Reconstruction of limbal stem cell deficient corneal surface with induced human bone marrow mesenchymal stem cells on amniotic membrane

CHE MAN ROHAINA, KONG YONG THEN, ANGELA MIN HWEI NG, WAN HASLINA WAN ABDUL HALIM, AIDA ZAIRANI MOHD ZAHIDIN, AMINUDDIN SAIM, and RUSZYMAH B. H. IDRUS

KUALA LUMPUR AND SELANGOR, MALAYSIA

The cornea can be damaged by a variety of clinical disorders or chemical, mechanical, and thermal injuries. The objectives of this study were to induce bone marrow mesenchymal stem cells (BMSCs) to corneal lineage, to form a tissue engineered corneal substitute (TEC) using BMSCs, and to treat corneal surface defects in a limbal stem cell deficiency model. BMSCs were induced to corneal lineage using limbal medium for 10 days. Induced BMSCs demonstrated upregulation of corneal stem cell markers; β1-integrin, C/EBPδ, ABCG2, and p63, increased protein expression of CK3 and p63 significantly compared with the uninduced ones. For TEC formation, passage 1 BMSCs were trypsinized and seeded on amniotic membrane in a transwell co-culture system and were grown in limbal medium. Limbal stem cell deficiency models were induced by alkaline injury, and the TEC was implanted for 8 weeks. Serial slit lamp evaluation revealed remarkable improvement in corneal regeneration in terms of corneal clarity and reduced vascularization. Histologic and optical coherence tomography analyses demonstrated comparable corneal thickness and achieved stratified epithelium with a compact stromal layer resembling that of normal cornea. CK3 and p63 were expressed in the newly regenerated cornea. In conclusion, BMSCs can be induced into corneal epithelial lineage, and these cells are viable for the formation of TEC, to be used for the reconstruction of the corneal surface in the limbal stem cell deficient model. (Translational Research 2014;163:200–210)

Abbreviations: $ABCG_2$ ATP = binding cassette, sub family G; AM = Amniotic membrane; BMSCs = Bone marrow mesenchymal stem cells; CK_3 = Cytokeratin 3; DAPI = 4′,6-diamino-2-phenylindole; EDTA = Ethylenediamine-Tetra-Acetic acid; FITC = Fluorescein isothiocyanate; NaCl = Natrium chloride; NaOH = Natrium hydroxide; NIH = National Institutes of Health; NT = No treatment; OCT = Optical coherence tomography; PBS = Phosphate buffer saline; qRTPCR = Quantitative real time polymerase chain reaction; SPSS = Statistical package for the social sciences; TEC = Tissue engineered cornea

From the Tissue Engineering Center, Universiti Kebangsaan Malaysia Medical Center, Kuala Lumpur, Malaysia; Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; Ear, Nose, and Throat Consultant Clinic, Ampang Puteri Specialist Hospital, Selangor, Malaysia; Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

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Reprint requests: Ruszymah B. H. Idrus, MD, PhD, Tissue Engineering Center, Universiti Kebangsaan Malaysia Medical Center, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Kuala Lumpur, Malaysia; e-mail: ruszy@medic.ukm.my or ruszyidrus@gmail.com.

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AT A GLANCE COMMENTARY

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Background

Cornea can be damaged by a variety of clinical disorders or injuries. These conditions may cause loss of limbal stem cells and can lead to blindness. Corneal transplant is the gold standard treatment but shortage of donors is still a problem.

Translational Significance

Bone marrow mesenchymal stem cells were induced to corneal lineage in a transwell co-culture system and used for the reconstruction of corneal surface in limbal stem cell deficiency model. Serial slit lamp evaluation revealed remarkable improvement in corneal regeneration whereas histologic and optical coherence tomography analyses demonstrated corneal histo-architecture and thickness resembling that of normal cornea.

Corneal damage can result from a variety of clinical disorders, including aniridia, Stevens-Johnson syndrome, or chemical, mechanical, and thermal injury. In severe injury, both the limbal and central epithelial cells of the eye can be lost, which is accompanied by inflammation, neovascularization, and conjunctivalization. The prognosis of corneal damage in many cases depends on the extent of the deficiency in the limbal epithelial stem cells.[1] In these cases, limbal tissue transplantation is necessary to restore eye function.[2] Pellegrini et al[3] have been culturing corneal stem cells from small biopsies of human limbal tissue for the past decade. Xu and et al[4] proved that *in vitro* reconstructed tissue engineered human corneal epithelium, from an untransfected human corneal epithelium cell line on a denuded amniotic membrane, has similar structures and functions to those of rabbit corneal epithelia. Higa et al[5] reported the novel method of carrier-free epithelial sheets from primary cultures of rabbit limbal epithelial cells by using biodegradable fibrin sealant. Silk fibroin membrane and film had been used in corneal transplantation because of its potential utility *in vivo*, including transparency, mechanical integrity, biocompatibility, and slow degradation.[6-8] The limbal epithelial cell is the best cell source for corneal tissue engineering because of its stem cell location, proliferative capacity, and ability to maintain epithelial cell morphology when cultured without the presence of serum[9,10] and co-cultured with 3T3 fibroblast feeder layer cells.[9,11] Masruddin et al[12] indicated that bilayered corneal construct that was kept *in vivo* for 90 days showed good development and re-epithelization similar to the normal cornea. However, the variation in the use of the amniotic membrane (AM) and 3T3 fibroblast, media requirements, and explants or suspension culture techniques can be employed in different combinations depending on the original reason for culturing the limbal epithelial cells.[13]

The limitation of good quality donor tissue is a major problem especially in Asia,[14,15] which makes corneal tissue engineering a very demanding field. Disease transmission is a major concern.[16,17] The use of mesenchymal stem cells as a cell source for a corneal substitute has attracted attention as a more effective treatment option with little or no immunogenic potential.[2,18] Research has shown that adult bone marrow is easy to isolate, is multipotential, and can be directly induced to differentiate into cardiac cells,[19,20] epithelial cells,[21,22] endothelial cells,[23,24] nerve cells,[25-27] bone cells,[28-30] cartilage cells,[31,32] and insulin-producing cells,[33]under different conditions *in vitro*. Many reports have suggested that the transplantation of bone marrow mesenchymal stem cells (BMSCs) could reconstruct the damaged cornea.[34-36] Ma et al[1] demonstrated that transplantation of a patient's own mesenchymal stem cells could successfully reconstruct the damaged corneal surface. Jiang et al[35] reported that BMSCs induced by corneal epithelial cells and seeded on the AM have significant effects on the treatment of damage caused by alkaline burn on the corneal surface of rats according to the scoring system in Table I.

The first objective of this study is to differentiate human BMSCs into corneal epithelial lineages and to construct a corneal substitute using induced human BMSCs via tissue engineering technology. A final objective is to prove the concept of using nude rat as an animal model of limbal stem cell deficiency. We evaluated the characteristics of differentiated BMSCs by detecting the gene expression of corneal stem cells and corneal-specific markers. Immunocytochemical analysis was performed to detect the presence of p63 and CK3 protein expression. The optical clarity and corneal vascularization were determined by slit lamp examination whereas the corneal thickness was measured by an optical coherence tomography (OCT) scan. At the end of the experiment, had a histologic analysis was performed on the harvested cornea and the presence of p63 and CK3 was evaluated.

METHODS

All tissues that were obtained in this study were approved by Universiti Kebangsaan Malaysia Research