OCULAR REGENERATION

Ocular surface diseases and disorders including chemical or burn injuries, Stevens-Johnson syndrome and neurotrophic keratopathy continue to be major challenges

by Sean Henahan

With several new stem cell strategies and tissue engineering techniques now close to clinical utility, and numerous innovative biotech approaches in the pipeline, research groups around the world report being tantalisingly close to the goal of producing effective new treatment options while solving the limitations of current approaches.

“When I began residency some 36 years ago we had very little knowledge of the pathogenesis of devastating ocular surface disorders such as Mooren’s ulcer, Stevens-Johnson syndrome, and meibomitis-related severe keratoconjunctivitis. Now we have seen much progress and have a better understanding of the pathogenesis of corneal disorders, which is leading to some sophisticated therapeutic modalities,” said Shigeru Kinoshita MD, PhD, professor and chair, Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan, in a keynote lecture at the 2nd Asia Cornea Society conference in Kyoto.

Ocular surface reconstruction using regenerative medical approaches and tissue engineering aims to restore the anatomic and physiologic ocular surface, and ultimately to prevent recurrence of the ocular surface pathology.

Japanese corneal specialists pioneered some of the key approaches in the treatment of ocular surface disease, such as cultivated mucosal epithelial cell transplantation. Dr Kinoshita, a leader in the field, continues to be involved in some of the most leading-edge research.

Dr Kinoshita and colleagues are also interested in producing human corneal endothelial cell regeneration through implantation of cultivated corneal endothelial cells. His lab is currently looking at three types of therapies to treat corneal endothelial disease: cultivated corneal endothelial cell sheet transplantation, cultivated corneal endothelial cell injection therapy, and eye drops for promoting corneal endothelial cell proliferation and migration.

He has reported promising results in animal experiments with transplantation of corneal endothelial cell sheet on type 1 collagen. This produced high endothelial cell density with long-lasting clear corneas.

“This research showed that the cells could easily migrate out of the transplanted cultivated cell sheet and proliferate. They migrated to peripheral cornea, eventually creating endothelial cells on the entire cornea,” he told a session of the XXIX Congress of the ESCRS.

Problems with sloughing of the cell sheet led to the search for an alternative way to deliver the cells. Animal experiments using human DSAEK flaps demonstrated that it was possible to transfer cultivated cells into the anterior chamber in this manner, with transparent corneas as far out as eight months.

Dr Kinoshita and colleagues are also evaluating direct injection of cultivated corneal endothelial cells. He believes this would ultimately prove a better approach than transplanting cultivated cornea endothelial cell sheets. Several researcher groups are now working on this.

While doing this research Dr Kinoshita and colleagues, notably Drs Naoki Okamura, Noriko Koizumi and Morio Ueno, discovered an interesting molecule, ROCK (rho kinase) inhibitor (Y027632). This molecule enhances corneal endothelial cell survival, promotes cellular attachment and proliferation, and inhibits apoptosis.

Ocular regeneration with an eye drop

The combination of direct cell injection with ROCK inhibitor offers an intriguing potential treatment of ocular surface disease. In a recent landmark publication (N Okamura et al., Br J...
growth factors that enhance proliferation and fraction. Platelets contain great reservoirs of coagulated, so platelets are not present in the blood with sodium citrate. It is very rich in bioactive proteins in the blood.

Platelet-rich plasma (E-PRP) could prove a useful new way to approach these problems. Practical application of a new emerging therapy

Stevens-Johnson syndrome, persistent epithelial defects, dormant corneal ulcers, and chronic severe dry eye are some of the most common, and most challenging ocular surface disorders. Jorge Allo MD, PhD, chair, Department of Ophthalmology, Alicante Ophthalmology Institute, believes that biological activation of the ocular surface by platelet-rich plasma (E-PRP) could prove a useful new way to address these problems.

Blood-derived products have been used for a long time in ophthalmology. Blood derived products have demonstrated their capacity to enhance healing and stimulate the regeneration of different tissues. This healing effect is attributed to the growth factors and bioactive proteins in the blood.

"Autologous platelet-rich plasma (E-PRP) is easy to prepare. It is an amber coloured and slightly turbid blood fraction obtained after centrifugation of uncoagulated total blood with sodium citrate. It is very rich in platelets, growth factors and clotting proteins. It is different from autologous serum because it is easy to prepare. It is an amber coloured bioactive proteins in the blood.

Dr. Kinoshita said he is preparing a clinical study protocol to test this approach in humans. It may take some years to gain approval according to Japanese government guidelines for clinical research using somatic stem cells, he noted.

The drop regimen also improved the conjunctival and corneal surface in cases of micropunctate keratitis, decreasing inflammation, and promoting wound healing, lubricate. A study of patients with severe dry eye showed that at three months 89 per cent had improvement in subjective symptoms, and 86 per cent had significant decreases in hyperaemia, he reported.

Ocular surface syndrome post-LASIK can be very distressful for patients and refractive surgeons, he noted. It is characterised by symptoms of dry eye, micropunctate keratitis, decreased and unstable tear film and decreased visual acuity. Of neurotrophic aetiology, it is a long-lasting problem that is difficult to treat, he commented.

"In our hands E-PRP is a reliable and an effective therapeutic tool to enhance epithelial wound healing in ocular surface disease,“ Jorge Allo MD, PhD

Dr. Kinoshita reported treating more than 1000 patients in the last five years with autologous platelet-rich plasma (E-PRP). Depending on the situation, he uses either a topical eye drop or a solid form of the blood product.

"Patients receiving E-PRP for chronic epithelial defects and for corneal ulcers did exceedingly well. The drops promoted healing, improved the anatomy and symptoms, closing the wound, and restoring visual function in most cases. This appears to be a very promising and practical approach," he said.

"Autologous platelet-rich plasma (E-PRP) in our hands E-PRP is a reliable and an effective therapeutic tool to enhance epithelial wound healing in ocular surface disease. Platelet rich plasma provides a high concentration of essential growth factors and cell adhesion molecules by concentrating platelets in a small volume of plasma. It has a major role in wound healing and enhances physiological processes at the site of injury or surgery," he said.

The E-PRP is made from patients blood an hour before the procedure. Nothing needs to be added, and it requires no special preparation. This means it can easily be used in any practice, he added.

Improving limbal cell transplantation

One goal of ocular surface reconstruction strategies is repairing limbal epithelial stem cell deficiency, a problem common to many disorders including thermal or chemical injury, Stevens-Johnson syndrome, persistent epithelial defects, chronic severe dry eye, or in those at high risk for perforation. Platelet-rich plasma is a biosynthetic mimic that restores refractive function with optical clarity, and resists enzymatic degradation.

Bruce Jackson MD

"In our hands E-PRP is a reliable and an effective therapeutic tool to enhance epithelial wound healing in ocular surface disease.” Jorge Allo MD, PhD

MEMBRANE DEGRADATION

The primary substrate for limbal epithelial cell transplantation, but several alternatives now in the pipeline may help overcome some of the limitations of amniotic membrane.
Amniotic membrane has a number of positive characteristics as a substrate, particularly its anti-inflammatory and anti-angiogenic properties. However, disadvantages include the biological variability between donors and even within donors. Moreover, it lacks the transparency of an ideal substrate.

**Biosynthetic corneal substitute**

Speaking at the same meeting, Bruce Jackson MD, University of Ottawa Eye Institute, Ottawa, Canada, described a promising research approach that could allow the use of recombinant collagen for corneal substitution.

In addition to helping cope with the chronic shortness of human corneal donors, a safe source of synthetic implant material could reduce concerns about disease transmission and might be useful when donor grafting is not appropriate, such as in cases of previously failed grafts, autoimmune diseases, and alkali burns, he noted.

"The ultimate goal is a biosynthetic mimic that restores refractive function with optical clarity, and resists enzymatic degradation. It could be sutured, would be biocompatible, would allow regeneration of host tissue, be non-toxic, with no inflammation or immunogenic problems," he said.

Recombinant type I human collagen might prove an ideal scaffold to recreate the natural corneal microenvironment, allowing for the colonisation of the central cornea by keratocytes. Type III human collagen, which is found in the skin, rather than type II collagen, which is found in the cornea, appears to be a better choice because of its superior mechanical properties and lower cost.

Dr Jackson’s institution is involved in collaborative research with Per Fagerholm MD, University Hospital, Linköping, Sweden, to evaluate the clinical potential of this approach. He cited a recent clinical study in which 10 patients, nine with keratoconus and one with a deep corneal scar, underwent anterior lamellar keratoplasty and implantation of the biosynthetic cornea. At 24 months, the biosynthetic implants remained stably integrated and avascular, with evidence of nerve ingrowth. The epithelial surface barrier was re-established and remained stable.

The next step will be to strengthen these implants and make them more resistant to enzymes. The researchers are now investigating additional applications including embedding the transplants with nanoparticles for the delivery of drugs, and creating collagen implants suitable for refractive correction.

**Gene therapy with a contact lens**

Looking farther ahead, the future of corneal disease treatment is likely to include gene therapy. The successful treatment of humans with a rare form of retinal disease, Leber’s congenital amaurosis, with gene therapy, suggests that treatment of corneal disease may not be that far in the future.

Leber’s congenital amaurosis was a particularly good candidate for gene therapy because its pathogenesis was associated with a very small number of genes, and the gene defect was well understood.

Akira Murakami MD, professor of ophthalmology, Juntendo University School of Medicine, Tokyo, Japan, has identified a potential candidate corneal disease for gene therapy, and a potential treatment. Gelatinous drop-like corneal degeneration (GDLD) is a rare refractory corneal disease that generates sub-epithelial deposition of amyloid. Mutations in the TACSTD2 gene have been identified in Japanese families with GDLD, leading researchers to believe it may be amenable to gene therapy.

Presenting at the 2nd Asia Cornea Society conference in Kyoto, Dr Murakami noted that he and his team wanted to use a non viral vector approach to deliver the corrected gene. He reported the transfer of the TACSTD2 gene into the corneal epithelial cells encapsulated in a hydrogel contact lens. In animal studies the team reported being able to successfully introduce the gene into epithelial cells, and to record expression of the relevant protein.

"There are still a lot of problems to solve. But we have transfected a gene using this approach, which is an important first step. We believe that some day the use of phosphate groups containing hydrogel as a gene transfer device could be applied to non-invasive novel therapeutic method for GDLD," said Dr Murakami.