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Ocular surface reconstruction with suspended limbal stem cells delivered by sutureless fixated amniotic membrane patch

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Corneal epithelium is important for maintaining ocular integrity and visual function. For total limbal stem cell deficiency (LSCD), cell-based therapy is necessary because the cell source of corneal epithelium disappeared. In previous study, we designed a sutureless technique, using a polymethyl methacrylate ring and fibrin sealant, to fixate amniotic membrane patch on ocular surface in rabbits. In this study, we used the sutureless fixated amniotic membrane (AM) as cell delivery system for ocular surface reconstruction in a rabbit model with total LSCD. A population of cells including limbal stem cells was selected from primary cultured limbal epithelium by adhesion to Matrigel™ for 30 min. Fluorescent immunohistochemistry showed MUC5AC(–), Cx43(+), Ki67(+), integrin α6(+), integrin β1(+), P63(+), and ABCG2(+) in the adhesive cells. The selected cells were then injected into the space between injured ocular surface and the AM patch. The AM patch was removed and a new one was added at 24 h. Ocular surface was reconstructed in 5/8 rabbits at 7 days. 8/8 cases showed corneal inflammation and peripheral neovascularization in rabbits with only AM patch at 7 days. The reconstructed stratified epithelium showed K12(+), MUC5AC(–), Cx43(+), Ki67(+), and integrin β6(+) detected by fluorescent immunohistochemistry, which indicated a normal phenotype of corneal epithelium. This cell delivery system, based on suspended single cells, for ocular surface reconstruction might make it easy to evaluate the in vivo effects of various seeding cells and could have the potential to shorten waiting time in patients with LSCD or persistent epithelial defect.

***Keywords*:** limbal stem cell, ocular surface reconstruction, amniotic membrane

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