The story behind the first stem cell therapy

People can be visually impaired or experience a loss of sight for any number of reasons. Advancing years can lead to age-related macular degeneration while diabetes can give rise to diabetic retinopathy – a condition in which high blood sugar levels damage the retina. As serious as these conditions are, they do not compare to the sudden shock a person experiences when the eye is injured by an accident involving an acid or a chemical.

When the eye is injured by a burn, the corneal surface which contains a stem cell reserve is irreversibly damaged. In the healthy eye this reservoir renews itself approximately every six to nine months. In the damaged eye, it stops regenerating and the conjunctiva gradually begins to cover the cornea, impairing vision and causing chronic pain and inflammation.

Until recently the long-term management of chemical or other serious accidents to the eye involved reconstructing the damaged tissue with allografts or autografts. This works in some cases, but it falls short of the holy grail of regeneration.

Now regeneration is a possibility for patients with severe eye injuries following European regulatory approval in February 2015 of a stem cell therapy for adults with ocular burns. The product, Holoclar, consists of corneal epithelial cells containing stem cells which have the capacity to repair and regenerate damaged tissue. The stem cells are the drug's substance and the number of cells has been precisely determined. It is these cells, which reside in the limbus, that regenerate the damaged tissue.

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>
> *Michele De Luca*

Holoclar is an autologous treatment, which means that stem cells are harvested from the patient, expanded in a culture and then transplanted back into the same patient. One of the unique features of the therapy is that it takes very little tissue to supply the laboratory sample: the developers estimate that a 1-2 mm^2 biopsy is enough to create a product for transplantation.[1]

The family company

Holoclar is manufactured by Holostem Terapie Avanzate, a spin-out of the University of Modena and Reggio Emilia in Italy, and commercialised by Chiesi Farmaceutici SpA, a family-controlled pharmaceutical company based in Parma. The business model that led to the successful production of Holoclar is a collaboration going back more than five years among the university, the company and a non-profit foundation, Fondazione Cassa di Risparmio di Modena. But the research history is much longer. It revolves around the decades-long professional relationship between two scientists, Michele De Luca and Graziella Pellegrini.

Professors De Luca and Pellegrini are co-founders of Holostem and hold senior positions in regenerative medicine at the University of Modena. As scientists they have worked together in laboratories across Italy and published, with colleagues, scholarly articles on epithelial stem cell biology in numerous publications.

In separate interviews with *MedNous*, Profs De Luca and Pellegrini explained how an early association with stem cell pioneer Howard Green and subsequent work with burn patients in Italy led to the discovery of Holoclar.

The story starts in 1985 when Prof De Luca was a visiting scientist in Professor Green's laboratory at the Harvard Medical School in Cambridge, Massachusetts. Prof Green was chairman of the Department of Cellular and Molecular Physiology, and had achieved notoriety two years earlier for helping to save the lives of two young boys who had sustained third-degree burns from a fire at a vacant house in Wyoming. The burns were so severe that there was very little tissue to use for a skin graft. In desperation, the doctors turned to Prof Green for help. The Harvard academic had developed a method for growing epidermal cells in the laboratory. This involved taking cells from a small piece of epidermis (the outer skin) and growing them into a large number of cultured epithelia. Epithelial cells line the surface of many structures in the body including the skin and cornea of the eye. Prof Green had previously shown that it was possible to culture epithelia in the laboratory and create an epidermal structure which looked like, but was superior to, a skin graft.[2] According to the university, he turned his laboratory into a skin-manufacturing facility, and his postdoctoral researchers worked as epithelium engineers.[3] They produced enough material to save the boys' lives.

The event was transformative for Prof De Luca. Returning to Italy, he and Prof Pellegrini joined the National Cancer Institute in Genoa where they worked in a laboratory culturing epithelial cells from skin for the treatment of burn patients.

Dr Pellegrini recalled: "We saw people who were clinically dead, become alive. They were saved with a transplantation of the epidermal surface. This was the reason for our big interest in the field."

Types of epithelial cells

In the period leading up to the mid-1990s, Profs Pellegrini and De Luca spent a lot of time characterising different types of epithelial cells in the body. For example, Prof De Luca became the first scientist to establish a human urethral stem cell culture to treat a congenital defect known as posterior hypospadias.[4] Both scientists were also interested in the eye, and in particular understanding how different epithelial cells in the eye function separately, or together. In 1996 they moved to a hospital in Rome where they developed a protocol for the reconstruction of the eye's ocular surface.

The scientists hypothesised that a small area of the eye at the border between the cornea and the conjunctiva, or the limbus, contained stem cells capable of regenerating the cornea. They tested this hypothesis on two patients whose eyes had been injured from alkali burns. The first patient had sustained burns 10 years earlier, and the second, 22 years earlier. A biopsy was taken from the limbus, cultured in a laboratory to produce sheets of corneal epithelium and then grafted onto the damaged eyes of both patients. Two years after the procedure, both patients were stable and had improved vision. The results were written up in *The Lancet* in 1997.[5]

The procedure is repeated

One year later, the scientists repeated the procedure, but this time using 'in-process controls' to ensure that each cell culture yielded the same drug substance even if the cells themselves had been taken from different patients. The patients in this round of investigations had suffered from burns leading to a depletion of stem cells in the limbus of their eyes. Biopsies of limbal stem cells in the healthy eyes were taken and cultured onto a fibrin substrate. The fibrin cultures were then grafted onto the damaged corneas. The results were published in *Transplantation* in 2001.[6]

In 2002, the science team moved to Venice from Rome, and then in 2007 they moved to Modena. At the University of Modena, they set up the Centre for Regenerative Medicine where Holostem Terapie Avanzate operates. In addition to being a co-founder of Holostem, Prof De Luca is director of the regenerative medicine centre. Prof Pellegrini, also co-founder of the company, is the centre's coordinator of cell therapy.

At a ceremony marking the opening of the centre in 2008, the two scientists met Andrea Chiesi, director of research and development at Chiesi Farmaceutici. The company liked what it saw and decided to invest in the project. Mr Chiesi soon became chief executive of Holostem.

The decision by the Italian scientists to commercialise their work took place years after Prof Green's invention had been put on the market in the US. The Harvard professor spun out his technology into a company called Biosurface Technology Inc, which was acquired by Genzyme in 1994. Biosurface's main asset is a cultured epidermal autograft, known today as Epicel. Epicel is indicated for patients with serious skin burns.

Holoclar's path to the market has been different. This was due to a decision by the inventors to prove, beyond a shadow of a doubt, that the cells which were restoring sight to burn victims were in fact stem cells. After much work they established that the stem cells were holoclones.[7]

"We did a lot of basic science, trying to understand holoclones at a molecular level. The formal demonstration that in a human clinical setting the holoclones were responsible for the regeneration of the cornea *in vivo* was the *New England Journal of Medicine* paper in 2010. That is the basis of the Holoclar product because in that paper we formally demonstrate that the entire clinical procedure, the entire clinical success was strictly dependent on holoclones, so formally proving that they were the stem cells responsible for the long-term regeneration of the limbal/corneal epithelium," Prof De Luca said.

Holoclar has been approved in Europe as an advanced therapy medicinal product (ATMP) which means that it was reviewed by a special panel of scientific experts before receiving a positive opinion by the Committee for Medicinal Products for Human Use.

The European Commission has given it a green light to be marketed for the treatment of adult patients with moderate to severe limbal stem cell deficiency due to physical or chemical ocular burns.

According to Chiesi, patients in need of the treatment will have biopsies taken at designated clinics throughout Europe where medical personnel will be trained to handle the material. Once a biopsy has been taken, it will be transferred by courier from the clinical centre to the Holostem/Chiesi Farmaceutici premises in Modena. The courier will be expected to deliver the sample within 24 hours. The sample will then be processed, which could take a few weeks. Depending on the condition of the patient, a date for the transplant will be set and the therapy will be delivered to the clinical centre within 36 hours. Part of the tissue will be cryopreserved and will be available for a possible second transplant.

Prof Pellegrini said that the company is prepared to do up to 700 procedures per year, which is approximately the number of patients in Europe suffering from limbal stem cell deficiency.

The project continues

Meanwhile, back at the lab bench, Prof de Luca and colleagues are working on another project. This is to see if they can develop a gene therapy for epidermolysis bullosa, which is an inherited connective tissue disease causing blisters in the skin.

"We are working on the genetic modification of the epidermal stem cell, to try and help the so-called butterfly children or those with epidermolysis bullosa. We are performing a Phase 1/2 clinical trial using genetically modified, genetically corrected epidermal stem cells to restore the skin of these patients," Prof de Luca said.

Time passes, but there are also moments to reflect on the past. The Italian researchers are still in regular contact with Howard Green who is now in his 90s. The US academic has been supportive of their work, and of Holoclar.

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