Guest Editorial

Cutting corners, or simplifying technology to reach more patients; using the body as its own incubator for epithelial regeneration

Why some 10 years after investing heavily in the technique of laboratory expanded corneal epithelial cells for the treatment of limbal stem cell deficiency (LSCD) did the group of Virender Singh Sangwan (VSS) apparently turn their back on this and started using small explants of the cornea to treat LSCD? This technique of using small limbal explants was called "SLET or simple limbal epithelial transplantation."

The technique of cultivated limbal epithelial stem cell transplantation (CLET) works quite well in those groups who have established it. However, the bottom line is that despite some 17 years of its use with reasonably good success by all groups involved, it fails one critical test: it fails to reach a large number of patients because it requires a high level of infrastructure investment. To culture autologous corneal cells requires highly trained specialist cell culture staff working in dedicated premises under highly regulated conditions that meet the standard of the local regulatory authority. As such, it is beyond the reach of the majority of ophthalmic surgeons and patients suffering from LSCD who could benefit from it.

When Sheila MacNeil (SM) and VSS started writing a grant application together in 2009, it was against a background of knowing that the technique of expanding cells and transplanting them back to the patient on the amniotic membrane worked well, and their initial plan was to develop a synthetic off-the-shelf alternative to the human amniotic membrane (hAM).

However, it became obvious that although this was a worthwhile problem to tackle (and is still under development) there was a bigger issue, no matter how good the staff, of culturing the cells and preparing them under clean room conditions for transplanting them back to the patient and that this technique is far too resource-intensive to be adopted except by a few dedicated centers.

In thinking about it, SM was aware that some of the assumptions that had been made over the last 30 years about growing layers of epithelial cells for transplantation with respect to skin cells were worth challenging, such as: does one need to grow a continuous stratified layer of epithelium before you can graft it successfully to the patient? She had previously shown that transfer of sub-confluent populations of keratinocytes on a simple carrier membrane worked, as well as culturing cells into a layer of several cells.[1]

The next question was related to the area of expansion. In expanding keratinocytes for patients with extensive burns, it is really important to achieve a major expansion of cells in the laboratory before grafting them back to the patient, in order to provide something which can be a useful adjunct to split-thickness skin grafting for surgeons to use.

In the case of the cornea, the area to be covered is both much smaller and there is not the clinical need for rapid barrier cover to prevent fluid and electrolyte loss and bacteria entry. Sheila hypothesized that small explants taken from a nondamaged cornea could generate a new epithelium in a damaged denuded cornea over several weeks. Indeed, it has been shown in skin that small pieces of split-thickness skin, fairly widely spaced from each other, will lead to the outgrowth of a continuous epithelium. This technique known as Meek grafting is known to work but is not widely adopted in the burns community where extensive skin grafts are often required rapidly, whereas this particular technique is very labor-intensive.[2]

On discussion with VSS, he agreed that this hypothesis merited investigation and suggested testing this cautiously and safely in a few patients to assess the hypothesis that corneal explants placed on a denuded cornea would regenerate a corneal epithelium using hAM as a substrate. His first pilot study on a few patients who were followed up for a year was very promising and was published in 2012.[3] This clearly showed that a new stratified corneal epithelium could be regenerated within 6–8 weeks.

The authors were aware that this technique could provide a solution for surgeons who did not have access to the technique of cultured limbal epithelial stem cells. The key question then was that how effective would this technique be compared to CLET?

The next few years led to very successful clinical dissemination of this technique which VSS achieved through publications, presentations at meetings, and running informal training courses and then workshops for clinical colleagues.

The current issue of the journal carries an excellent review on SLET, collates outcomes from peer-reviewed literature, and provides practical tips for learning the technique.[4] It is now well documented that the outcomes of SLET are as good or better than CLET in adults for LSCD because of chemical burns and much better than CLET in pediatric LSCD.[4]

Why Does SLET Work So Well? The Role of Mesenchymal Stem Cells in SLET

In CLET, the technique selects for the laboratory expansion of a particular population of corneal limbal stem cells (LSCs). In SLET, on the contrary, the "limbal explants" allow for expansion of LSC with their supporting niche to grow and multiply in their natural environment. This is a complex interplay of stromal matrix, growth factors, corneal nerves, tear film, and the supporting cells such as stromal mesenchymal cells which grow in an ocular surface ecosystem. We have reported mesenchymal stem cells (MSCs) to be present in limbal cultures and characterized them by gene-expression analysis and compared them with bone marrow-derived mesenchymal cells.[5,6] The group of Sunil Chauhan *et al.* reported that bone marrow-derived MSCs are capable of restoring corneal transparency after injury involving corneal stroma in an animal model.[7] During ocular injury,

inflammation-induced transforming growth factors beta (TGFs-β), particularly TGF-β1 and TGF-β2, drive the differentiation of corneal fibroblasts (activated keratocytes) into α-smooth muscle actin expressing myofibroblasts, which are opaque and produce disorganized extracellular matrix, leading to the development of corneal opacity and scarring. In CLET there is an expansion of MSC *in vitro*. Similarly, we believe that in SLET there is an expansion of MSC *in vivo* contributing to corneal transparency post SLET. The mechanism for this seems to be that MSCs secrete high levels of hepatocyte growth factor (HGF), which inhibits the generation of opacity-inducing myofibroblasts. Thus, the suggestion is that MSC-secreted HGF seems to contribute to corneal transparency post SLET. Because of this, we suggest that there is a significant reduction in the number of patients with significant corneal scarring requiring penetrating keratoplasty or deep anterior lamellar keratoplasty post SLET.

Is CLET Now an Obsolete Procedure?

We do not believe so and suggest that CLET is specifically indicated in patients with LSCD caused by epidermolysis bullosa, where with gene editing it may now be possible to correct defective genes while cells are growing in cultures. Moreover, CLET in an animal model that offers an excellent tool for studying the surface epithelial physiology by tracking the labeled epithelial cells. Where do these cells go? How do they interact with other elements of surface ecosystem? How long do they last? In the case of allo-CLET how long will donor cells survive in the host? Such questions cannot be studied in SLET in humans or animal models but can be studied in CLET.

Some Surgical Advice on the Use of SLET

Although the authors of the review provide clear tips and techniques for doing autologous and allo-SLET, we advise novice surgeons to be careful in attempting allo-SLET in Stevens Johnson Syndrome/ocular cicatricial pemphigoid (immune-mediated LSCD indications) patients as they present very complex challenges and such cases should be taken up only by surgeons who are experienced in the use of immune modulation.[4] Similarly, patients with total LSCD (irrespective of the underlying cause) and associated extensive symblephera should only be taken up once the technique has been mastered for simpler cases, and when undertaken should be done in stages.

Summary and What Next?

The introduction of SLET has simplified the surgical treatment of LSC defects, making this technique available to many more surgeons and hence also to patients. As the review attests, more is still being learnt about it as more surgeons report on their experience.

At present, surgeons still need to be able to access a safe source of hAM to use in SLET. At the time of writing, the challenge of producing a synthetic alternative to the hAM, which can be made available for surgeons without the need to access amniotic membrane through an established tissue bank, is ongoing.[8] The authors hope that within 2 years they will be able to offer a prepacked synthetic biodegradable membrane to surgeons as a convenient and safe alternative to the amniotic membrane.

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About the author

Virender Singh Sangwan

Dr. Sangwan is a renowned ophthalmic surgeon of international repute with over twenty two years of rich experience. He has been involved with cutting-edge research in eye care and his major contribution has been in the field of translational research in Ophthalmology involving limbal stem cells. After mastering the technique of cultivation of stem cells for treatment of corneal blindness, he has treated over 1000 patients with the same. This represents the largest human application of adult stem cells anywhere in the world. He developed a cost effective technique of growing cells on the patient's ocular surface which is called Simple Limbal Epithelial Transplantion (SLET).

During his career, Dr. Sangwan has been longest associated with LV Prasad Eye Institute, Hyderabad, India (March 1998 to August 2018), where he worked as Director, Center for Ocular Regeneration (CORE) and Srujana- Center for Innovation. He has also been associated with the Department of Ophthalmology, Harvard Medical School, MA (USA) and with the DC-10 Flying Eye Hospital of ORBIS International, Inc. New York USA earlier in his career. Currently he is working as the Director, Innovations at Dr. Shroff's Charity Eye Hospital, Daryaganj, New Delhi, India.