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TISSUE ENGINEERING CONSTRUCTIONS USE IN THE TREATMENT OF TROPHIC ULCER DISEASE

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ABSTRACT

The implementation of new biotechnological analogues (equivalents) of tissues and organs, in particular, human skin equivalents (SE), designed to temporarily or permanently replace damaged or destroyed tissues in trophic ulcer disease, remains an urgent task of regenerative medicine to develop and clinical practice. Currently, there are different types of living cells in the full-layer TE and its individual layers are being created and examined.

A comparative analysis of existing SEs at the commercial and clinical pre-trial stage was carried out. The features of their structure and the possibilities of their use in solving experimental and clinical problems were analyzed. The characteristics of the three main variants of SE were considered. Examples of the use of stem cells to create skin equivalents are given. The main advantages of using stem cells as the tissue component of EC are described.

Key words: bioengineering skin replacement tissues; skin equivalents; biomaterials; tissue engineering; wound healing; trophic wound.

INTRODUCTION

The skin is the largest organ of mammals and serves as a protective barrier between the human body and the environment.Due to the location of the border, the skin is constantly exposed to potentially harmful microbiological, thermal, mechanical and chemical factors [1]. In case of skin damage, the restoration of the barrier properties of the body becomes its main task. The first is due to the partial or complete restoration of the structure of the skin. Because the structure and functions of this organ are closely related [2].

Violation of the normal biological response to skin damage due to illness, injury or surgery will inevitably lead to significant complications. The wound healing process is very complex and is often multifactorial in chronic injuries [3].

The regenerative capacity of humans is very limited: unlike animals, the skin cannot be restored with basic intent, and marginal epithelization is difficult. Currently, a complete lack of understanding of the molecular, cellular and physiological mechanisms that regulate wound healing is a common cause of disappointing treatment outcomes.

The most important and rapidly developing area of modern regenerative medicine is the use of tissue technology in the treatment of trophic ulcers. The task of Tissue Technologies in this case is not only to move living cells to the defective area, but also to fully restore the structure and function of the skin, stimulate regenerative processes and create a microenvironment for the implementation of the capabilities of their own tissues and cells. Tissue engineering techniques are used to solve such problems.

Structure of skin equivalents

Human skin equivalents are bioengineering constructions-consisting of a skin substitute, a cellular component, i.e. a substrate (Matrix, skaffold) that is an analogue of cultured human skin cells and extracellular matrix [4].

Most substitutes produced in tissues for living skin are created by laboratory cultivation of skin cells and their combination with podlojka. SE is used to restore the structure of the skin and, therefore, the barrier function (the main purpose of treating patients with burns), as well as to initiate wound healing (in chronic non-healing wounds). The use of SE accelerates healing, eliminates wounds, reduces pain, inflammation, and prevents the appearance of scars, contractures, or pigment defects [4].

The main requirements for SE are their biological and toxicological safety, effectiveness and lack of immunogenicity. It should be taken into account that any tissue material carries the risk of contracting a viral, bacterial or other infection, and the patient may have individual intolerance to backing or they may cause a strong inflammatory reaction.

In addition, it is advisable that SE biodegradates and substitutes contribute to the restoration of normal tissues with physical and mechanical properties similar to human skin. Also important are the economic efficiency of biomaterial production, its ease of finding and its long shelf life [2, 5].

Tissue component

The main cell types in mammalian skin are fibroblasts and keratinocytes. Accordingly, most wound healing research uses one or both of these cell types as the cellular component of SE.

Epidermal keratinocytes are the main part of skin cells. In the process of keratinization, special proteins are synthesized in them - acidic and alkaline types

of keratins, filagrin, involucrine, keratoline and others resistant to mechanical and chemical influences [6, 7]. Keratinocytes of the basal layer are able to actively divide, and when they differ from each other, the cells move from the basal layer to the superficial layers of the skin. Thus, every 3-4 weeks, renewal of the epidermis (physiological regeneration) occurs [8, 9].

For the first time, keratinocyte culture was established in 1975 by D. Reinwald and G. Taken by Green [10]. The first keratinocytes were cultured using fibroblasts of the Swiss mouse - 3T3. The feeder cells were then replaced by the addition of growth factors and extracellular matrix proteins to the culture environment. It turns out that some of them contribute to the growth of keratinocytes and can be used as a substrate for their cultivation [11-13].

Dermal fibroblasts are heterogeneous populations of mesenchymal cells and play a major role in regulating cell interactions and maintaining skin homeostasis. Dermal fibroblasts produce all the main components of the extracellular matrix - collagen, glycosaminoglycans, proteoglycans-and are also responsible for the continuous process of Matrix remodeling [14]. Due to these properties, fibroblasts are widely used to create TE.

Types of skin equivalents

The skin equivalent, depending on the purpose, can be monoculture and includes only the epidermis layer or only the dermis layer, or has a fully layered structure [2]. Thus, existing TE species can be divided into three main groups: epidermal, dermal, and full-fledged.

Epidermal equivalent type

Keratinocytes are used to create this type of SE. Depending on the source of the cells, such equivalents can be otological (the source of the cells is the patient's own skin) or allogenic (the cells are derived from the donor skin). 1-2 cm^2 of the skin to separate keratinocytes. With the help of enzymes and mechanical effects, the epidermis is isolated from the dermis, and then a suspension of individual keratinocytes is obtained through additional enzymatic treatment. Primary keratinocytes are grown in the laboratory for up to several weeks, thanks to which layers of keratinocytes are obtained, several times larger than the size of the donor skin layer [1, 16]. For transplantation in two patients with large burns, the otologist was the first to use sheets of cultured epithelium made from keratinocytes.E. O'conner used [17, 18].Epidermal autotransplantants (EA) were later used to permanently cover large-scale burns in two additional patients [18].

One of the main disadvantages of EA is poor transplant survival, even when keratinocytes are grown properly, mainly in wounds that lack dermal elements [19]. In the mid-1980s. C.B. Cuono et al. the presence of a dermal component has

shown importance, they have reported a good survival rate of EA placed in a healthy vascularized allogenic dermis [20, 24]. However, their proposed method has its drawbacks. First, in some countries where organ and tissue transplantation is still not common, skin allografts may not exist [21, 22].

Second, allogeneic (murine) dermis loscutlaris contain risks associated with infection or rejection.

Thirdly, it is difficult to coordinate two successive stages of transplantation: first, the placement of dermal allografts on the wound, and then EA. It turns out that if the allogenous dermis is rejected before the use of cultured EA, this method of treatment becomes impossible for the patient [22]. Finally, the high cost of EA production is often cited as one of the main obstacles to their widespread use in many areas [23-24].

Dermal equivalent type

As a rule, it represents connective tissue cells - fibroblasts in combination with a collagen matrix (podlojka). Cells can fill the surface and / or the entire size of the substrate. The Dermal equivalent can be created on the basis of other connective tissue cells - mesenchymal stem cells (MSC), and as an extracellular matrix, almost any three-dimensional podlojka currently available can be used. According to the literature, there are now many marketed skin equivalents, and most of these products have been well analyzed and tested at the pre-clinical and clinical trial levels [15, 25,26]. Many modern biologically compatible dermal grafts are able to to to some extent mimic the basic properties of human connective tissue, ensuring the integrity, elasticity and blood vessels of the structure. Fibroblasts are easy to distinguish and grow technologically easily, while they are an active cellular component that can repair the collagen of the dermis, stimulate wound granulation and secrete a number of growth factors that accelerate skin regeneration. It is not surprising that dermal equivalents from fibroblasts are common all over the world.

However, when applying skin equivalents, the problem of epithelization of extensive skin lesions remains, and in many cases the use of such products is combined with the use of skin autografts for permanent coating [27].

Skin equivalents using synthetic materials began to be developed in the 1990s, but are now less commonly used. These include a Dermagraft of cryosalmed allogenic human fibroblasts, derived from the skin of a Transsitic and biodegradable neonatal foreskin, made up of fibroblasts of allogenic human neonatal foreskin, bound to a silicon membrane and grown in pig collagen covering a nylon web. polyglactin (vicrill) net [28]. It is known that both of these products are not currently on the market, but these technologies are licensed by

Advanced BioHealing to further improve the product [15]. Unlike transsitis, Biobrane is still widely used as a synthetic skin equivalent to treat secondary burns in many centers [29,30]. It is structurally similar to Transsitis, but contains fewer neonatal human fibroblasts. It is also used in complex wound topology as a dressing material along with autogrefts, as well as for the cultivation of keratinocytes [30, 31]. The popularity of Biobrane is due to its versatility and low cost compared to Transcyte, which has high efficacy in treating secondary burns [32].

The original skin equivalent is N.K. Developed at the Koltsov Institute of Developmental Biology in Russia. Fibroblasts grown in gelatin or collagen microcrackers were wrapped in three-dimensional collagen gel. Microtashers are also a type of three-dimensional matrix in the form of small (50-70 μm) smooth or porous spheres with a longer biodegradation period compared to a gel. This better complements deep connective tissue defects of full thickness such as equivalent fistulas [33].

Full layer equivalent type

A fully layered type of SE, or usually the living equivalent of skin, consists of the epidermis and skin layers.

The promising autological skin-epidermal equivalent is a composite skin replacement developed in Cincinnati, United States. This Te consists of collagen-glucosaminoglycan podlojka, which contains otological fibroblasts and keratinocytes. Now known as PermaDerm [2], this product can be created in 30 days and is able to provide a permanent replacement of the dermal and epidermal layers of the skin. It has indications for the treatment of major skin defects, but has not yet received FDA (Food and Drug Administration) approval for clinical use [22, 34, 35].

In 2009, one German scientist reported that a composite autogreft was developed using MatriDerm as a matrix for growing otological fibroblasts and keratinocytes [36]. It has been claimed that the developed full-thickness skin equivalent is homologous to healthy human skin. The basics were the features of the epidermis layer, comparison of signs of differentiation and proliferation of cells, the presence of a functional basal membrane. Transplantation of this equivalent showed a positive result with the complete closure of relatively small wounds (up to 9 × 6 cm) [37, 38].

The most famous of full-layer TES is Apligraf. It is a TE made up of a dermal component - an epidermal layer formed by a bovine type 1 collagen matrix collected by neonatal human fibroblasts and keratinocytes grown on an equivalent Surface. Several multicenter randomized clinical studies have shown the

effectiveness of Apligraf in the treatment of long-term incurable wounds, venous and diabetic trophic ulcers [39], resulting in the drug being one of the first equivalents created in tissues. FDA approved for the treatment of skin wounds.

In Russia, in the late 1990s, research began on the development and application of live EKS. last century [40]. The work was carried out mainly in two directions: live EC with the otological cells of the patient embedded in the affected tissue and live EC with allogenic cells, which are enough to normalize the engraving process for a short time at the transplant site. the reparative process and stimulates the regeneration of the receiving tissue [41,42]. Similar cell structures are used to correct various epithelomesenchyma defects. In addition, their use is not limited to skin wounds [43].

The survival rate of the described full-thickness SEs can be limited by the absence of blood and lymphatic vessels, as well as skin appendages. That is why active work is being carried out at the level of pre-clinical research on the development of equivalents similar in structure and properties to the skin of an ordinary person [8, 44].

Despite the fact that the use of commercial SEs already available has made significant progress in the field of regenerative medicine, their use is not yet regular due to the high cost, limited efficiency and the inability to regenerate skin supplements [45].

Conclusion

As can be seen from the information provided, there is currently no ideal skin equivalent for wound healing. All epidermal and dermal bioengineering products require a multi-stage application procedure or autogrefts for final epithelization of the wound.

However, the rapid progress in the field of tissue engineering and the development of various approaches to creating the use of human skin substitutes, including stem cells, gives hope that such a product will be created in the near future.

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