SURVEY OF OPHTHALMOLOGY VOLUME 52 • NUMBER 2 • SEPTEMBER–OCTOBER 2007

ELSEVIER

DIAGNOSTIC AND SURGICAL TECHNIQUES

MARCO ZARBIN AND DAVID CHU, EDITORS

Transplantation of Ex Vivo Cultured Limbal Epithelial Stem Cells: A Review of Techniques and Clinical Results

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Abstract. Ex vivo cultured limbal epithelial stem cells have been used successfully to treat corneal limbal stem cell deficiency. We identified 17 reports of the application of this novel cell-based therapy in humans. In addition we identified four reports of the use of culture oral mucosal epithelial cells to treat limbal stem cell deficiency. We examined these reports to discern the success rate, complication rate, visual outcome, whether there is an optimal technique and which patients are the most likely to benefit. We also discuss the different culture methods employed and the regulations governing cell banks that are providing this service. We found that the techniques used to cultivate and transplant cells varied, but that no individual method was clearly superior. The reported success rate is similar across all studies for both allografts and autografts. The clinical indications for this treatment are not clearly defined as indicated by the variety of disorders treated. Follow-up is limited and the long-term success rate is yet to be established. Nonetheless, we conclude that there is sufficient evidence to support the continued use and refinement of this procedure as a treatment for corneal stem cell deficiency. (**Surv Ophthalmol 52**:483–502, 2007. © 2007 Elsevier Inc. All rights reserved.)

Key words. amnion • cornea • cultured cells • deficiency • stem cell transplantation • stem cells • tissue engineering

The use of stem cells to regenerate and repair tissues and organs has been the subject of intense scientific and media interest. There are practical and ethical obstacles to the use of embryonic stem cells and the alternative of using somatic or adult stem cells has major advantages in terms of immediate clinical application. The adult human eye harbors stem cells in the limbal region adjacent to the cornea,[13,58,75] in the conjunctiva,[75] the pars plana and pars plicata of the retinal ciliary margin,[50,53,111] and the adult human retina.[10,61] The successful use of ex vivo cultured limbal epithelial stem cells (LESCs) to treat corneal limbal stem cell deficiency (LSCD)[18,37,55,56,63,64,67,76,80,87,89–91,94–96,102] and

0039-6257/07/$–see front matter
doi:10.1016/j.survophthal.2007.06.013

investigations into cell-based therapies for retinal degenerations[16,54,59,68,72,97,110,113] has put ophthalmology at the forefront of this biotechnology.

The use of ex vivo cultured LESCs to treat corneal LSCD in humans was first described by Pellegrini et al in 1997.[76] Since then 16 additional reports of the use of this technology to treat patients have been published.[18,37,55,56,63,64,67,80,87,89–91,94–96,102] In addition, four further studies have reported the transplantation of ex vivo cultured autologous oral mucosal epithelial cells to treat LSCD.[46,47,62,71] Numerous reviews have dealt with the scientific theory and evidence behind this treatment;[14,23,26,38,57,69,81,103] however, these reviews have not specifically addressed several key questions which are relevant to the clinician considering referral of patients for this procedure. This review aims to examine the published clinical reports to determine whether there is an optimal method and group of patients for whom this technique is most likely to be beneficial, as well as the likely success rate, complication rate, and visual outcome. We also discuss current regulations governing cell banks that are providing it.

In this review we define LESCs as a population of epithelial cells within the basal cell layer of the limbal epithelium that have been shown experimentally to have characteristics of and behavior of stem cells.[5,13,19,58,75,108] In contrast, by the term ex vivo cultured limbal epithelial cells (LECs) we refer to a mixed population of cells, a certain proportion of which have the characteristics of limbal stem cells.[5,19,76,80,95,108]

Limbal Epithelial Stem Cells and Corneal Epithelial Homeostasis

The corneal epithelium is essential in maintaining the clarity and the regular refractive surface of the cornea. As with other epithelial surfaces there is a continuous loss of cells from the surface of the corneal epithelium.[52,103] Desquamated cells are replenished by a small population of LESCs located in the basal layer of the limbal epithelium, which also play a central role in corneal epithelial regeneration and repair following injury.[13,14,24,26,57,81,103] Clinically LSCD is associated with loss of the limbal palisades,[52,70] suggesting that LESCs reside in or on the limbal palisades. However, there is uncertainty regarding the exact distribution of stem cells within the limbus. Recently Chen et al demonstrated that the entire basal cell population at the superior limbus demonstrate characteristics consistent with a stem cell phenotype.[8] However, expression of the putative limbal stem cell marker ABCG2 within the palisades is not uniform but rather is expressed by clusters of limbal basal cells.[5,19,108] In contrast to these studies, Dua et al described distinct limbal structures, termed limbal epithelial crypts, which the authors propose are a niche structure and the location of LESCs.[25] This study reported an average of six limbal epithelial crypts per cornea but the number of corneas examined (5) was small and detail on the circumferential distribution and exact number present in each cornea was not reported.[25]

Limbal Stem Cell Deficiency

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A deficiency of LESCs occurs in a variety of disorders. Some, such as aniridia, are the result of a genetic disorder and are heritable.[41,70] More commonly, however, LSCD results from acquired factors such as chemical or thermal injury, ultraviolet and ionizing radiation, Stevens Johnson syndrome, advanced ocular cicatricial pemphigoid, contact lens wear, multiple surgeries, radiation, antimetabolites, or extensive microbial infection.[9,24,27,29,33,35,40,42,92] If LESCs are depleted by trauma or disease, conjunctivalization of the cornea occurs as conjunctival epithelial cells and blood vessels peripheral to the limbus migrate onto the corneal surface.[43,79] This results in persistent epithelial breakdown and superficial corneal vascularization. Patients complain of chronic discomfort and impaired vision.[105]

DIAGNOSIS AND CLASSIFICATION

Conjunctival epithelial ingrowth onto the cornea (conjunctivalization) is central to the diagnosis of LSCD.[26,79,103,105] Clinical signs observed are epithelial haze, superficial subepithelial vascularization, persistent or recurrent epithelial defects, epithelial and stromal inflammation, late fluorescein staining, and loss of the limbal palisades of Vogt.[22,26,43,44,70,105] The clinical diagnosis may be confirmed using Impression cytology. Specimens can be stained with periodic acid Schiff stain, to identify goblet cells, or monoclonal antibodies to cytokeratin 3 and cytokeratin19 to confirm a conjunctival phenotype.[6,15,70,79]

LSCD may be classified as partial or total.[26] In partial deficiency there is localized deficiency of LESCs in a region of limbus but an intact population of LESCs in other areas. This results in sectoral ingrowth of conjunctival epithelium in areas of SC deficiency.[40,42,79] In total stem cell deficiency there is dysfunction or destruction of the