Advances in stem cell-based regenerative medicine

Despite setbacks and failures, therapies to replace damaged tissue are making their way into the clinic

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Stem cell-based regenerative medicine has experienced turbulent times in recent years that have involved several scandals implicating prominent institutions and numerous retractions of papers from leading journals. But it has also seen real progress being made on the way to the clinic. It reflects the tension between human ambition and hope on one side and the need for hard evidence and solid data on the other side in a field where early expectations ran far ahead of reality. This tension may help explain the recent scandal involving stem cell surgeon Paolo Macchiarini, who was found guilty of scientific misconduct regarding an article in *The Lancet*, which has since been retracted. It is an ongoing saga as Macchiarini is still publishing in leading journals, even though his reputation as a surgeon and stem cell researcher has been compromised.

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The story dates back to 2004, when Macchiarini's team extracted some pig intestine and removed the cells to leave a collagen matrix that was then repopulated with muscle and fibroblasts to generate connective epidermal tissue. The patch was implanted in the airway of a 58-year-old patient suffering from respiratory problems

after surgery for lung cancer [1]. Macchiarini then expanded this technique to whole tracheas using plastic scaffolds in addition to natural tissue from human donors. In another clinical case, the bare plastic scaffold was populated with stem cells taken from the patient's own bone marrow and implanted in a young female patient in 2008.

This propelled Macchiarini almost to rock star status and an appointment by the Karolinska Institute, where he refined his technique by replacing donor windpipes with plastic scaffolds made to order. A number of operations were performed, but in many cases led to complications and even death. While the exact reasons varied and were not always clear, it emerged that the operations had been performed without the usual preparatory studies, including prior evaluation in animal models. In at least some of the cases, it seems that the implanted tracheas failed to integrate properly with the bronchi, larynx and oesophagus.

The Karolinska Institute investigated these cases, which led to Macchiarini's dismissal and finding seven researchers in total responsible for scientific misconduct. In a decision published in June 2018, the institute announced that six articles contained serious inaccuracies (<https://ki.se/en/news/seven-researchers-responsible-for-scientific-misconduct-in-macchiarini-case>). The main points were that the articles contained fabricated and distorted descriptions of the patients' conditions before and after surgery, that justification for treatment as a last resort for survival was lacking and that reference to relevant animal experiments—which must precede human studies—was missing. In

addition, ethical approvals and appropriate informed consents had not been obtained. The case is still ongoing, as the Swedish Prosecution Authority reopened its own earlier investigation in December 2018 (<http://news.cision.com/aklagarmyndigheten/r/investigation-concerning-surgeries-resumed-after-review,c2697275>).

Scientific and regulatory challenges

Yet, the underlying technique—the use of scaffolds, on which stem cells extracted from the patient's bone marrow are implanted—has not itself been condemned. “In principle this is not conceptually wrong,” commented Michele de Luca, Director of the Centre for Regenerative Medicine Stefano Ferrari at the University of Modena and Reggio Emilia in Italy. “Other people have taken a similar approach to the oesophagus, although no data has been published yet.” However, he did express concern that the whole field of regenerative medicine had been tainted by a number of cases where—although no gross misconduct was involved—researchers had taken short cuts and neglected basic science.

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Inevitably, given the complexities involved in tissue regeneration, never mind whole organs, work that lacks due diligence is almost destined to fail, sometimes with devastating or fatal consequences for patients. “There are many examples in the fields of regenerative medicine of improper use of stem cells, or presumed stem cells, in clinical situations,” De Luca said. “In order to do proper regenerative medicine, you need a proper disease, a proper rationale to treat that disease with that stem cell and a proper knowledge of the biology of that stem cell that is used to treat that specific disease. Then you need to define a clinical protocol in order to minimize adverse events and assure clinical success.” This advice has often been overlooked, sometimes out of desire to short-circuit the long process from conception to clinic, according to Anthony Atala, Director of the Wake Forest Institute for Regenerative Medicine in the USA. “It is interesting to note that there have been a number of clinical trials performed where there was sparse scientific and preclinical data, and not surprisingly, these efforts did not succeed,” he said. In addition, the field is plagued by clinics that advertise dubious or fraudulent therapies. “Unfortunately, a number of businesses have sprouted touting the benefits of stem cells as a cure for all ailments, where unknowing patients are spending large amounts of money for unproven therapies,” Atala added.

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More regulation is clearly needed to constrain such businesses, but Atala also touched on the desire of the public for new treatments to previously incurable diseases. He emphasized that regenerative medicine has registered a number of significant advances over the past 15 years, some of which are almost certain to make it to the clinic, but patience is required. “The public at large often may not realize the typical timeline for developing medical technologies,” Atala said. “It takes years of benchtop and preclinical data to elucidate the potential of these therapies.”

A long history

In fact, while scandals may have hogged the headlines, significant breakthroughs have been made in several areas, notably for skin and eye diseases. De Luca and his team for instance have developed new treatments for rare genetic skin diseases and eye injuries that cause the cornea to deteriorate.

To put these developments in context, it is worth taking a look at the history of stem cell medicine, which began almost by accident over 60 years ago with the first bone marrow transplants to treat leukaemia [2]. This treatment is still given in more advanced forms today and has saved more than 1 million lives since its first applications in the 1960s. Admittedly, the role of stem cells in bone marrow transplantation was poorly understood at the time and the treatment was not described as regenerative medicine.

There was also an air of serendipity over the next big step in the 1980s when laboratory-grown skin patches from patients were first used to treat burn victims, a technique pioneered by Howard Green at the Massachusetts Institute of Technology. Even then, the role of stem cells was not properly acknowledged and the technique was discovered almost by accident during a failed experiment to replicate a rare mouse tumour. Nonetheless, Green developed the ability to grow sheets of skin up to desktop size from extracts no bigger than a postage stamp [3]. The initial proof of concept came in 1983 when Green applied the technique for the first time at full scale on a boy who had suffered life-threatening third-degree burns. Although skin transplants had been used to treat burn victims, Green’s technique worked much better because it minimized the risk of tissue rejection as the skin was derived from the patient’s own stem cells. Since, it has been widely applied and saved many thousands of lives, and improved quality of life for many more.

The first serious attempts to regenerate whole organs came in the early 1990s and led to the ill-fated trachea operations but also a lot of animal work on more complex solid organs such as heart, liver, kidney and pancreas. But the next clinical breakthrough came again with skin replacement, involving a patient suffering from a rare and previously incurable group of skin diseases collectively called epidermolysis bullosa

(EB). It is caused by mutations that affect proteins crucial for anchoring the outer layer of skin, the epidermis, to the underlying subcutaneous tissue. This results in skin sloughing off after minor injuries or even causal abrasion, causing blistering and susceptibility to infections. Severe sufferers have wounds that require daily redressing and continuous risk of infections.

A treatment for epidermolysis bullosa

In 2015, De Luca received a call from doctors in Germany who had a young male patient with a severe form of EB, caused by a mutation in a gene encoding part of the protein laminin 332, which De Luca was targeting in an ongoing clinical trial. This case was particularly severe as the boy had contracted multiple infections and was suffering from life-threatening sepsis. Like the earlier technique pioneered by Green, the treatment involved culturing and growing skin from a small patch taken from the patient, but with the crucial difference that the defective genes were replaced before the cells extracted from the boy were grown into larger sheets and attached to the patient. The boy fully recovered, returned to school and now leads a normal life, with the skin continuing to settle down into a normal state, according to de Luca. “We are now preparing and running initial trials for other forms of EB,” he said. “We are using the genetic technologies of CAS9 for those cases where it is important to do gene correction rather than gene addition.”

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However, the retrovirus technique to deliver the corrected gene that was applied in the first case is closer to clinical deployment, de Luca added. This case is strengthened by the fact the procedure is similar to one of the first approved stem cell-based gene therapies for corneal regeneration in the eye. This technique, marketed under the name Holoclar, became the first advanced therapy medicinal product (ATMP) containing stem cells to receive conditional marketing authorization from the European Commission in 2015 following

recommendation by the European Medicines Agency (<https://www.ema.europa.eu/en/medicines/human/EPAR/holoclar>).

Repairing eye damage

Holoclar uses limbal stem cells to repair the cornea after injury and has been successfully used in a number of patients. These stem cells are located in a part of the eye called the limbus, at the boundary between the sclera, or white part of the eye, and the cornea, the clear part at the front. Under normal conditions, limbal stem cells repair damage to the outer layer of the cornea, but more excessive injury can compromise the stem cells themselves. The Holoclar therapy can be applied in a similar way to EB skin treatments, by extracting a tiny portion of the undamaged limbus where healthy stem cells still reside and growing it into a sheet of cornea that can be transplanted back onto the eye.

Eye conditions in general are likely to be among the first clinically approved regenerative treatments on a large scale, given significant progress in treating some cases of age-related macular degeneration (AMD). AMD is a major cause of impaired vision, the fourth most common cause of blindness after cataracts, preterm birth and glaucoma. The prevalence of advanced AMD in the UK among people aged 50 years or over is 2.4%, rising to 4.8% for those over 65 years and 12.2% over 80 years (<https://www.nice.org.uk/guidance/ng82/chapter/Context>).

There are two principal forms of AMD: wet and dry. The wet form, which accounts for 10% of all AMD cases, is caused by abnormal growth of blood vessels in the macula, the middle part of the retina responsible for central vision, which leads to scarring of the macula and rapid loss of central vision. Dry AMD involves slow deterioration of the cells of the macula without any abnormal vasculature. It is for a subset of wet AMD where the initial breakthrough has been achieved in a trial sponsored by Pfizer and led by Pete Coffey from the Institute of Ophthalmology, University College London.

As with de Luca's case of EB, it was a small study involving just two patients with severe vision loss, but as Coffey argued, it demonstrated the potential of the treatment. The two patients had suffered large haemorrhages beneath the retina involving the fovea, a small pit in the central macula

associated with clearest vision. This can cause small rips in the retinal pigment epithelium (RPE) as fluid progressively accumulates, leading to larger tears as the membrane is stretched beyond its elastic limit. Eventually, the RPE separates from the overlying photoreceptor cells, which causes degeneration of these cells now exposed to blood components [4]. Previous animal studies had indicated good prognosis for these cases if treatment is carried out before significant degeneration of photoreceptor cells.

The UCL team engineered a RPE patch comprising a fully differentiated, human embryonic stem cell-derived monolayer on a synthetic basement membrane. This patch was implanted into the subretinal space of one eye in each of the two patients. "Those two patients are basically reading again," Coffey described the outcome. "The first lady couldn't read more than a word and a half a minute and can now manage 80 words a minute. The second patient who couldn't even see the book is reading 50 words a minute." This is leading to an effort to recruit more patients and finish the original trial by the end of May 2019.

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While the results are promising, much larger and long-term trials will be needed to establish both safety and efficacy, and to determine whether other side effects will emerge, such as slow-growing tumours. There was also no control group to compare with the current gold standard treatment, primarily injections of anti-vascular endothelial growth factor (VEGF) to block the abnormal blood vessel development.

Meanwhile, Coffey commented, the success achieved so far has brought on a new problem, namely to scale up the procedure for larger trials and make it affordable for health agencies. The first step would be to extend treatment to the remaining wet AMD cases and to early-stage dry AMD. It was easier to establish safety and efficacy for this severe form of

wet AMD, partly because the damage is limited to the cells of the RPE, which can be regenerated with the technique the team developed. "Other trials elsewhere are going into late-stage dry AMD when it's not just the RPE that's died, but also the overlying neural retina with light sensitive cells and the vasculature behind the RPE, the choroid, has started to deteriorate," Coffey commented. "So you effectively need three layers, which is not what current trials are doing."

Advances for treating Parkinson's

Unfortunately, only few studies have established clinical efficacy and given hope to patients with previously incurable diseases, according to Giulio Cossu, Professor of Regenerative Medicine at the University of Manchester, UK, who works on treating muscular dystrophy. He pointed out that there has been exponential growth in experimental therapies, broadly defined as regenerative medicine, "[b]ut results vary from unequivocal clinical efficacy for previously incurable and devastating diseases to, more frequently, a modest or null effect." As Cossu further explained, regulators are caught between pressure from patients and the need to see more data, which takes time. He also urged that clinical trials should compare treatments with the gold standard in a field, rather than just placebo, in order to establish sufficient efficacy to justify the risks of treatment and costs of further development.

Yet, Cossu noted the spectacular results of these recent cases and the prospect of more to come. He nominated Parkinson's disease as the one most likely to benefit from the next breakthrough in regenerative medicine. There is indeed a long history of cell transplantation to treat Parkinson's dating back to the 1990s, which have produced highly variable results without any clear indication why. Researchers were drawn to Parkinson's early on because it involves loss of dopamine (DA) neurons, which makes it a natural target for stem cell-based regeneration. Remission has already been achieved at early stages through dopaminergic drugs, but these cause side effects and do not replace natural dopamine production at the site of greatest loss.

Targeted neuronal replacement therapies clearly had the potential to address these shortcomings, although with questions over

whether that would lead to sustained remission, never mind a cure. Proof-of-principle studies using foetal brain tissue to create grafts capable of releasing dopamine showed long-term efficacy and improvements in quality of life and recovery of some non-motor features [5]. However, these transplants had mixed results and generated side effects, such as disease-related pathology, years after being implanted. Efforts to identify underlying intractable issues led to formation of TRANSEURO, a new trial in Europe that has now grafted 11 patients with these foetus-derived grafts (<http://www.transeuro.org.uk/>). Yet, the use of foetal tissue raises concerns both over ethics and issues linked to acquisition and broader use, and difficulties in standardizing it for more widespread clinical application. In TRANSEURO, only 20 out of a planned 90 or more surgeries actually took place, with the rest abandoned owing to lack of tissue supply. This approach was clearly not sustainable given the large number of Parkinson's sufferers.

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This led to using human pluripotent stem cells, derived either from early pre-implantation embryos or from reprogrammed adult somatic cells, as these can be robustly differentiated into midbrain dopaminergic neurons [5]. This is the underlying approach of new trials, one of the largest of which is now being set up between Sweden, the UK

and the USA. This is now possible as a result of painstaking work to address shortcomings of previous approaches, according to Cossu. “If I had to bet money that something would work, I’d put it on those trials,” he said.

Overcoming the blood/brain barrier

There is also substantial progress towards treating lysosomal storage diseases (LSDs), a group of conditions where inherited enzyme deficiency leads to accumulation of various compounds and toxins in lysosomes. Individual LSDs are rare but collectively occur in around 1 in 5,000 births [6]. Most of these affect development and the central nervous system; while some LSDs can be treated by enzyme therapy, the blood/brain barrier bars entry of enzyme preparations into the brain.

There is no immediate prospect of any gene therapy correcting this deficiency in neurons, but there is potential for boosting production of the relevant enzymes in the CNS itself. According to Cossu, this can be achieved by using gene-editing technology on haematopoietic stem cells. These include microglia, which act as the primary immune defence in the CNS, given that most antibodies produced elsewhere are unable to cross the blood/brain barrier. They also produce various enzymes, which makes them therapeutic targets for some LSDs. “So we engineer microglia to make 10 times the amount of a lysosomal enzyme as normal cells,” Cossu explained. “What is known is that the microglia then release enough enzyme into the medium that the neurons take it up.” He added that LSDs are an area of rapid advances with trials underway, as the gene-editing approach has shown it can reverse the disease if applied early enough [7]. “We have learnt that you have to do it when the children are six months or less, because if

you wait another six months, the neurons have degenerated to the point you can’t revive them anymore,” Cossu said. “You need to intervene before the disease has compromised the integrity of the tissue.”

Overall, the field of stem cell-based regenerative medicine is poised to come up with major therapeutic advances, while it is still bogged down by too many false promises and misguided attempts that ultimately failed. Progress relies, as in many medical fields, on rigorous and careful work over long time and persistence in light of early failures, if the underlying scientific principles are sound.

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