Ocular Surface Disorders
—Reconstruction of transparent tissues—

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Abstract: With the establishment of human embryonic stem (ES) cell lines, the idea of transplanting stem cells differentiating into blood cells, nerve cells, and muscle cells is gradually being realized. If stem cells established from human ES cells become available, not only transplantation medicine, but also the quality of medicine as a whole would be expected to change. If extracorporeal generation of tissues or organs by means of tissue culture succeeds, it will pave the way for regeneration medicine, in which not only transplantation of cells, but also replacement of whole diseased organs or tissues may become possible. Stem cell transplantation that heralds the beginning of regeneration medicine has been widely applied clinically in the form of blood stem cell transplantation (bone marrow transplantation). In the case of somatic cells, progress has been made in research on stem cells differentiating into epithelial cells, including those of the skin and intestinal mucosa. Recently, the presence of stem cells in corneal epithelial cells of the eye was identified, and transplantation of the corneal epithelial stem cells has been initiated. In the year 2000, the production of epithelial sheets by in vitro incubation of stem cells and its clinical application to transplantation were reported. Another ongoing project attempts to produce each of the three layers comprising the structure of the cornea separately, and to combine these to produce an artificial cornea. Clinical application of reconstruction of transparent tissues based on research on corneal epithelial stem cells is rapidly expanding. In the near future, transplantation of corneas that were regenerated using ES cells or autogenous stem cells may become possible, without the need for dependence on the supply of stem cells from eye banks.

Key words: Regenerated cornea; Stem cell; Eye bank; Corneal transplantation

Corneal Epithelial Stem Cells

The corneal epithelium consists of five to seven layers of squamous cells. Unlike the skin, the corneal epithelium is not keratinized. While the basal cells have long been known to
undergo cell division, they are now referred to as transient amplifying cells, since they are believed to have a definite life span. They can be compared to peripheral blood cells. The corneal epithelial stem cells are to the corneal epithelium as bone marrow cells are to the peripheral blood cells. The corneal epithelial stem cells have been confirmed to be slow cycling cells. Transient amplifying cells arise from the stem cells as a result of very slow cell division, and these cells undergo repeated active cell division to maintain the cellular layers.

According to the XYZ theory of Prof. Thoft, the cell loss from the surface (Z) is balanced by the sum of the supply of basal cells (X) and the centripetal movement of peripheral cells (Y). The X component is equivalent to the dividing transient amplifying cells, and the Y component is accounted for by the slow supply from the stem cells (Fig. 1). The loss of epithelial cells that composes the Z component has long been attributed to friction between the eyelids. However, at present, programmed cell death or apoptosis is believed to explain this loss. The thickness of the superficial layer of epithelial cells is kept constant by such a mechanism.

**Stem Cells of the Corneal Epithelium Are Present in the Corneal Limbus**

The presence of stem cells itself and the location of these cells have long been controversial. In recent years, however, the stem cells have been revealed to be present in the corneal limbus. This finding explains various phenomena that could not be understood before, such as why the corneal epithelium is severely invaded by blood vessels after alkali injury, why corneal transplantation fails in such cases, and why prolonged use of contact lenses leads to vascularization of the cornea.

These questions are now explained by the concept of disorder of the stem cells of the corneal epithelial cells. When the stem cells of the epithelial cells are depleted for some reason, a group of cells with the characteristics of corneal epithelium is depleted from the ocular surface (OS), and the corneal surface becomes covered by peripheral conjunctival epithelial cells. Conjunctival epithelial cells require a blood supply, and because the tight junctions between the cells are weak, they are strongly stained with fluorescein dyes. This is the reason for vision disorder in these individuals. The research group of Puangsricharern and Tseng divided the patients with failure of the corneal limbal function into two groups, i.e., one in which the stem cells were depleted and the other with abnormality of the stroma surrounding the stem cells.

**Corneal Epithelial Stem Cell Transplantation**

In the conventionally performed corneal transplantation, only the central cornea could be transplanted, which means only the transient amplifying cells could be transplanted. However, these cells have a definite life span. Therefore, although the donor cornea is covered with corneal epithelial cells for a while, within two or three months, the central cornea becomes covered again with conjunctival epithelial cells invading from the periphery.

At present, corneal limbal transplantation is attracting attention as a new approach for cor-
neal transplantation. While corneal epithelio-plasty and conjunctival transplantation serve as other treatment options, the success of these techniques can be considered to be related to transplantation of the corneal limbal cells. Corneal limbal transplantation originally began with transplantation of the autogeneous limbus. This is because rejection was anticipated due to the presence of numerous antigen-presenting cells, including Langerhans cells, in the corneal limbus. At present, the availability of immunosuppressive drugs such as cyclosporin has made it possible to inhibit rejection to a significant extent, and allogeneic corneal limbal transplantation has become possible.

**Surgical Technique of Corneal Limbal Transplantation**

Although it would be unrealistic to expect doctors other than ophthalmologists to perform actual transplantations, the method employed by us is briefly introduced for the sake of information. This technique has been adopted by the Department of Ophthalmology of Tokyo Dental College for 9 years, from 1992, and numerous clinical reports have been published. Today, our technique is globally recognized as a standard technique.

**1. Treatment of the recipient cornea**

The cicatricial tissues on the ocular surface are eliminated as thoroughly as possible. Then, the tissues on the sclera are also eliminated (Fig. 2). In the peripheral areas, abnormally proliferating connective tissue is often present under the conjunctiva. Since abnormal fibroblasts are considered to be present in these tissues, it is considered important to eliminate these tissues also as thoroughly as possible.

Usually, the sclera is not damaged. In the superficial type of Stevens-Johnson syndrome and genuine corneal stem cell disorders, often the cornea itself is still transparent. Therefore, caution needs to be addressed not to injure the Bowman’s membrane when detaching the cornea. If the transparency of the cornea itself is severely impaired, lamellar keratoplasty should be performed concomitantly. Needless to say, penetrating keratoplasty should also be performed when endothelial disorder is present.

**2. Preparation of corneal limbal graft**

There are three possible approaches, i.e., allograft transplantation using an eye bank cornea or a volunteer-donated cornea by HLA-matching relatives, and autograft transplantation in which a part of the limbus of the patient’s contralateral eye is transplanted. In all the cases, it is crucial to excise the corneal stroma to the maximal extent possible to leave as thin a corneal graft as possible, so as to eliminate unwanted Langerhans cells while preserving sufficient stem cells in the corneal limbus. As described later, the humoral factors produced by the stroma cells are also important for differentiation and division of the epithelium, but the amount of these factors remaining in the thin graft is believed to be sufficient.

In allograft transplantation using an eye bank cornea, the central part is punched out with an ordinary trephine. When penetrating or lamellar keratoplasty is performed concomitantly, the punched out central portion can be used for corneal transplantation. The remaining limbal area is treated as described below:

i) Eliminate the sclera while avoiding, as much as possible, damage to the epithelium.

ii) Then, remove the corneal stroma and prepare thin sections.

iii) During the operation, provide sufficient moisture and viscous substances, such as hyaluronic acid, to protect the corneal and limbal epithelia from becoming damaged.

When using a volunteer-donated cornea or the contralateral eye of the patient, rectangular sections about 5 mm wide and 10 mm long obtained from the upper and lower corneal limbus are transplanted.

**3. Transplantation of corneal limbus**

The limbal tissue is placed at the position of
the original limbus. The microenvironment is believed to be important for the survival of the stem cells of the corneal limbus. Although it remains to be clarified in detail, at least factors derived from the aqueous humor, factors derived from blood, and the presence of abundant nerves are known to be important. If the donor limbal tissue is placed on the scleral side, the factors from the aqueous humor are not supplied. If it is placed too close to the corneal side, blood vessels do not reach it. Accordingly, the original position appears to be the most suitable (Fig. 2).

**Concept of Amniotic Transplantation and Serum Replacement Therapy**

In recent years, surgical approaches have been attempted for several conditions, such as ocular pemphigoid and Stevens-Johnson syn-
drome, that were until now considered to be intractable. Surgery is considered even in cases that cannot be successfully treated with corneal limbal transplantation alone, and total reconstruction of the ocular surface is required. The ocular surface damaged by the disease is completely reconstructed. The problems associated with such conditions as ocular pemphigoid are listed below.

i) Absence of stem cells of the corneal epithelium (and occasionally of the conjunctival epithelium)

ii) Ocular surface covered with cicatricial tissues and absence of normal basal membrane and connective tissues

iii) Serious symptoms of dry eye

iv) Palpebral problems including ciliary entropion

The following countermeasures are taken to resolve each of these problems when reconstructing the ocular surface.

i) Corneal limbal transplantation is performed using an eye bank cornea to compensate for the loss of stem cells. This allows corneal epithelial stem cells to be transplanted.

ii) The cicatricial tissues are eliminated and amniotic membranes are transplanted to provide new basal membranes and connective tissues.

iii) Tear fluid is supplied by frequent instillation of autologous serum.

iv) Palpebral entropion is treated by aggressive tarsorrhaphy, and the exposed ocular surface area is reduced to prevent evaporation of tear fluid.

Figure 3 illustrates the concept of ocular surface reconstruction. Normal division and differentiation of corneal epithelial cells are compared to the blooming of flowers in the garden. Rain (tear), flowers (epithelium), seeds (stem cells), and soil (substrate; stroma and amniotic membrane) are necessary for new flowers to bloom. Needless to say, like bone marrow transplantation, immunosuppression and management of the whole body are required in the case of transplantation of stem cells also. Since instillation of autologous serum at intervals of 15 to 60 minutes may be very troublesome for the patients, adequate information must be provided before the operation.

A Typical Example of Amniotic Membrane Transplantation and Serum Instillation

Figure 4A shows the eye of a woman with corneal opacity and markedly declined vision due to Stevens-Johnson syndrome. In the tear fluid test, production of tear fluid was not observed at all despite repeated Schirmer’s testing with nasal stimulation, suggesting that the lacrimal glands had been destroyed. Instillation of the patient’s autologous serum was essential to replace EGF and vitamin A, which are present in the tear fluid, for the purpose of allowing the epithelial wound to heal. By ocular surface reconstruction, we succeeded in reconstructing the corneal epithelium (Fig. 4B). This patient had been followed up only for 30 months and a more prolonged period of monitoring of the postoperative course is necessary. Nonetheless, it is remarkable that effective surgical approaches have become possible even against intractable severe dry eye, which in the
past was considered impossible to treat.

**Regeneration Cornea Project**

Based on the technology of reconstructing the corneal epithelium, an attempt has been made to create the three-layer structure of the cornea, consisting of the corneal epithelium, corneal stroma, and corneal endothelium, with the purpose of reconstructing the entire transparent tissue of the cornea. This is the new regeneration cornea project. We have already started animal experiments by producing stromal tissues with collagen and implanting them in rabbits (Fig. 5). In the future, using corneal epithelial cells, conjunctival epithelial cells, corneal endothelial cells, and corneal stroma cells differentiated and induced from ES cells, we intend to regenerate the cornea for use in anyone. At present, instillation of autologous serum, as described earlier, is required in cases with severe dry eye. However, once we succeed in the regeneration of lacrimal glands, taking into consideration the aqueous channel, including aquaporin, regeneration of the anterior ocular segment including the tear fluid, may also become possible.

**Conclusion**

The presence of corneal epithelial stem cells has been discovered, and transplantation of these cells has enabled treatment of serious ocular surface diseases, which until now could
not be treated by conventional methods. Development of techniques to incubate stem cells and to implant them in the body is under way. Since 1999, we have started transplantation of corneal epithelial stem cells incubated in the amniotic membrane.

In the 21st century, Japanese medicine is expected to make dramatic progress toward regeneration medicine. With the Bioventure Grant from the former Ministry of Education, we have begun a project for the regeneration of cornea from stem cells. The cooperation of researchers in experimental medicine and clinicians is essential for clinical ophthalmologists to perfect the development of the regeneration cornea.

**REFERENCES**


