

Mucosal stem cells used for restoring severely damaged corneal epitheliums

**Daithí O hAnluain
in London**

CORNEAL autografts are possible in patients with bilateral injury by seeding an amniotic membrane with buccal mucosal epithelial stem cells to provide a new transparent epithelial cell sheet, delegates to the Moorfields Bicentenary Scientific Meeting heard.

Shigeru Kinoshita MD, corneal graft specialist at the Kyoto Prefectural University of Medicine in Japan, outlined his relative success with the technique in 19 patients. "The transparency is not 100%, but it is quite good," he said.

Dr Kinoshita and his team first developed the technique in his laboratory in rabbits. Initially the team performed an oral biopsy and put the tissues in the limbal corneal junction after superficial keratectomy. This cured the ocular surface but massive scarring with neovascularisation developed later. However, when they used the cultivated oral epithelial stem cells the surface remained clear.

Histological analysis showed the rabbit graft contained K3 cells, but no K12. These are thought to be the quite unique keratins for cornea. The rabbit results

encouraged the team and they received permission to proceed with clinical trials in June 2002.

The following month he enrolled his first patient, a 32-year-old male with acute phase chemical injury. The team tried a standard amniotic transplant with allogeneic cultivated corneal stem cells, but it failed, suffering from persistent corneal defects. The cultivated oral epithelial cell sheet succeeded, however. At 18 months, vision improved from HM (hand movements) to 20/40. As with rabbits, the oral graft shows K3 cells but not K12.

Epithelial transplants successful in most cases

So far, the team used the technique on 22 eyes in 19 cases. He treated 15 eyes in 12 patients for ocular surface reconstruction; five cases of fornix reconstruction, and one case each of pterygium and tumour resection. The oral epithelial transplant survived in 80 per cent of the cases, with widely varying visual acuity results.

Some cases are a comparatively modest success. In one case a Stevens Johnson Syndrome patient saw her vision improve from HM to a BCVA of 20/200.

"It's enough for her to use her mobile phone. We've seen this

case for two years and the cornea is still clear," said Dr Kinoshita.

The oral epithelial grafts survived in 12 (80%) patients, though five (33%) suffered from persistent epithelial defects. Three of these responded to treatment. Dr Kinoshita said 13 (87%) cases were a clinical success. Most failures occurred in patients with Stevens Johnson Syndrome and the technique may not be the best choice for these patients.

The treatment is for patients who routinely reject corneal allografts and suffer some bilateral injuries by chemical or burns, though three Stevens Johnson Syndrome cases were treated successfully. Four other Stevens Johnson Syndrome cases and one acute burn case suffered from persistent epithelial defects.

"The current clinical problem is infection and immunological epithelial rejection. If there are no epithelial stem cells and few conjunctival stem cells, we could potentially use buccal, nasal or tracheal stem cells."

The team creates corneal grafts by taking oral mucosal epithelial stem cells from the patient. Given that the mouth is not germ-free, a separate department is in charge of cleaning the patient's mouth

and harvesting the cells.

Once harvested, the cells are placed in suspension on an amniotic membrane, which is itself in suspension in a 3T3 cell solution. The 3T3 solution is itself treated with Mitomycin C.

Air-lifting technique enhances tissue integrity

The team developed a special air-lifting technique for recovering the cultivated epithelial stem cell sheet, which encourages a tight adhesion between the epithelial cells. This tight adhesion enhances cell maintenance. Simply blinking can lose cells in corneas with comparatively loose adhesion.

The membrane is applied through a standard graft procedure and Dr Kinoshita noted that it is impossible to tell whether one is using a regular cornea or an oral mucosal epithelial stem cell sheet at that point.

Dr Kinoshita told delegates that the mucosal stem cells cultured on amniotic membrane could be one weapon in the armoury of corneal graft specialists dealing in difficult cases. He believed it was particularly appropriate for bilateral injury in young people, because of the problems associated with

immunosuppressive drugs. In cases of unilateral injury, he said autograft from cultivated corneal epithelial stem cells was the treatment of choice, while he recommended an allograft for bilateral injury in patients over 50 years old.

Corneal grafts are beginning to benefit from a number of new approaches. In April 2005 *EuroTimes* reported a new technique using cultured limbal stem cell allografts. Ultimately, they may overcome the need for long-term immunosuppression in patients who do not require subsequent full thickness allografts.

skinoshi@ophth.kpu-m.ac.jp