Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОБЗОРЫ

DOI: 10.19163/2307-9266-2022-10-6-515-524

FEATURES OF QUALITY CONTROL STRATEGY FOR DRUGS BASED ON VIABLE SKIN CELLS

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Received 28 Apr 2022 After peer review 07 July 2022 Accepted 15 Sep 2022

The aim of the study was to research the international experience in quality assurance of the products based on skin cells in order to identify the features of the quality control strategy in the development, production, as well as during an expert quality assessment as a part of the state registration procedure in the Russian Federation.

Materials and methods. The article provides an analysis of the materials presented in the assessment reports of the USA and Japanese regulatory authorities, as well as on the official websites of manufacturers, in review and scientific papers on the study of the structure and properties of tissue-engineered skin analogs.

Results. The manufacture of products containing human skin cells is associated with such risks as the possibility of contamination of the preparation with infective agents transmitted by materials of the animal origin, feeder cells, donor cells, or during the manufacturing process; a small amount of biopsy materials; a complexity of a three-dimensional product structure when combining cells with a scaffold; continuity of the manufacture process and a short product expiry date. The raw materials and reagents control, the creation of cell banks, using animal feeder cells only from qualified cell banks, an in-process control and release testing in accordance with the requirements of the finished product specification, make it possible to obtain a preparation with a reproducible quality. The specification should contain information about the identity, safety and potency of the product. For each preparation, the choice of approaches for assessing the quality is individual and depends on its composition and mode of action.

Conclusion. The features of the quality control strategy for the drugs based on human skin cells, consist in the implementation of control measures in order to obtain a proper quality of cellular (viability, sterility, identity, potency, et al) and non-cellular (physico-chemical scaffold properties) components or the whole graft (bioburden, barrier properties). The approaches and methods for determining the potency should be selected individually for each product and reflect the number, viability and identity of cells, a proliferative activity and secretable ability of the cellular component.

Keywords: skin substitute; keratinocytes; skin fibroblasts; organic and synthetic scaffolds; product quality control; quality attributes

Abbreviations: SS – skin substitute (complete full-thickness skin replacement); ES – epidermal substitute; DS – dermal substitute; MSC – mesenchymal stem cell; FDA – U.S. Food and Drug Administration; PMDA – Pharmaceuticals and Medical Devices Agency; MCB – Master Cell Bank; WCB – Working Cell Bank; PCR – Polymerase Chain Reaction; VEGF – Vascular Endothelial Growth Factor.

ОСОБЕННОСТИ СТРАТЕГИИ КОНТРОЛЯ КАЧЕСТВА ПРЕПАРАТОВ НА ОСНОВЕ ЖИЗНЕСПОСОБНЫХ КЛЕТОК КОЖИ

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Получена 28.04.2022 После рецензирования 07.07.2022 Принята к печати 15.09.2022

For citation: O.A. Rachinskaya, E.V. Melnikova, V.A. Merkulov. Features of quality control strategy for drugs based on viable skin cells. *Pharmacy & Pharmacology.* 2022;10(6):515-524. **DOI:** 10.19163/2307-9266-2022-10-6-515-524

Для цитирования: О.А. Рачинская, Е.В. Мельникова, В.А. Меркулов. Особенности стратегии контроля качества препаратов на основе жизнеспособных клеток кожи. *Фармация и фармакология.* 2022;10(6):515-524. **DOI:** 10.19163/2307-9266-2022-10-6-515-524

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Цель. Изучение международного опыта обеспечения качества препаратов на основе клеток кожи с целью выявления особенностей стратегии их контроля качества при разработке, производстве, а также при экспертной оценке качества в рамках процедуры государственной регистрации в РФ.
Материалы и методы. В статье приведен анализ материалов, представленных в экспертных отчетах регуляторных органов США и Японии, а также на официальных сайтах производителей, в обзорных и научных работах по исследованию структуры и свойств тканеинженерных аналогов кожи.
Результаты. Производство препаратов, содержащих клетки кожи человека, сопряжено с такими рисками, как возможность загрязнения продукта инфекционными агентами при использовании материалов животного происхождения, фидерных клеток, клеток донора или в процессе производства; небольшой объем биопсийного материала; сложность трехмерной структуры препаратов при комбинировании клеток с носителем; непрерывность процесса производства и небольшой срок хранения продукта. Контроль сырья и материалов, создание банков клеток, использование фидерных клеток животных только из аттестованных банков, внутрипроизводственный контроль и тестирование препарата при выпуске в соответствии с требованиями спецификации на готовый продукт позволяют получить продукт с воспроизводимым качеством. Спецификация должна содержать сведения о подлинности, безопасности и активности продукта. Для каждого препарата выбор подходов для оценки качества индивидуален и зависит от его состава и механизма действия.
Заключение. Особенности стратегии контроля качества препаратов на основе клеток кожи человека заключаются в проведении контрольных мероприятий с целью получения надлежащего качества клеточного (жизнеспособность, стерильность, подлинность, активность и другие) и неклеточного (физико-химических свойств носителя) компонентов или целого графта (бионагрузка, барьерные свойства). Подходы и методы для определения активности должны выбираться индивидуально для каждого продукта и отражать число, жизнеспособность и подлинность клеток, пролиферативную и секреторную способность клеточного компонента.
Ключевые слова: эквиваленты кожи; кератиноциты; фибробласты кожи; органические и синтетические носители; контроль качества препарата; показатели качества
Список сокращений: ЭК – эквивалент кожи (полнослойный); ЭЭ – эпидермальный эквивалент; ДЭ – дермальный эквивалент; МСК – мезенхимальные стволовые клетки; FDA – Управление по санитарному надзору за качеством пищевых продуктов и медикаментов (США); PMDA – Агентство по фармацевтической продукции и медицинским приборам (Япония); МБК – мастер банк клеток; РБК – рабочий банк клеток; ПЦР – полимеразная цепная реакция; VEGF – фактор роста эндотелия сосудов.

INTRODUCTION

Skin substitutes (SSs) are tissue-engineered analogs of the skin, which are three-dimensional constructs based on *in vitro* cultured skin cells and various synthetic or organic carriers (scaffolds, matrices, matrixes) used in medicine for temporary or permanent replacement of damaged epidermal, dermal or full-layer skin areas [1-3]. For the same purpose, it is possible to use cultured skin cells, for example, fibroblasts or keratinocytes, without creating a three-dimensional structure on their basis using a carrier in the form of a cell suspension [4].

The use of SSs and skin cells without a carrier is aimed at restoring the structure and functions of the skin, primarily the barrier one (protecting the body from infection by pathogens from the environment and preventing the loss of water and mineral salts by the body through the wound surface). It is also important to note the acceleration of healing processes and the reduction of pain in burns, acute and chronic wounds, scars, diabetic ulcers, nevi, skin structure disorders as a result of genetic and other diseases [5–9].

A key step in the development and production of SSs is the isolation of cells certain types from a donor skin, followed by the cultivation of these cells *in vitro* in order to obtain the amount of cellular material necessary for a therapeutic effect. All the SSs currently approved for a clinical use in the world, contain only two types of cells (either individually or in tandem): keratinocytes and fibroblasts. The use of keratinocytes able of forming a layer of outer cornified epithelium underlies the creation of epidermal grafts (epidermal tissue-engineeed equivalent, epidermal substitute – ES), and the use of fibroblasts in combination with organic or synthetic carriers makes it possible to create an analogue of the dermis – a dermal graft (dermal tissue engineered equivalent, dermal substitute – DS), which increases the probability of the subsequent successful engraftment from 15 to 45–75% [10]. There are products given a permission for a clinical use in different countries of the world. They combine both ESs and DSs, and are composite two-layer SSs (full-layer SSs) [11]. Full-thickness skin equivalents are an alternative to skin grafts obtained both from healthy skin areas of the patients themselves (autografts) and from healthy donors (allografts).

The developments were carried out to improve the functionality of tissue-engineered structures and achieve greater similarity with healthy human skin through the use of other cell types: endothelial cells, Langerhans cells, melanocytes [12–14]. The inaccessibility and complexity of cultivating keratinocytes and fibroblasts led to attempts to use mesenchymal stem cells (MSCs) of various origins. They have a high proliferative potential and the ability to differentiate, either alone or in tandem with a carrier [15]. However, all these studies are under development or clinical trials: currently, there are no registered SSs in the world based on the use of these cell types.

DOI: 10.19163/2307-9266-2022-10-6-515-524

Among the preparations containing the skin cells that have been approved for a clinical use, there are products based on the cells of both autologous (more often ESs) and allogeneic origin (more often DSs and full-thickness SSs) [16]. In this case, the product can be a suspension of cells and be applied to the wound surface by spraying (in the form of a spray), or in the form of a tissue-engineered graft to cover the wound as a result of an application [4].

In a number of preparations, intradermal injections of cells using a needle and syringe are used. To create a graft, SSs cells are placed on a carrier (matrixes, scaffolds, matrices). This is most often used as collagen of the animal origin (bovine, porcine, murine) and synthetic carriers (silicone, hyaluronic acid, and others). This makes it possible to obtain a multilayer structure with a well-defined barrier function, a biomechanical stability, stratification of keratinocytes, formation of intercellular interactions, synthesis of the basement membrane and important components of the extracellular matrix [17, 18].

All SSs can be used as temporary bioactive barrier dressings, however a number of products, mostly containing biodegradable carriers, can be used as a permanent replacement for a damaged skin area.

During the production and implementation of the registration procedure for SSs, the question of an adequate and comprehensive assessment of their quality arises. This is complicated by a composite of these preparations, which include both a component of viable cells and, often, a non-cellular component – a carrier.

THE AIM of the study was to research the international experience in quality assurance of the products based on skin cells in order to identify the features of the quality control strategy in the development, production, as well as during an expert quality assessment as a part of the state registration procedure in the Russian Federation.

MATERIALS AND METHODS

To analyze the characteristics of quality control strategies for the products based on viable skin cells, the basis of the study was the materials presented in assessment reports of the regulatory authorities of the United States (Food and Drug Administration – FDA) and Japan (Pharmaceuticals and Medical Devices Agency – PMDA) for the following products that had received approval for a clinical use by Fda.gov and Pmda.go.jp. They were: Apligraf and GINTUIT (Organogenesis, Inc., USA)[1]; LAVIV (Fibrocell Technologies, Inc., USA)[2]; STRATAGRAFT (Sratatech Corporation, USA)[3]; Epicel (Genzyme Biosurgery, USA)[4]; Invitrx (Ortec International, Inc., USA)[5]; JACE (Japan Tissue Engineering Co., Ltd., Japan)[6].

Information on the other products used in the countries of the European Union (EU), the Republic of Korea, the Russian Federation (RF) and other countries of the world, was obtained from the official websites of manufacturers, as well as from review and scientific papers on the study of the structure and properties of tissue-engineered skin substitutes.

The following electronic resources were used to conduct the study: PubMed, Scopus, Google Scholar, eLibrary, Ema.europa.eu., Fda.gov, Pmda.go.jp. The queries were conducted on combinations of the following keywords: “skin substitutes”, “scaffolds for skin repair and regeneration”, “skin tissue engineering”, “skin cells products quality control”, “skin cells products quality attributes”, as well as on the trade names of the products, approved for a medical use. The search was carried out for the period from Oct 2021 to Ap 2022.

The logical methods of the system analysis and modeling were used in the work.

RESULTS AND DISCUSSION

Currently (dated Apr 2022), more than two dozen products containing viable human skin cells have been approved for clinical use in the EU, USA, Australia, Japan, the Republic of Korea and the Russian Federation (for some products, the license term expired has expired and has not been renewed).

Table 1 presents preparations based on human skin cells used for skin resurfacing (ESs, DSs, full-thickness SSs and the preparations based on skin cells without a carrier) that have received a clinical approval in different countries of the world. Information on them is presented on the manufacturers’ official websites and in assessment reports of regulatory authorities (see “Materials and methods”).

[1] Food and Drug Administration (FDA). Gintuit – Summary Basis for Regulatory Action, 2012. Available from: http://wayback.archive-it.org/7993/20170723023240/https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM297753.pdf

[2] Food and Drug Administration (FDA). LAVIV – Summary Basis for Regulatory Action, 2011. Available from: https://wayback.archive-it.org/7993/20170723023939/https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM262780.pdf

[3] Food and Drug Administration (FDA). SRTATAGRAFT – Package Insert, 2021. Available from: https://www.fda.gov/media/150129/download

[4] Food and Drug Administration (FDA). Epicel – Summary of safety and probable benefit, 1998. Available from: https://www.fda.gov/media/103308/download

[5] Food and Drug Administration (FDA). Invitrx – Summary of safety and probable benefit, 1998. Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf/H990013B.pdf

[6] Pharmaceuticals and Medical Devices Agency (PMDA). JACE – Review Report, 2007. Available from: https://www.pmda.go.jp/files/000223079.pdf

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ISSN 2307-9266 e-ISSN 2413-2241
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Table 1 – Examples of products based on human skin cells that have received approval for clinical use in the world

Product name	Manufacturer and country of registration	Cellular component	Carrier type	Origin of cells	Intended diseases for treatment
EPIDERMAL SUBSTITUTES AND PREPARATIONS CONTAINING KERATINOCITES					
Epicel (Cultured epidermal autografts)	Genzyme Biosurgery, USA	Keratinocytes layer (2–8 cells thick) cultured in the presence of non-dividing mouse fibroblasts (feeder layer)	–	Autologous	Deep burns (more than 30% of the body surface)
JACE (Human autologous epidermal cell sheet)	Japan Tissue Engineering Co., Ltd. (J-TEC), Japan	Keratinocytes layer (several cells thick) cultured in the presence of irradiated 3T3-J2 cells derived from a mouse embryo (feeder layer	–	Autologous	Extensive (more than 30% of the body surface) burns, the 2nd-3rd degree
KeraHeal	Biosolution Co., Ltd., Republic of Korea	Suspension of cultured keratinocytes	–	Autologous	Deep burns, the 2nd degree (more than 30% of the body surface), and the 3rd degree (more than 10% of the body surface)
KeraHeal-Allo	Biosolution Co., Ltd., Republic of Korea	Suspension of keratinocytes	Thermosensitive hydrogel	Allogeneic	Deep burns, the 2nd degree
Holoderm	Tego Science, Inc., Republic of Korea	Cultured keratinocyte progenitors with epidermal flap formation	–	Autologous	Burns, the 3rd degree (more than 50% of the body surface)
Kaloderm	Tego Science, Inc., Republic of Korea	Cultured keratinocytes with epidermal flap formation	–	Allogeneic	Deep burns, the 2nd degree; Diabetic foot ulcers
EpiDex	Modex Therapeutiques, Switzerland	Cultured keratinocytes	Not available	Autologous	Not available
EPIBASE	Laboratoires Genevrier, France	Keratinocytes cultured to a confluent monolayer	–	Autologous	Not available
MySkin	CellTran Ltd., United Kingdom	Keratinocytes cultured to a subconfluent monolayer	Surface Coated Silicone Matrix	Autologous	Neuropathic, decubitus, diabetic foot ulcers; Burns
Laserskin (Vivoderm)	Fidia Advanced Biopolymers, Italy	Keratinocytes cultured to a confluent monolayer	Microperforated hyaluronic acid membrane	Autologous	Diabetic and venous ulcers of the lower extremities; Shallow burns; Vitiligo
Bioseed-S	BioTissue Technologies GmbH, Germany	Keratinocytes cultured to a subconfluent monolayer	Matrix on fibrin sealant	Autologous	Treatment-resistant chronic venous ulcers of the lower extremities
CellSpray	Clinical Cell Culture (C3), Australia	Keratinocytes uncultivated / cultured to a subconfluent monolayer	–	Autologous	Not available
Multi-layered sheet of keratinocytes	Institute of Cytology RAS, Russia	Cultured keratinocytes	Not available	Not available	Burns of varying severity, including critical and supercritical; Ulcers of different etiology; Wounds resulting from trauma; Fistulas, bedsores
DERMAL SUBSTITUTES AND PREPARATIONS CONTAINING FIBROBLASTS					
LAVIV (Azficel-T)	Fibrocell Technologies, Inc., USA	Cultured fibroblasts	–	Autologous	Medium-deep and deep wrinkles of the nasolabial folds

DOI: 10.19163/2307-9266-2022-10-6-515-524

Product name	Manufacturer and country of registration	Cellular component	Carrier type	Origin of cells	Intended diseases for treatment
TransCyte (DermagraftTC)	Advanced BioHealing, Inc., USA	Cultured neonatal fibroblasts	Silicone film backing, porcine collagen nylon mesh	Allogeneic	Superficial and deep burns; Chronic ulcers of the lower extremities, including diabetic and venous ones; bedsores
Dermagraft	Shire Regenerative Medicine, Inc., USA	Cultured neonatal fibroblasts	Bioresorbable collagen on polyglactin or polyglactin-910 sponge	Allogeneic	Chronic diabetic ulcers of the lower extremities, affecting the dermis, but not reaching the tendons, muscles and bones; Chronic and infected wounds
CureSkin Inj.	S.Biomedics Co., Ltd., Republic of Korea	Cultured fibroblasts	–	Autologous	Post-acne depressive scars
Rosmir	Tego Science, Inc., Republic of Korea	Cultured fibroblasts	–	Autologous	Reduction of the nasolacrimal trough
Hyalograft 3D	Fidia Advanced Biopolymers, Italy	Cultured fibroblasts	Microperforated hyaluronic acid membrane	Autologous	Diabetic and venous ulcers of the lower extremities; Shallow burns; Vitiligo
Cell technology SPRG therapy	Human Stem Cell Institute	Cultured fibroblasts	–	Autologous or allogeneic	Correction of age-related skin changes; Recession and mucosal deficiency in the area of teeth and dental implants
Dermal equivalent	Institute of Cytology RAS, Russia	Cultured fibroblasts	Collagen gel	Allogeneic	Burns of varying severity, including critical and supercritical; Ulcers of different etiology; Wounds resulting from trauma; Fistulas, bedsores
			FULL-THICKNESS HUMAN SKIN EQUIVALENT		
Apligraf	Organogenesis, Inc., USA	Layers of cultured keratinocytes and fibroblasts	Matrix of bovine collagen and extracellular matrix proteins	Allogeneic	Soft tissue wounds of the oral cavity
GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen)	Organogenesis, Inc., USA	Layers of cultured keratinocytes and fibroblasts	Type I bovine collagen	Allogeneic	Defects and lesions of the mucous membrane of the gums
STRATAGRAFT (Allogeneic Cultured Keratinocytes and Dermal Fibroblasts in Murine Collagen)	Sratatech Corporation, USA	Stratified epithelial layer containing differentiated keratinocytes deposited on a dermis-like structure formed by fibroblasts	Type I mouse collagen	Allogeneic	Thermal burns with intact dermal elements (deep burns, the 3rd degree.)
Invitrx (Composite Cultured Skin)	Ortec International, Inc., USA	Layers of cultured keratinocytes and fibroblasts	Type I bovine collagen sponge with a thin gel-like layer of bovine collagen	Allogeneic	Dystrophic epidermolysis bullosa (after arm reconstruction surgery)
OrCel	Ortec International, Inc., USA	Layers of cultured keratinocytes and fibroblasts	Type I bovine bollagen sponge	Allogeneic	Dystrophic epidermolysis bullosa (after hand reconstruction surgery); Burns and wounds
PolyActive	HC Implants BV, Netherlands	Cultured keratinocytes and fibroblasts	Synthetic matrix from terephthalate derivatives	Autologous	Shallow wounds

Note: Laserskin and Hyalograft 3D preparations can be used together. Such a complex preparation has the trade name TissueTech Autograft System; SPRG – Service for Personal Regeneration of Gum.

ISSN 2307-9266 e-ISSN 2413-2241
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Table 2 – Some qualification of keratinocytes and fibroblasts production cell banks

Indicator	Approach to testing	Methods of analysis
Virus safety	In vitro and in vivo testing for viruses (including retroviruses) specific to humans, pigs, cattle	Transmission electron microscopy; Reverse transcription
Sterility, mycoplasma contamination	Detection of all types of microbiological contaminants: bacteria and fungi	Microbiological methods; PCR
Tumorigenicity	In vitro studies of cell genome stability; In vivo studies of tumor formation	Karyotyping; Isoenzyme analysis; Cell culture aging tests; Tumors formation tests
Identity	Expression of involucrin by keratinocytes; Biosynthesis of collagen by fibroblasts	Isoenzyme analysis; Immunochemical methods; PCR
Activity	Viability; Cell culture growth parameters	Cell counting with an automatic counter or with a hemocytometer; Flow cytometry; Morphological analysis
Comparability (with previously characterized cells)	In vitro tests, including confirmation of cellular purity, degree and intensity of percutaneous absorption, cytokine profile analysis and VEGF quantitation; In vivo studies on immunodeficient animals of the involucrin expression level, engraftment, integration, morphology and deformation of the graft, reduction of the wound surface through a wound contraction	Histology; Immunochemical methods; MTT test

Note: PCR – polymerase chain reaction; VEGF – Vascular Endothelial Growth Factor; MTT – Mitochondrial Tetrazolium Test (colorimetric method using tetrazolium salt).

It should be notified that not all skin cell products had been originally registered as drugs. A number of products, such as Epicel (Genzyme Biosurgery) and Invetrx (Ortec International), received the FDA approval for a clinical use as medical devices (Humanitarion Device Examption, HDE), but then were reclassified as biological tissue based products -"Tissue & Tissue Products"- regulated by the FDA's Center for Biologics Evaluation and Research (CBER). In the Russian Federation, there is currently no experience of products state registration containing viable human skin cells as biomedical cell products within the framework of the Federal Law dated Jun 23, 2016, No. 180-FZ "On Biomedical Cell Products" currently in force in the Russian Federation, or as advanced therapy medicinal products within the framework of the Decision of the Eurasian Economic Commission Council dated Nov 3, 2016, No. 78 "On the Rules of marketing authorization and assessment of medicinal products for human use". Previously, three tissue-engineered products (SPRG cell technology from the Institute of Human Stem Cells, a dermal substitute and multilayer keratinocyte layer from the Institute of Cytology of the Russian Academy of Sciences) [6] received a permission from the Federal Service for Surveillance in Healthcare (Roszdravnadzor) for clinical use in medical practice as cell technologies for a personalized skin treatment[7]. Now, the validity of permits for the use of cellular technologies and registration certificates for medical devices have expired, or their commercial use has been suspended due to the changed legislative framework of the Russian Federation in the field of development and registration of the products based on viable human cells.

Currently, only expert reports on the products registered by the FDA and PMDA often containing incomplete information regarding the conduct of a quality assessment during an in-process control and at the release control of the product are available in the public domain. Based on the available data, the following features of the quality control strategy for SSs and the products based on viable skin cells as well as the main problems associated with their production, can be identified.

Input control of raw materials and reagents

The use of raw materials of the human and animal origin (cell culture serum, bovine pituitary extract, collagen for matrix) is associated with such a safety problem for the product use as the risk of microbiological and viral contamination, and in case of the materials obtained from cattle, there is an additional risk transmission of transmissible spongiform encephalopathy. In order to reduce these risks, materials and reagents of the animal origin are tested for sterility (the presence of bacteria, fungi, mycoplasma), the presence of the viral contamination

[7] Federal Service for Surveillance in Healthcare of Russian Federation. List of medical technologies approved for use in medical practice as of December 30, 2011. Available from: https://roszdravnadzor.gov.ru/documents/12545.

DOI: 10.19163/2307-9266-2022-10-6-515-524

and bacterial endotoxins. All the materials from cattle should be obtained from the countries where cases of transmissible spongiform encephalopathy have not been reported [22].

In case of the most preparations production, cell donors are tested for the presence of pathogens of infectious diseases. However, for the autologous product Epicel (Genzyme Biosurgery), donors of cellular materials are not subjected to such testing, which leads to the risk of a possible infection of the personnel working with the biomaterial and product. This requires special precautions in the production[8].

In addition, the materials used to create cell carriers and form a three-dimensional structure of the product must be tested for physical and chemical properties.

Creation of cell banks

Another feature of the products production based on human cells intended for an allogeneic use is the need to create cell banks.

For the production of ESs, DSs and full-thickness SSs for an allogeneic use, human keratinocytes and fibroblasts are used. They are obtained from the biopsy materials of healthy donors, cultured in order to develop the cellular material necessary for the therapeutic effect. Obtaining a required number of cells, which can be used for one or even several patients from a small donor area of the skin, would be difficult without a creation of the production cell banks: a master cell bank (MCB) and a working cell bank (WCB). As a rule, a number of passages between MCB and WCB is small9. The cell banks should be cvalificated and the banked cell lines should be characterized by quality attributes such as sterility, absence of mycoplasma contamination and introduced viral agents, a cytogenetic stability, tumorigenicity, purity, potency and identity, a proliferative activity and viability. The features of testing cell lines for some quality attributes, given in the assessment reports of the regulatory authorities that have issued the permission for the clinical products use (section “Materials and methods”), are shown in Table 2.

Risk of using animal cell feeder layer

For the cultivation of human skin cells in the production of a number of preparations, for example, Epicel (Genzyme Biosurgery), STRATAGRAFT (Stratatech Corporation) and JACE (Japan Tissue Engineering Co.), a mouse cell feeder layer, which may be present in a residual amount in the finished product, is used. The FDA classifies these products as xenografts, and despite the possibility of using feeder cells only from qualified cell banks and recognizing a low risk of transmitting infectious agents through these cells, it recommends product recipients to refuse to donate blood, plasma, tissues, eggs, breast milk and other biomaterials.

In-process control

The main characteristic of the production of preparations containing viable human cells is the implementation of all processes under aseptic conditions. The impossibility of sterilizing the resulting finished product leads to the need to control the product for sterility and the absence of mycoplasma as an in-process control.

The structural features of the preparations[10,11] based on skin cells, which are multilayer structures on a carrier, lead to the need to track the morphology of the product using a visual assessment and histological examination, an investigation of physical properties after the graft washing stage, a proliferative activity of keratinocytes, and viable cells counting [23, 24]. Reporting of cell morphology in the form of photographs, control of stratification in the cell culture, and characterization of other cell types (other than fibroblasts) present in the cell culture, was also requested by the FDA advisory committee for Laviv containing skin fibroblasts without a carrier.

The results of testing for the above-listed quality indicators, along with some others, such as “bioburden”, may be included in the specification for the finished product, but obtained during the production process. This is due to the short shelf life of the finished product (without cryo-freezing) [25], which does not allow long-term tests during the drug release. Therefore, the shelf life of Apligraf and Gintuit (Organogenesis, Inc.) after thawing is 15 days[13], and the shelf life of STRATAGRAFT (Stratatech Corporation) is 4 h.

Release testing of finished products

All the products currently registered in the world and containing viable human skin cells are obtained as a result of a continuous production process, and therefore, have only one final specification for the finished product. Depending on the structure of the product, the presence of a multilayer structure, or carrier, the specifications may differ in the set of quality attributes by which the product is release tested. Such testing may be performed partly before cryopreservation the finished product and

[8] Food and Drug Administration (FDA). Epicel – Summary of safety and probable benefit, 1998.
[9] Food and Drug Administration (FDA). Gintuit – Summary Basis for Regulatory Action, 2012.
[10] Ibid.
[11] Pharmaceuticals and Medical Devices Agency (PMDA). JACE – Review Report, 2007.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

partly after packaging on thawed samples, as outlined in the assessment report for LAVIV (Fibrocell Technologies, Inc.).

In general, the specification for a finished product containing viable human skin cells includes the following quality attributes:

– Visual assessment of the product appearance (description);
– Viability and total number of cells;
– Sterility;
– Mycoplasma;
– Bacterial endotoxins;
– Identity (for fibroblasts and keratinocytes);
– Purity;
– Activity (efficiency);
– Container closure integrity.

The quality attribute "Description" should reflect such parameters as color, transparency of the product, the appearance of ESs or Dss (if applicable): the presence of irregularities on the surface of the structure, wrinkling, deformation, changes in the thickness of the layers[12].

The assessment of sterility is mainly carried out using a standard microbiological test, followed by Gram staining of microbiological preparations[13,14]. The test for mycoplasma at the release control of JACE (Japan Tissue Engineering Co., Ltd.) was carried out by a cytochemical method by staining DNA with a fluorescent dye (using indicator cell lines). It is assumed that the results of testing for sterility and mycoplasma can be obtained after the clinical use of the product, due to the excess of the duration of the test over the expiration dates of some preparations[15]. The possibility of the product clinical use without results of the sterility assessment and mycoplasma contamination leads to the requirement to provide information for the doctor with a treatment plan for the patient in case of infection with product-contaminating agents on the package insert of the finished product.

The assessment report on the JACE preparation (Japan Tissue Engineering Co., Ltd.) indicates the need for the content control of the residual amount of bovine serum albumin, determine the residual number of feeder cells and the physical properties of the flap (possibly at the stage of in process control). Such tests can be attributed to the quality attribute "purity" and prove the absence of impurities in the finished product resulting from the technological process.

One of the most difficult and controversial points in the selection of adequate methods of analysis in assessing the quality of products used to heal wounds and skin lesions is the confirmation of their potency. Most often, the mechanism of products action is not demonstrated (not established) or is not given in the regulatory documentation for the product. A number of assessment reports on products indicate the possibility of wound healing due to the activation of a recipient cell division during the secretion of biologically active substances by the cells of the product (platelet, fibroblast, vascular endothelial, epidermal growth factors, cytokines, type IV collagen, tenascin, fibronectin, and others). A similar mechanism of action has been notified for Apligraf, GINTUIT, STRATAGRAFT, KeraHeal-Allo, OrCel, TransCyte and Dermagraft. The ability of fibroblasts to accelerate the mechanisms of tissue regeneration due to the secretion of biologically active substances by them is considered proven [26, 27]. At the same time, such products are temporarily on the wound surface, with a gradual elimination of cells and a resorption of non-cellular material (if applicable). For another group of products (KeraHeal, TissueTech Autograft System, CellSpray), a decrease in the wound surface was shown due to the proliferation and differentiation of the cells of the product itself with the formation of the skin structures necessary to perform a barrier function. These preparations are used as permanent applications to close the wound surface due to the possible proliferation, migration and differentiation of cells with the formation of a stratified epithelium [28].

The choice of methods and approaches for assessing the potency (which is a measure of the effectiveness of a product in clinical use) of each preparations containing human skin cells occurs on an individual basis and is agreed by the product manufacturers with the regulatory authorities of the countries where their clinical use will be carried out. For example, the effectiveness of LAVIV (Fibrocell Technologies, Inc.) was concluded based on the determination of the total cells number in the preparation, the confirmation of the fibroblasts identity, and the analysis of the collagen secreted by the cells. To evaluate the effectiveness of GINTUIT (Organogenesis, Inc.), a histological examination of the graft was performed. However, the advisory committee accepted this approach only as the evidence of the structural product integrity, and suggested that an additional analysis of cell-secreted cytokines be carried out.

Besides, if necessary, additional quality attributes can be added to the specification for the finished product: pH, bioburden[16], barrier properties of grafts, which

[12] Food and Drug Administration (FDA). SRTATAGRAFT – Package Insert, 2021.
[13] Food and Drug Administration (FDA). Epicel – Summary of safety and probable benefit, 1998.
[14] Food and Drug Administration (FDA). LAVIV – Summary Basis for Regulatory Action, 2011.
[15] Food and Drug Administration (FDA). Gintuit – Summary Basis for Regulatory Action, 2012.
[16] Ibid.

DOI: 10.19163/2307-9266-2022-10-6-515-524

confirm the formation of the outer cornified epithelium and are examined on the basis of a histological analysis[17]. The barrier function confirmation and studies of graft permeability are carried out using different approaches, for example, by determining the thickness of the epidermis and studying the lipid profile [29]. A formation of the correct basement membrane is possible by confirming the presence of type IV collagen at the dermo-epidermal junction.

CONCLUSION

Due to the continuity of the technological production process, a short shelf life and the impossibility of carrying out sterilization procedures for the finished product containing viable human skin cells, as well as the complexity of combined preparations (applicable to grafts), which consists in the presence of a non-cellular component-carriers (matrixes, scaffolds, matrices) in the finished product) which is often difficult to separate from cells, it is of great importance to comply with a number of requirements for a technological process and take into account the peculiarities of the quality control strategy for such drugs. These requirements are as follows: creation of a qualification program for raw materials and reagents, which would include the risks of their use; a control of materials of the animal origin; creation of skin cell banks used for the production of allogeneic products; the use of feeder cells only from qualified banks; conducting a number of studies on quality attributes during in process control with the inclusion of the results of these studies in the specification for the finished product. In addition to standard tests (description, viability, total number of cells, sterility, mycoplasma, bacterial endotoxins, identity, purity, potency) characteristic of a quality control of any preparations containing viable human cells, a quality control of preparations containing skin cells includes additional studies of physical and chemical properties of the carrier and/or the whole graft (bioburden, barrier properties of the graft).

Due to the difficulty of demonstrating the potency of products used for wound healing, and the incomplete knowledge of the nature of their action, approaches and methods for determining this quality attribute should be selected individually for each product and reflect such properties of the cellular component as the number, viability and identity of cells, as well as their proliferative and/or secretory capacity.

FUNDING

The work was carried out within the framework of the state task of the Scientific Centre for Expert Evaluation of Medicinal Products of the Ministry of Health of Russia No. 056-00052-23-00 for applied scientific research (State Registration of Research Work No. 121021800098-4).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Olga A. Rachinskaya – information and analytical search on the investigation topic, data processing, article writing;
Ekaterina V. Melnikova – aim and objectives setting, text correction;
Vadim A. Merkulov – concept planning, consulting for legal acts regulating drugs circulation.

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