

Stem cells for sore eyes

Dr Virender Singh Sangwan from the LV Prasad Eye Institute in Hyderabad, India has pioneered a simple and affordable stem cell-based technique to repair eye damage in collaboration with **Professor Sheila MacNeil** of Sheffield University's Department of Materials Science and Engineering in the UK



What are the main foci and goals of your research efforts?

VS: Our main goal is to develop a scalable stem cell-based treatment for damage to the outer ocular surface. Damage can be caused by a variety of injuries, most notably chemicals, infections, allergies and autoimmune diseases. To treat damage to one eye, we have developed a very good technique of autologous cultivated limbal stem cell-based reconstruction. With further simplification of the technique by growing stem cells on the surface of the eye using the natural environment – simple limbal epithelial transplantation (SLET) – it can now be scaled up and any corneal surgeon could do this procedure with little training.

Are there particular advantages of working at the LV Prasad Eye Institute (LVPEI)? Could you describe the scope of the work conducted in the different districts?

VS: There are several positives to working at LVPEI. Firstly, the Institute has excellent clinical and surgical research, and community outreach programmes, giving us unparalleled access to clinical material and the ability to take cutting-edge advances to grassroots level in rural communities. Secondly, LVPEI is not-for-profit which means all communities can access our services; this way, the impact of our stem cell-based treatment can really reach the masses. And finally, LVPEI's mission is to create, practice and disseminate new knowledge. Limbal stem cell research is conducted at very few other eye centres across India.

As a practicing ophthalmologist you are able to bridge research and applications. Is this ability to directly see the benefits of your efforts a motivating factor for you?

VS: Yes, certainly. Several of my patients who had a damaged outer ocular surface were told

by their previous doctors that nothing could be done about their eye condition (limbal stem cell deficiency – LSCD), even in the US. When our surgery (cultivated limbal epithelium transplantation/SLET) restored their sight, their joy was immense and I felt a great sense of achievement in making such a difference to their lives. Many of them went back to their previous eye doctors just to tell them that they can see again and that their eyes look almost normal!

What are the advantages of your method over existing limbal transplantation techniques?

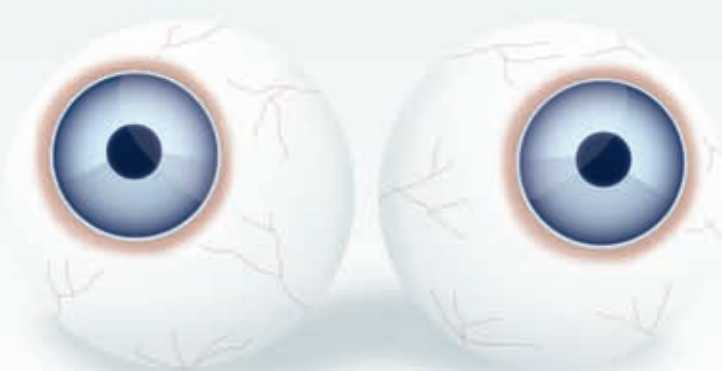
VS: The most obvious advantage of cultivation over direct limbal transplantation is that less limbal tissue is required for the procedure, thus reducing iatrogenic LSCD at the donor site. As well as this, our limbal explants culture technique is simpler, more cost-effective, safer and can easily be replicated in a current good manufacturing practice (cGMP) laboratory. The method only requires a glass slide with a Petri dish to grow the cell and no complex culture inserts. With the introduction of *in situ* cultivation using SLET in 2010, we made cell-based therapy a surgical technique with no need for a laboratory and at a significantly reduced cost. It was a real game changer for the regenerative field.

You are aiming to develop a synthetic, biodegradable carrier membrane. How close are you to realising this goal?

SM: We have now completed the laboratory development of a cobweb-like membrane made out of microfibrils of polylactic glycolic acid, a polymer that is approved for use by the US Food and Drug Administration (FDA) in dissolvable sutures. This membrane can be sterilised, vacuum-packed and stored for at least 18 months before being combined with either cultured cells or, more excitingly, very small pieces of limbal tissue. We can regenerate an epithelium in the laboratory (using an *ex vivo* rabbit cornea model) within a few weeks, starting either from cells that have been expanded under cleanroom conditions first, or from these small pieces of limbal tissue.

Could your stem cell treatment technique find applications outside ophthalmology?

SM: I am investigating how this technique could be applied in elective surgery for skin reconstruction and exploring whether small biopsies of the patient's tissue can be disaggregated and combined with biodegradable membranes to regenerate new skin for patients who require reconstructive surgery for burns contractures and scarring. This is at an early stage of development but does show signs of potential. Our previous work on the cornea has strengthened our conviction that it is possible to regenerate small areas of patient tissue using the patient as the incubator if one has a suitable membrane or scaffold.



The clear impact of his work on society remains the biggest motivation for Sangwan to continue developing ocular surgical techniques

So that all may see

Innovative research based in India has brought state-of-the-art stem cell eye therapy to rural communities in the country. The new and simple treatments are making a huge impact on society at a remarkably low price

'SO THAT ALL MAY SEE' is the motto of the L V Prasad Eye Institute (LVPEI) in Hyderabad, India which strives to provide eye care to all who need it, regardless of social status or income. This philosophy has driven 25 years of remarkable research, treatment and an impressive expansion across the southern state of Andhra Pradesh. Through its innovative Eye Health Pyramid structure and community-based approach to eye care, this not-for-profit organisation has developed a highly effective network to ensure that the expertise advanced at its main centre in Hyderabad reaches those most in need via 95 rural community care centres. For Dr Virender Singh Sangwan, a practising clinician at LVPEI, this structure has enabled his pioneering research into stem cell-based treatments for corneal diseases or injuries to impact the lives of people who live at great distances from his clinic.

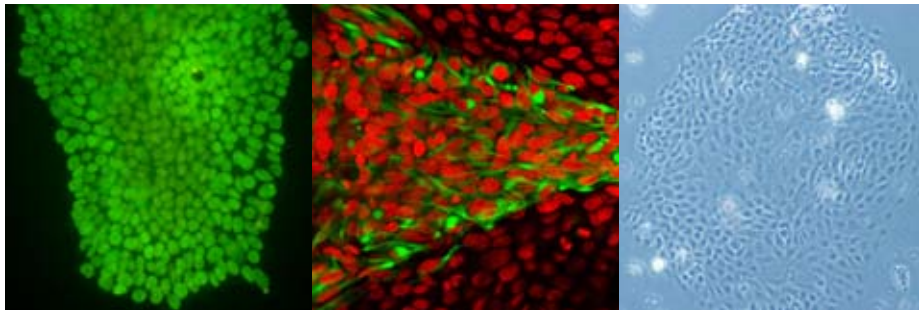
STEM CELL DEFICIENCY

The cornea is the clear protective layer that protects the front of the eye. It plays an important role in focusing light onto the retina. As it is such an exposed surface, its cells must be continually replaced from a reserve of limbal stem cells (LSCs). If damage occurs to the eye and the cornea can no longer reproduce itself, then there is a high risk of inflammation, scarring and loss of sight due to limbal stem cell deficiency (LSCD). In collaboration with Professor Sheila MacNeil who is based at the University of Sheffield's Department of Materials Science and Engineering in the UK, Sangwan has developed a cutting-edge treatment for LSCD that can be administered on a wide scale with remarkably little training.

FROM SMALL BEGINNINGS

The story of how Sangwan developed his technique is all the more inspiring when one considers how it all began. As a clinician he found himself too busy to spend enough time in the laboratory, so he enlisted the help of his colleague Geeta Vemuganti, a pathologist with no previous experience in culturing cells. Vemuganti's input was vital to making Sangwan's vision a reality, although they faced many challenges along the





CULTIVATED ADULT LIMBAL STEM CELLS UNDER PHASE CONTRAST MICROSCOPY IN THE LABORATORY

way: “We started our journey without a stem cell laboratory or the right kind of wares required for culturing. We innovated and designed our own systems from scratch and faced plenty of criticism from within and outside the country,” Sangwan explains. Despite these obstacles, Vemuganti and Sangwan continued undeterred.

Their efforts resulted in a groundbreaking technique which enabled them to grow transparent, stichable epithelium – a barrier of tissue which protects the cornea – in the laboratory. This can then be transplanted to a damaged cornea to restore vision lost after burns or damage to the outer surface of the eye. To reduce the risk of rejection, the technique uses blood serum from the patient or a related donor in order to culture the required stem cells. Previously, it was thought that multiple layers of epithelium cells were necessary to repair corneal damage, however Sangwan and his team proved that a monolayer can be used effectively. This simpler technique, using monolayer rather than multilayers, meant the procedure could be replicated with just a glass slide and a Petri dish. Once enough cells have been cultured from a sample of the patient’s healthy eye tissue – a process that usually takes 10-14 days – surgery can take place. This involves cleaning up the abnormal tissue and then grafting on the membrane and cells from the Petri dish by simply pasting them onto the surface of the eye with biological glue.

Through rigorous testing in various clinical situations, Sangwan proved that this new procedure – cultivated limbal epithelium transplantation (CLET) – was both a safe and effective way to treat LSCD. Over 200 eyes were treated using the procedure and their good progress monitored for a decade.

COLLABORATIVE PROGRESS

Building on the success of CLET, Sangwan collaborated with MacNeil to develop the technique even further: “In our first Skype call, Sheila explained that we could develop a synthetic scaffold with micropockets where explants would sit and cells grow out of them on the ocular surface without the need for laboratory cultivation,” he recalls. “Suddenly I realised that we can do this surgery in a completely different manner, reduce the cost for patients and create a new treatment model to be practiced outside major eye hospitals.” These initial conversations led the collaborators to a technique that could be

carried out *in situ*, with no need for a laboratory at all. It came to be known as simple limbal epithelium transplantation (SLET) and has transformed the technique’s efficacy in terms of the number of patients that can now be reached.

CUTTING-EDGE AND COST-EFFECTIVE

Not only is SLET a safe and simple procedure but, as there is no need for expensive laboratory equipment, it has the additional benefit of being much cheaper than its predecessor; the equivalent treatment would cost a patient \$15,000 in the US as opposed to Rs 15,000, or roughly \$250 in India. For Sangwan and his colleagues at LVPEI, this means that they can extend free treatment to more of those who are in need.

Through the use of Sangwan’s CLET and SLET techniques, more than 1,000 procedures have been carried out to restore damaged tissues and lost vision. This represents the largest successful human trial of stem cell technology ever undertaken.

LOOKING AHEAD

The clear impact of his work on society remains the biggest motivation for Sangwan to continue developing ocular surgical techniques. Although the majority of procedures have been successful, not all patients have responded in the desired way. This poses an important challenge to Sangwan and other researchers to improve understanding of the biological processes that determine whether treatment will work in each individual. Further research into the cellular mechanisms behind this already very successful technique is underway at a new Center for Ocular Regeneration (CORE) at the Kallam Anji Reddy (KAR) campus, Hyderabad. The goal is to find ways to increase the success rate of limbal cell transplants and determine new avenues for the treatment of eye conditions using cell therapy.

Beyond ophthalmology, Sangwan is confident that the method he and his co-researchers have developed can also be used in other areas: “I believe that this technique could be applied to grow parts of other solid organs which are composed mostly of epithelial cells, like the liver and pancreas,” he states. From very small beginnings, he and his team have pioneered a life-changing treatment that has made a real difference to thousands of lives. Looking ahead, it is clear that the researchers have only scratched the surface of the method’s potential, and that many more lives are likely to be improved in the future.

INTELLIGENCE

STEM CELL RESEARCH AT LV PRASAD EYE INSTITUTE

OBJECTIVES

To develop a cost-effective and simple-to-perform stem cell-based treatment for currently incurable blinding conditions.

KEY COLLABORATORS

Professor D Balasubramanian; Geeta Vemuganti; Dr Sayan Basu; Dr G N Rao; Indumathi Mariappan, LV Prasad Eye Institute, Hyderabad, India • **Professor Sheila MacNeil**, Sheffield University, UK • **Professor May Griffith**, Linköping University, Sweden • **Professor James Funderburgh**, University of Pittsburgh, USA

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DR VIRENDER SINGH SANGWAN is a practicing ophthalmologist from Haryana, who first trained at the Maharshi Dayanand Medical College and Hospital, Rohtak, Haryana, then at the LV Prasad Eye Institute, Hyderabad, and later at Harvard Medical School, Boston, USA. He is currently Associate Director at the LV Prasad Eye Institute. Among many achievements, Sangwan was awarded the Dr Shanti Swarup Bhatnagar Prize in Medical Sciences in 2006 and the National Technology Prize by the Department of Biotechnology in 2007. Last year, *India Today* named him one of the top 20 scientists in India. He is married to Vandana, a dentist, and they have two children – a daughter Sonalika and son Sahil.

