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Transplantation of cells of the oral mucosa in the treatment of limbal stem cell deficiency

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Abstract

The actual problem of treatment of patients with limbal cell stem deficiency is reviewed. We summarized practical experience in various fields of medicine, aimed at tissue reconstruction using cells of the buccal mucosa. In ophthalmology, an effective method has long been searched to treat patients with the limbal stem cell deficiency causing an intense opacification and vascularization of the cornea and followed by a significant decrease in visual acuity. Recent studies have shown that the transplantation of epithelial cells of oral mucosa can significantly improve the treatment of patients with this disease. Although the mechanisms of oral mucosa epithelial cells' action are still insufficiently studied, the existing positive experience of oral mucosa using for tissue repair has great interest to practitioners, giving potential possibilities of its use, therapeutic effectiveness and ease of obtaining. A brief review of the literature presents the description of the morphological features of

the. buccal mucosa and the analysis of published data about the use of buccal epithelium in various branches of medicine and in ophthalmology, in particular.

Keywords: cornea, limbal stem cell deficiency, oral mucosa, cultivated cells of the oral mucosa

Conflict of interests Authors declare no conflict of interest

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AM, amniotic membrane

BCVA, best-corrected visual acuity

LSCD, limbal stem cell deficiency

NV, neovascularization

Introduction

Currently, the problem of blindness and poor vision is one of the socially and economically significant problems. Blindness due to corneal pathology according to World Health Organization (2010) accounts for 5% of all causes of blindness in the world [1]. According to the data from the Russian Federation regions, in 2012, corneal blindness accounts for 5.9% of all blind and visually impaired people in Russia. In the structure of corneal blindness of the Russian Federation, corneal ulcers account for 9%, and corneal scars and opacities of various etiologies account for 21%.

In this regard, the search for methods of treating eye diseases and injuries that cause disturbances in regeneration processes and lead to a

significant decrease in visual acuity and blindness remains a topical issue in ophthalmology [2-4].

Reconstructive interventions are required to restore visual functions in patients with corneal lesions. For the reconstruction of the surface of the anterior segment of the eye, therapeutic soft contact lenses, adhesives and adhesives, autoconjunctival plastic surgery, as well as transplantation of the donor cornea and amniotic membrane (AM) have been used [2]. However, the functional results of such operations are not always favorable, especially in patients with recurrent corneal epithelial lesions and vascularized opacities, the main cause of which is the death or functional deficiency of limbal stem cells, leading to impaired corneal tissue regeneration [4]. In addition, there is still a high risk of allograft rejection and the need for a long-term use of immunosuppressive drugs. In this regard, the search for the optimal surgical technique for corneal reconstruction remains relevant. Methods using cellular technologies, such as the use of limbal progenitor cells and buccal cells (epithelial cells of the oral mucosa) are becoming popular [5].

The use of conjunctival limbal autograft transplantation (CLAU) and cultivated limbal epithelial transplantation (CLET) are most commonly used in the treatment of unilateral limbal stem cell deficiency [6, 7] the main disadvantages of these techniques is their inability to be used with total bilateral lesion of the limbus area, and the persisting risk of LSCD of the unaffected eye, as well as limited quantities of material if re-transplantation is necessary [8, 9]. Therefore, for the treatment of bilateral LSCD, various options of allogeneic limbal transplantation have recently been used, in which good functional results are observed, but a long-term use of immunosuppressants is required, and the risk of graft rejection remains [8, 10].

In recent years, the focus of scientists' attention has shifted towards the use of stem cells from autologous tissues of other structures. One of these options was the oral mucosa, which is maximum similar in structure to the corneal epithelium.

Structure of the corneal epithelium and oral mucosa

The corneal epithelium is represented by a multi-layered flat non-keratinizing epithelium, up to 50 microns thick, consisting of 5-6 layers of regularly arranged cells connected by desmasomes [11, 12]. Cells of the innermost layer (basal, germ, and germinal) arranged in a single row have a prismatic shape and a large oval nucleus located close to the top of the cell [11, 12]. Due to the proliferation of cells in this layer, the epithelium is renewed, and defects on the corneal surface are closed [11]. Basal cells are located on a structureless anterior limiting lamina - the Bowman's membrane of 6-9 microns thick. It is an acellular modified hyalinized part of the stroma comprised of randomly oriented collagen fibrils and associated proteoglycans [12]. Adjacent to the basal layer are 2-3 layers of polyhedral cells having rounded nucleus; their processes are embedded between neighboring epithelial cells, like wings (winged or spiny cells) [12]. Then there are 2 layers of greatly flattened cells that have no signs of keratinization, with a flat outer face. Elongated narrow cell nuclei of the epithelium outer layers are located parallel to the surface of the cornea and have a flat outer face [11, 12].

The oral mucosa is formed by two layers: a multi-layered squamous epithelium located on the basement membrane, and its own mucosal lamina [13-17].

The proper lamina of the mucosa is represented by cells (fibroblasts, macrophages, mast cells, lymphocytes, leukocytes),

intercellular matter containing collagen (types: III, IV, V, VI), elastic and reticular fibers, as well as amorphous matter [13, 14, 16].

The basement membrane consists of a light fine-grained layer – a light lamina formed by glycoproteins, and a deep-lying layer – a dense lamina containing collagen fibers (types I, III, and IV collagen) [13, 16].

The epithelium of the oral mucosa is represented by keratinizing and non-keratinizing epithelium, depending on the oral cavity department [13-15].

Thus, the epithelium of the buccal mucosa is multilayered and non-keratinizing, with the exception of the line along the closing of the teeth, and consists of the following layers: basal, spiny, granular, and superficial [13-17].

The basal layer is represented by cubic or prismatic cells lying on the basement membrane. Among these cells, there are poorly differentiated (progenitor) cells, which proliferation and differentiation contribute to a continuous formation of epitheliocytes and maintenance of the epithelium integrity. [14, 16].

The spiny layer consists of several layers of large irregular-shaped cells with numerous processes [13, 16].

The granular layer is formed by several layers of flattened, spindle-shaped cells with a high ability to synthesize proteins [14].

The superficial layer is represented by densely packed flattened cells, which are constantly peeling off [13, 16].

Considering all the above, we can conclude that the structure of the epithelial tissue of the buccal mucosa and the cornea of the eye are similar. This suggests considering buccal epithelium as a culture for creating tissue-engineered structures for the treatment of the affected anterior segment of the visual organ.

Experience of using the oral mucosa epithelium in other areas of medicine

The oral mucosa is most commonly used in medical fields such as urology and gynecology. For many years, the buccal mucosa has been used in reconstructive urology for urethral strictures. Since the early 1990s, the buccal mucosa autograft has been the most commonly used material for replacement urethroplasty. A single buccal mucosal autograft can be used to treat strictures up to 4-7 cm long, depending on the size of the oral cavity [18].

In 2012, S.B. Kulkarni et al. published the results of a retrospective analysis of the treatment of 117 men with urethral stricture of 10-18 cm long of various etiologies by unilateral urethral dissection and dorsal placement of two buccal mucosal grafts [19]. The result of the operation was considered successful if the patient did not require any additional interventions, dilation or urethrotomy. Of 117 patients, the treatment was successful in 98 (83.7%) and unsuccessful in 19 (16.3%). Of the 104 cases without prior treatment, 90 (86.5%) were successful, and 14 (13.5%) were unsuccessful. Of the 13 patients with repeated urethroplasty, 8 (61.5%) had a favourable outcome.

In addition to traditional flaps, alternative materials are currently being developed using the tissue engineering of the autologous buccal mucosa for replacement urethroplasty. A buccal graft, which is considered the best replacement for urethral tissue in reconstructive surgery, providing 80-90% of satisfactory long-term results, can now be cultivated using tissue engineering. This makes it possible to avoid massive tissue harvesting in complex and extended urethral strictures [20, 21].

Experience of using the oral mucosa in ophthalmology

The first references to the use of oral mucosa in ophthalmology date back to the beginning of the XX century. R. Denig in 1912 and P.H. Ballen in early 1963 conducted the study aimed at determining the possibility of using a multi-layered, flat, non-keratinizing epithelium of non-ocular origin as an autograft to cover the ocular surface defects caused by chemical (alkaline) burns. The investigators used the lip mucosa as a tissue source [22-24]. In the experimental part, Ballen used a mucous membrane graft consisting of epithelium and native mucosal lamina in healthy rabbits' eyes burned with sodium hydroxide; and in the clinical part he used such for fresh moderate to severe burns. Transplant was performed as soon as possible after the burn was sustained, and it was preceded by the removal of the necrotic conjunctiva and subconjunctiva. The graft was then applied on the affected ocular surface and sutured to the sclera. Rabbit grafts remained adherent when placed on a wound that was completely free of necrotic tissue and was in contact with at least one edge of the area where the blood vessels were functioning. However, the grafts were rapidly fibrotic and highly vascularized [23]. Autotransplant with the lip mucosa was performed in 8 of 15 patients: in 2 of 2 moderate alkaline burn cases and in 6 of 13 severe alkaline burns. A conservative therapy alone was performed in 7 patients with severe burns. At follow-up, a corneal perforation occurred in all patients who received a conservative therapy alone, whereas among patients who underwent transplantation with the lip mucosal graft, only in 2 patients with severe burns the graft sloughed, and later on, the perforation occurred [23]. P.H. Ballen suggested that the mucosal graft acted as a bridge for the limbus and cornea neovascularization that promoted cell infiltration followed by fibrosis, and further prevented corneal perforation. Thus, the proposed method reduces the risk of

corneal perforation in moderate and severe burns; however, due to severe fibrosis, it yields unsatisfactory functional results in the form of low visual acuity.

In the following years, the possibility of oral mucosal transplantation to the cornea surface continued to be studied. In 1986 I.K. Gipson et al. studied in vitro the possibility of adhesion of the epithelial layer obtained after treating a full-thickness flap of New Zealand rabbit oral mucosa with dispase II, followed by mechanical separation of the epithelium from the underlying layers with tweezers, to the de-epithelialized surface of the cornea [24]. During the experiment, they found that hemidesmosomes were formed between the corneal basement membrane and epithelial cells, which confirmed the adhesion of the epithelium to the de-epithelialized surface of the cornea. Given the results obtained, the scientists continued the in vivo study. The study was performed on the New Zealand rabbit corneas, which were previously either mechanically de-epithelialized over their entire area or only on the temporal side with the preservation of the basement membrane; or the surface keratectomy was performed. Next, an allo- or autograft (a prepared epithelial layer) was applied on the surface of the cornea in the central or corneal-limbal zone with four to six interrupted nylon sutures, placed in the conjunctival cavity, and lateral tarsorrhaphy was performed. In all cases, the graft size was smaller than the wound surface of the cornea. Eyelid sutures were removed after 24 hours, graft sutures were removed on the 4^{th} or 5^{th} day after grafting to the de-epithelialized corneal surface, and on the 7^{th} day after surface keratectomy. It was found that grafts located in the central avascular area of the cornea did not adhere to the corneal surface and were rejected, and the epithelium allo - and autografts fixed in the corneal-limbal zone remained adhered throughout the entire follow-up period in the animals, but newly formed vessels

appeared under the graft. Scientists have suggested that this is due to the fact that to maintain the transplanted epithelial layer adhered to the surface of the cornea, the nearby vessels are needed. Scientists have suggested that if the transplantation is performed on the surface of the vascularized cornea, this will allow the epithelium to remain viable and subsequently achieve complete closure of the corneal epithelial defect. Thus, the proposed method of treating corneal surface injuries allows for their regeneration, but the presence of corneal neovascularization during transplantation and superficial neovascularization that develops after transplantation limit the use of this treatment technique with optical purpose.

In general, in the 20th century, scientists studying the efficacy of oral mucosa transplantation for corneal surface damage proposed the techniques that prevented gross corneal morphology derangement, but visual functions remained low. In the following years, the investigators aimed both at saving cornea integrity, and also at improving visual functions, and started investigating the transplantation of ex vivo cultivated cells of the oral mucosa for corneal lesions.

In 2004, T. Nakamura et al. performed the first transplantation of autologous oral epithelial cells cultivated ex vivo on the human amniotic membrane in patients with LSCD syndrome [25]. The study included 4 patients (6 eyes) with Stevens-Johnson syndrome (3 eyes) and severe corneal burns (3 eyes). Samples of the oral mucosa were taken from each patient 2-3 weeks before transplantation, from which epithelial cells were subsequently isolated and cultivated on a de-epithelialized AM. Further, the oral mucosa epithelium cultivated on the AM was transplanted to the surface of the cornea of an affected eye. After removing the conjunctival tissue from the damaged cornea, the graft was applied and sutured to the sclera in the limbus region. Two days after transplantation, the corneal

surface of all the operated eyes was clean and smooth, and a fluorescein test showed that the entire corneal surface was epithelialized. The average follow-up period after transplantation was 13.8 months. In all cases, superficial peripheral neovascularization was detected directly under the AM. Postoperative visual acuity improved by two or more lines. During the entire observation period, the transplanted epithelium remained on the surface of the eye, and there were no cases of persistent corneal defects.

In 2004-2009, M. Hirayama et al. conducted a study comparing the efficacy of cultivated oral mucosal epithelial cell sheet transplantation of AM-based sheets with that of substrate-free cell sheets prepared on fibrin-coated dishes in 32 patients (16 pts in each group) with LSCD syndrome [26]. In the postoperative period, the stability of the ocular surface was assessed by the severity of symblepharon, neovascularization (NV), and corneal conjunctivization. Transplantation of substrate-free cell sheets provided a stable ocular surface in 10 (62.5%) of the 16 eyes after 12 months of follow-up, persistent epithelial defects were observed in 4 eyes, and corneal conjunctivization in 2 eyes. In the second group, stable ocular surface was achieved in 6 of 16 eyes (37.5%), persistent corneal epithelial defects were observed in 6 eyes, and corneal conjunctivization was observed in 4 eyes. Mean postoperative BCVA (best-corrected visual acuity) improved after 1 ($p=0.016$), 3 ($p=0.0061$), 6 ($p=0.041$), and 12 ($p=0.0090$) months in the substrate-free sheet group. An improvement in BCVA by more than two lines was observed in 11 eyes (68.8%). In the group with AM as a substrate for cultured oral mucosa cells, the mean postoperative BCVA significantly improved after 1 ($p=0.024$) and 3 ($p=0.023$) months, but was not observed after 6 or 12 months. Improvement of BCVA by more than 2 lines was registered in 7 (43.8%) of 16 eyes in this group. Thus, the mean postoperative BCVA was significantly better in the substrate-free group. In this study, 4 grades of

corneal NV were distinguished: grade 0 meant no NV on cornea; grade 1 meant the NV reaching the peripheral corneal region; grade 2 denoted the NV not reaching the optical center of the cornea, grade 3 denoted the NV reaching the center of the cornea. In the preoperative period, grade 3 NV was present in 7 (43.8%) eyes in the group without a substrate and in 10 (62.5%) eyes in the group with AM. The incidence of NV grade >2 significantly decreased at 1, 3, and 6 months in the substrate-free group and at 1 and 3 months in the AM group. After 12 months, the incidence of eyes with NV grade >2 was significantly less in the substrate-free group than in the AM group ($p=0.023$). No major postoperative complications were observed in any of the two groups. Thus, the results of the study indicate that autologous oral mucosal epithelial cell transplantation using substrate-free sheets cultivated on fibrin-coated dishes provides better midterm clinical results than those obtained with using the grafts with AM as the substrate.

In 2013, C. Sotozono et al. published a long-term study results on the effectiveness of cultivated oral mucosal epithelial cell sheet transplantation with amniotic membrane used as the substrate in 40 patients (46 eyes) with total LSCD [27]. With regard to the LSCD etiology, the patients were allocated into four groups: the first group included patients with Stevens-Johnson syndrome (21 eyes), the second group included those with ocular cicatricial pemphigoid (10 eyes), the third group comprised patients with chemical or thermal burns (7 eyes), the fourth group included those with other diseases (8 eyes) associated with the LSCD development: 3 eyes with idiopathic LSCD; 1 eye with radiation keratopathy, 1 eye with graft-versus-host disease, 1 eye with congenital aniridia, 1 eye with Salzmann's nodular corneal degeneration, 1 eye with LSCD induced by drug toxicity. The study evaluated the best corrected visual acuity (BCVA) and the ocular surface condition

(epithelial defects, conjunctivization, NV, opacity, corneal keratinization, symblepharon) before transplantation, at the 4th, 12th, and 24th weeks after transplantation. In all patients, at 24 weeks after transplantation, the BCVA was at least 0.01. In patients of the first group, BCVA significantly improved at 4, 12, and 24 weeks after surgery (p=0.0005, p=0.0010, and p=0.0117, respectively). The ocular surface condition also improved significantly at 4, 12, and 24 weeks after surgery (p=0.0001 for each time point). In the second group, BCVA significantly improved at 4 weeks after surgery (p=0.0156), but later this improvement was leveled. However, the improvement in ocular surface condition retained throughout the follow-up period (p=0.0020, p=0.0020, and p=0.0078, respectively, for weeks 4, 12, and 24). In patients of the third group, BCVA did not change until 24 weeks after surgery, but the ocular surface condition significantly improved in all 7 patients (p=0.0156 for each follow-up period). In the fourth group, BCVA significantly improved in 6 of 8 patients; the improvement in the ocular surface condition was also observed only in 6 patients at 24 weeks after transplantation. None of the patients experienced any serious systemic complications. The main postoperative complications included persistent corneal epithelial defects (in the eyes of 16 (40.0%) of 40 patients, more often in the patients with Stevens-Johnson syndrome); corneal ulcer (2 patients (5.0%)); a transient increase in intraocular pressure due to the use of local steroid drugs (4 patients (10.0%)), which normalized after reducing the steroid doses; corneal infection (2 patients), resolved after a week with the instillation of antibacterial drugs. In this study, the symblepharon and NV severity grades were also shown to be predictive factors for improved vision at 24 weeks after transplantation (p=0.0023 and p=0.0173, respectively).

In 2013-2018, the Traumatology and Reconstructive Surgery Department of the Helmholtz National Medical Research Center for Eye

Diseases together with the Scientific Department of Biotechnologies and Transfusiology of the N.V. Sklifosovsky Research Institute for Emergency Medicine studied the effect of transplanting a combined bioconstruct consisting of buccal epithelium cultivated cells, collagen matrix (type I collagen) and soft contact lenses on the corneal regeneration after injury [28, 29]. After an experimental study of the effect of this bioconstruct on the healing of corneal epithelial-stromal defect (acceleration of corneal regeneration and repair, recovery of its structure), a limited clinical trial was conducted, which included 10 patients (8 men and 2 women) diagnosed with thermochemical corneal burn (3 patients) and chemical corneal burn (3 patients) of varying severity. In all cases, there was a burn injury (necrosis) of the conjunctiva and cornea with superficial to stromal opacification on the background of LSCD. Visual acuity ranged from 0.01 to 0.1, with mean of 0.05. The bioconstruct was applied on the damaged cornea, followed by applying a tight bandage to the eyelids for additional fixation. After the bioconstruct removal 3-5 days later, all patients showed a reduction in the erosion area and its depth, decreased edema, and an increase in the corneal tissue transparency. Corneal edema was completely resolved at mean 3-4 days. After 3-6 days, a complete corneal epithelialization was observed in 7 patients, while erosion still remained in 3 patients, but its depth and area decreased. Superficial corneal opacities persisted in 8 patients. Visual acuity averaged 0.1-0.2. At 5-9 days after the first transplantation of the combined bioconstruct, the second one was performed in 3 patients, after which the cornea was completely epithelialized at 3-5 days. During the entire follow-up period (6 months–1.5 years), erosion relapsed in only 3 patients, and therefore, they were re-treated with autologous buccal epithelial cells as part of the combined bioconstruct. As a result, complete epithelialization of corneal erosion was achieved in all cases. The

investigators concluded that cultivated buccal epithelial cells in the bioconstruct stimulate the healing of corneal defects, contribute to the inhibition of vascularization and conjunctivization of the affected area.

In 2014-2016, Y.J. Kim et al. evaluated the efficacy of transplantation with biomaterial-free cultured oral mucosal epithelial cell sheets (COMETs)) in patients with total LSCD [30]. The study involved 8 people (8 eyes), 6 of whom had Stevens-Johnson syndrome as the cause of LSCD, 1 had ocular scarring pemphigoid, and 1 had a severe chemical burn as the LSCD cause. Preoperative visual acuity was equal to or less than seeing the movement of a hand near the face. The outcome was assessed as positive if at 6 months after surgery there was no corneal epithelial defect; the fibrovascular tissue invasion did not reach the cornea optical zone; there was no symblepharon relapse; and if visual acuity improved. After 6 months of follow-up, the visual acuity improved in 5 eyes (62.5%) by 2 or more lines. The ocular surface was restored in 6 eyes (75%) at 6 months after transplantation, without relapse of significant fibrovascular invasion. Complete stable epithelialization was achieved at an average of 53.6 days. Stable restoration of the ocular surface was not achieved in 2 eyes (25%): in 1 case (a patient with recurrent cicatricial pemphigoid), the fibrovascular tissue invasion reached the optical corneal zone after 3 months, in the other case (a patient with Stevens-Johnson syndrome), a persistent epithelial defect was observed throughout the entire follow-up period. No systemic complications were reported in any case; the most common local complication was recurrent epithelial defects (4 eyes (50%) with Stevens-Johnson syndrome) which epithelialization was achieved by applying an amniotic membrane or contact lens.

Thus, according to the literature, the treatment of corneal injuries in LSCD using ex vivo cultivated oral mucosal epithelial cells allows restoring the corneal structure and improving visual acuity.

Conclusion

Currently, cultured oral mucosal cells are the most common, but incompletely studied source of cells for transplantation in the treatment of patients with bilateral limbal stem cell deficiency syndrome of various etiologies. In addition, the use of uncultivated cells of the oral mucosal epithelium in ophthalmology remains poorly studied and debatable. The use of such cells has undoubted advantages over cultivated ones: the cultivation process is more financially expensive and lengthy. Thus, the study of the efficacy of using uncultivated cells of the oral mucosa in the treatment of patients with LSCD is a promising trend in ophthalmology.

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