses are provided for the comparison of skincare active ingredient treated samples vs. placebo to demonstrate the utility of the methods to quantify and provide numerical data for a procedure that is typically qualitative in nature and based on observations by a histologist (11).

Mujaj et al. (2010) have shown that the inclusion of a fibroblast cell support layer is required for the isolation and expansion of primary keratinocytes. They developed a medium that used recombinant proteins to support the serum-free isolation and expansion of human dermal fibroblasts and keratinocytes. Their technique is a valuable alternative for culturing fibroblasts and keratinocytes using recombinant proteins (12).

Rasmussen et al. (2010) used mixed populations of green fluorescent protein (GFP)-labeled NIKS and unlabeled primary keratinocytes to model the allogeneic and autologous components in chimeric monolayer and organotypic cultures. They observed that in monolayer coculture, GFP-labeled NIKS had no effect on the growth rate of primary keratinocytes and cell-cell junction formation between labeled and unlabeled keratinocytes (13).

Spörl et al. (2010) suggested a real-time, non-invasive method for the long-term observation of cholesterol reorganization in plasma membranes and the dynamic process of cholesterol depletion and repletion in primary human keratinocytes (14).

Zavan et al. (2010) demonstrated that multipotent adult precursor cell can be isolated and expanded from two accessible adult tissue

sources: skin and adipose tissue (15). They isolated adult stem cells from skin and from adipose tissue derived from the same adult donor were treated with epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2). Neurospheres obtained were first expanded and evaluated in term of proliferative ability, and then their neuronal differentiation potential was analysed. Adipose- and skin-derived neurospheres grew in suspension as spheres in the presence of the mitogens FGF2 and EGF. With this protocols, the spheres have been able to proliferate and to originate Schwann and glial-like cells.

Cell culture begins with the harvesting of the cells and ends with the application of the prepared culture. Dermal fibroblast/myofibroblast progenitors do not readily circulate in the blood and have to be harvested locally from the skin (16). With the development of the theory for the stem cells some try to produce cell cultures from them (8) and they offer the adipose and the epithelial tissue as potentially good source for obtaining of multipotent stem cells from adults (3, 15).

For the cell culture medium could be used liquid solutions or gels where the cells could grow as monolayer or a full thickness layer (10). 3d structured mediums have been developed for the purpose of full thickness cell cultures, and the based on collagen/elastin prove to be suitable for cultivation, transport and transfer of keratinocyte cultures (17). Many 3d matrixes are based on a collagen scaffolding (18) around with the cells organize. A quantity of Keratinocytes growth factor produced is increased compared to monolayer (5). Recombinant proteins (12) and hydrogel based 3d mediums (7) are developed and need further research. Except from the molecular structure of the medium, many substances are tested for they possible positive effect on proliferation and/or differentiation of the cells in the cultures. Methyl-beta-cyclodextrin (MbetaCD) suppresses the early differentiation of keratinocytes, but speeds up the late differentiation and increases the proliferative capacity (14). Presence of adipocytes accelerates keratinocytes proliferation - responsible growth factors already isolated (9). Melanocytes improve the esthetic outcome in transplants (19).
Electric field could be used during cell cultivation to guide their migration and organization (20).

The available information on the use of electro-activated water in living organisms, suggests that it could be used in the cell cultivation as well. Our hypothesis is that the electro activated water has a bi-polar potential to be used as a strong antiseptic preparation, as well as an additive for the cell cultures medium. Alkaline fraction of the electro activated water could be used as a harmless activator of the cell proliferation (2), and the acid fraction – suitable for sterilization of the laboratory instruments (1).

There are different methods for characterizing the cultivated cells - visually (11), imunohistochemically (21). Skin releases volatile organic compounds that could be markers for age (6). This could be used for testing of the skin for diagnostic reasons, and probably for development of a tests for the cell cultures observation.

Application of the cell cultures is wide and involves transplantations, new drugs and cosmetic products testing, in vitro study of physiological and pathological processes (22) and other, for example molecular changes in keratinocytes after UV exposure (23).

CONCLUSIONS

Promising directions in the cultivation and application of cell cultures from keratinocytes and epithelial cells:
1. Burns and other skin and mucosae injuries could be treated with vital or stale cell cultures.
2. Tissue engineering could be used for the creation of a close to the physiological skin and mucosae substitutes for vaginal construction, oesophagus construction, lymphatic tissue engineering, oral mucosa construction.
3. In the recovery of specific skin differentiations - nails, hair, glands and regenerative medicine keratinocytes biotechnologies could be used.
4. Cell cultures are alternative to animal models, which complies with the EU Directive 86/609/EEC for the protection of animals used for scientific purposes.
5. The production of a new and improved mediums for cell cultivation is substantial.

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