NEWS & VIEWS

REGENERATIVE MEDICINE

An eye to treating blindness

Elena Ezhkova and Elaine Fuchs

Work on stem cells is one of the hottest research areas in biology. But are such studies of any therapeutic value? Fortunately, yes, as is evident from successes in treating blindness.

Few people today dispute the enormous potential of stem cells for regenerative medicine. But, despite ever-increasing reports on the Internet of stem cells being used to treat various disorders — from Alzheimer's disease to spinal-cord injuries to severe heart conditions — proven stem-cell therapies remain few and far between. A paper published in the *New England Journal of Medicine* by Pellegrini, De Luca and colleagues[1] stands as a refreshing example of a scientifically documented advance in stem-cell therapies, in this case to treat certain types of blindness.

The eyeball is covered by the cornea — the eye's most important light-refracting structure (Fig. 1a). The cornea produces the initial image and casts this onto the lens behind it. The clearness of the cornea is essential to visual acuity and depends on both the integrity of the corneal epithelium covering the eye's surface and a lack of blood vessels in the underlying support tissue (the stroma)[2]. At its margins, the corneal epithelium is attached to the delicate mucous (conjunctival) epithelium that covers the whites of the eye (sclera) and the internal part of the eyelids. The narrow zone between the cornea and the conjunctiva is known as the limbus. Experimental and clinical evidence indicates that the limbus is the source of corneal epithelial stem cells in humans[2].

The limbus can be destroyed by ocular burns or infection, causing corneal stem-cell deficiency[3]. But, in one of nature's remarkable efforts to repair tissues at all costs, abnormal invasion by conjunctival cells provides the damaged cornea with a protective surface layer (Fig. 1b). The consequences are dire, resulting in vascularization of the cornea, chronic inflammation, stromal scarring and, ultimately, corneal opacity and loss of sight[2].

Allogeneic corneal transplantations, which involve transplanting cornea from a genetically non-identical donor, have to some extent been successful in restoring patients' vision. Eventually, however, conjunctival cells invade and replace the transplanted cornea[2]. What's more, two other factors make treating patients with ocular burns by corneal transplantation problematic: the number of available donors is insufficient to meet demand, and the increasingly popular corrective laser eye surgery often makes the cornea unsuitable for transplantation. Pellegrini, De Luca and colleagues[1] now report that limbal stem cells maintained in culture can be a viable alternative source of cells for transplantation to treat burned human corneas.

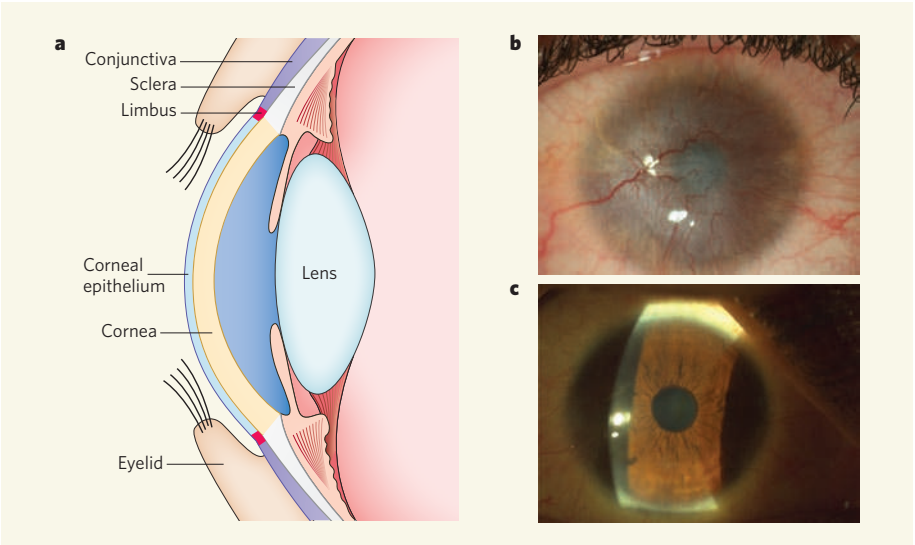

Figure 1 | Help from one eye to its neighbouring eye. a, The human ocular system. **b**, When the limbus is permanently damaged, as in the example of a patient shown, conjunctival cells invade the cornea to form a protective epithelial layer. This abnormal 'rescue' attempt leads to the formation of new blood vessels, chronic inflammation, stromal scarring and, finally, corneal opacity and loss of vision. **c**, Pellegrini, De Luca and colleagues[1] find that transplantation of corneal stem cells obtained by culturing cells taken from the limbus of the healthy eye regenerates a healthy cornea and permanently restores a patient's normal vision, as shown.

Stem-cell transplantation is not a new concept. More than half a century ago, E. Donnall Thomas showed that intravenous infusion of donor bone-marrow cells can repopulate the bone marrow and produce new blood cells[4]; he later won a Nobel prize for this first demonstration of the use of stem cells for regeneration of damaged or diseased tissues and organs. By the 1970s, physicians were successfully performing bone-marrow transplants, which are now used to treat blood disorders ranging from severe combined immunodeficiency to sickle-cell anaemia to leukaemias, as well as other cancers of the human immune system. By the early 1980s, human skin stem cells were being cultured to make epidermal sheets to repair the skin of badly burned patients.

Pellegrini, De Luca and colleagues have been culturing corneal stem cells from small biopsies of human limbal tissue for the past decade. The appreciable similarities between limbal and epidermal cells allowed the researchers to adapt methods[5,6] developed for human epidermal stem-cell cultures. Cultured epidermal-cell colonies can be classified according to cell number and capacity for growth. The smaller-sized colonies generate epidermal cells that stop growing over time. By contrast, the larger-sized colonies — referred to as holoclones — display quintessential features of stem cells, namely long-term self-renewal and the ability to regenerate tissue. This makes them suitable for burn therapy.

Pellegrini, De Luca and co-workers[7] discovered that human limbal cells cultured using a similar protocol also form small and large colonies. Interestingly, only the limbal holoclones and not the smaller colonies expressed p63, a transcription factor that is essential for the

proliferative potential of epidermal stem cells[8]. In the impressive accompanying clinical studies[9], the researchers obtained limbal stem cells from the healthy eye of 112 patients with ocular burns, cultivated them and then transplanted the cultured cells onto the patients' damaged eye. After an extensive 10-year monitoring period, the authors now report[1] permanent restoration of a transparent, self-renewing corneal epithelium in three-quarters of the study patients (Fig. 1c). Notably, 78% of the successful transplantations involved cultures in which p63-expressing cells constituted more than 3% of the cells capable of forming colonies. These observations unveil a direct correlation between the percentage of p63-positive corneal stem cells in a culture and their transplantability. The correlation presents a powerful diagnostic tool for predicting whether any given limbal culture is likely to be suitable for long-term transplantation.

This work[1] also offers hope for exploring alternative sources of limbal stem cells to treat patients who have suffered severe injuries to both eyes, and who therefore lack limbal stem cells. Indeed, in the future it might be possible to create corneal stem cells by culturing other cells from the patient — for instance, skin stem cells — and then either directly inducing their transdifferentiation to limbal cells, or transforming them first to an embryonic-stem-cell-like state (induced pluripotent stem cells, or iPS cells) before inducing their differentiation along the limbal lineage. To achieve such stem-cell therapies and improve on the existing ones, researchers will need to learn more about how corneal stem cells differ from their skin counterparts. Better protocols must be devised for purifying stem cells and for preserving and enhancing their self-renewal *in vitro*. And protocols for transdifferentiation and/or iPS-lineage differentiation will need to be established for the generation of limbal stem cells.

Pellegrini, De Luca and colleagues' work[1] elegantly demonstrates how the knowledge of one type of stem cell — in this case, the human epidermal stem cell — can be used to advance a clinical treatment for another, the limbal stem cell. Their paper sets the gold standard for the level of scientific proof that is needed for each new stem-cell therapy, and provides a blueprint that can be applied to the development of other adult stem cells for clinical therapies. Stem-cell therapy still has a long journey ahead, but the light is beginning to shine brightly on its path. ■

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1. Rama, P. *et al. N. Engl. J. Med.* **363,** 147–155 (2010).
2. Pellegrini, G., Rama, P., Mavilio, F. & De Luca, M. *J. Pathol.* **217,** 217–228 (2009).
3. Dua, H. S. & Azuara-Blanco, A. *Surv. Ophthalmol.* **44,** 415–425 (2000).
4. Thomas, E. D., Lochte, H. L. Jr, Lu, W. C. & Ferrebee, J. W. *N. Engl. J. Med.* **257,** 491–496 (1957).
5. Barrandon, Y. & Green, H. *Proc. Natl Acad. Sci. USA* **84,** 2302–2306 (1987).
6. Green, H. *Sci. Am.* **265,** 96–102 (1991).
7. Pellegrini, G. *et al. J. Cell Biol.* **145,** 769–782 (1999).
8. Senoo, M., Pinto, F., Crum, C. P. & McKeon, F. *Cell* **129,** 523–536 (2007).
9. Pellegrini, G. *et al. Lancet* **349,** 990–993 (1997).

EARTHQUAKES

Climate and intraplate shocks

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The heartland of the United States lies within a tectonic plate, certain regions of which have experienced large and geologically recent earthquakes. Explanations for those events are still being sought.

Over the past several decades, the earthquake cycle along tectonic-plate boundaries has become increasingly well understood. There is a consensus that geological, geodetic and palaeoseismic data can be combined to establish long-term earthquake probabilities, with a degree of certainty that improves as more and better data become available.

There is no such consensus when it comes to intraplate earthquakes. The reason is that there are no well accepted principles that account for why large earthquakes have occurred where they did in the recent past, where they are likely to occur in the future, or how large they might be. In this context, Calais *et al.*[1] (page 608 of this issue) provide a valuable contribution. Their study region lies in the central United States, around New Madrid, Missouri, which in 1811–12 experienced a sequence of three earthquakes estimated to be of magnitude 7 or larger.

Much still needs to be done to reduce earthquake hazards for those living along active plate boundaries. To recognize that, one needs only to look at the devastating consequences of the 2004 earthquake and tsunami in Sumatra (230,000 dead in 14 countries), or the earthquake in Haiti earlier this year (approximately 200,000 dead and 2 million left homeless). But the situation is even worse in intraplate regions, especially in the developing world. In the past decade alone, tens of thousands of people have died in each of the earthquakes that hit Bhuj, India (2001), and Bam, Iran (2003), as well as in the magnitude 7.9 Wenchuan event that occurred in China in 2008 (Fig. 1). We know that intraplate earthquakes result from plate-driving forces transmitted through plate interiors[2,3]. But without a better understanding of why intraplate earthquakes occur where they do, the potential for future damaging earthquakes must be considered 'high impact but low probability'. In the developing world, it is unlikely that much will be done to prepare for such events.

The New Madrid seismic zone is the best studied of locations that have been affected by intraplate earthquakes. One of the enigmatic features of this zone is the rate at which large earthquakes occur. Palaeoseismic data[4] indicate the occurrence of at least three, and possibly five, large earthquakes (or sequences of such earthquakes) in just the past few thousand years. However, faults seen on seismic reflection profiles show little cumulative deformation over the past few million years[5], during which time the regional geological processes have been essentially identical. Hence, the long-term earthquake rate seems to be much lower than that of the past few thousand years.

Moreover, unlike at plate boundaries, where over time the average rate of seismic-strain release in big earthquakes matches the rate at which strain energy accumulates as a result of relative plate motion, analysis of data from the Global Positioning System (GPS) has shown that the rate of strain accumulation in the New Madrid region is quite low[6]. The occurrence of multiple large events in a relatively short period of time seems to be due to the release of strain energy that accumulated over a very long period of time.

In turning to the new paper by Calais *et al.*[1], I should declare an interest in that the model used is conceptually similar to one proposed by Grollimund and myself a few years ago[7]. Both studies invoke the consequences of the retreat of glaciers from much of continental North America at the end of the last ice age. And both assume that the brittle crust is in a state of frictional failure equilibrium — that is, even in relatively stable plate interiors, stress levels are close to that at which slip could occur on faults that are appropriately oriented to the current stress field. This allows even a relatively small perturbation of stresses in the lithosphere to induce brittle faulting in the upper crust, and time-dependent flow in the viscous lower crust and upper mantle.

In Calais and colleagues' model[1], the perturbation is caused by localized erosion of approximately 12 metres in the past 16,000 years, produced by river incision. This induces upward flexure of the lithosphere in the New Madrid area, 'unclamping' some of the critically stressed faults in the region. In our paper[7] we argued that, consequent on the removal of ice-sheet load, seismicity is localized around New Madrid because of anomalously low