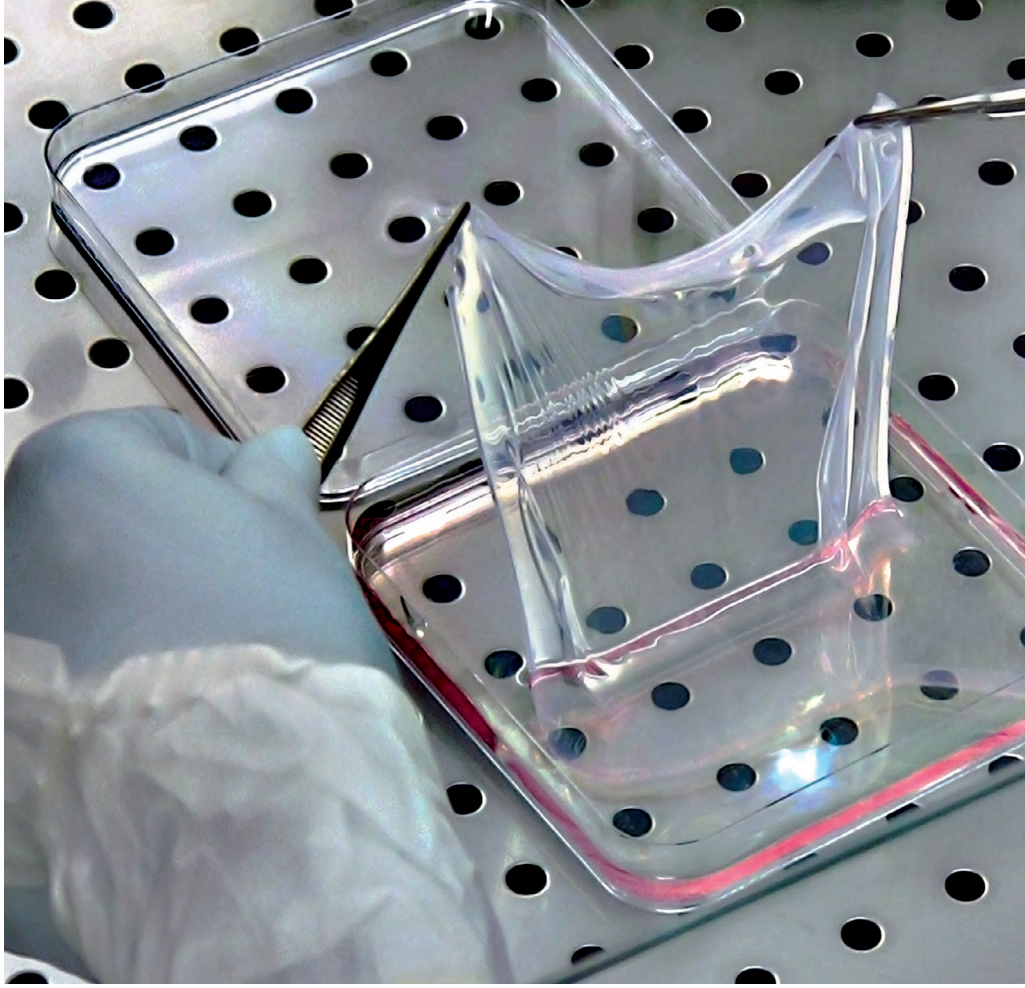
DERMATOLOGY

Under the skin

The largest organ in the body is a prime target for gene therapy.

BY KAT ARNEY



CMR UNIMORE

It's not often that a figure in a scientific paper can make you wince with pain. But it's impossible to look at figure 1a in Michele De Luca's 2017 *Nature* paper and not feel a sympathetic twinge at the sight of a young boy, Hassan, covered from head to toe with red-raw wounds[1].

The son of Syrian refugees who fled to Germany, Hassan was born with junctional epidermolysis bullosa (JEB) — a condition caused by a genetic fault in one of three genes (*LAMA3*, *LAMB3* and *LAMC2*) encoding subunits of the laminin-332 protein, which binds the surface of the skin to the underlying layers. Affected children rapidly develop large, painful blisters over their skin and internal mucous membranes, which can easily become infected.

By 2015, when Hassan was seven, his skin was almost entirely destroyed and he was suffering from severe bacterial infections. Doctors at Ruhr University in Bochum, Germany, could offer only palliative care to relieve his suffering. But Hassan's father enquired about experimental treatments, and the doctors got in touch with De Luca at the University of Modena and Reggio Emilia, Italy, who was working on a radical skin therapy.

De Luca's research builds on the life-saving work of cell biologist Howard Green at the Massachusetts Institute of Technology in Cambridge. Green was the first to discover that sheets of skin cells could be grown in the laboratory, creating personalized skin grafts that avoid the problems of immune rejection. De Luca worked with Green at Harvard Medical School in Boston, Massachusetts, in the 1980s, and he later decided to develop Green's approach for treating genetic skin conditions by genetically modifying the skin cells to fix the disease-causing mutation.

"We've been using epidermal skin-cell cultures for many years to treat hundreds of patients, carrying out a lot of work on basic stem-cell biology as well as gaining clinical experience, so it was obvious to try and genetically modify these cells for treating rare skin diseases like JEB," De Luca says.

The idea of growing genetically modified skin for therapeutic use was first proposed in 1994 by dermatologist Gerald Krueger at the University of Utah in Salt Lake City[2], and De Luca and his team reported the results[3] from an initial small clinical trial of genetically modified skin grafting back in 2006.

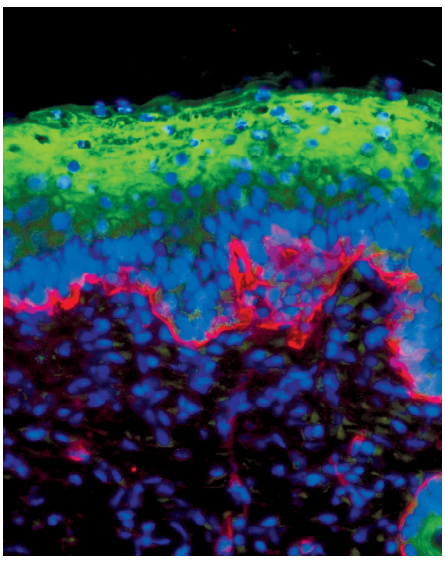
The recipient was a 36-year-old man with JEB caused by a *LAMB3* mutation. He was treated with nine small patches of skin that were grown from his own epidermal cells and modified with a viral vector expressing the missing gene. The grafts remained stable and healthy for more than a year, proving that the technique had the potential to provide long-term correction of the condition.

UNSCHEDULED INTERRUPTION

Despite this early success, De Luca's clinical work ground to a halt for nearly a decade owing to European Union legislation governing cell and gene therapies. "The regulations regarded our grafts as medical products, so they had to go through the same regulatory process," he sighs. "We had to stop all our activities, build up a compliant manufacturing facility and register the therapy — it was only in 2015 that we were finally able to start our trials again."

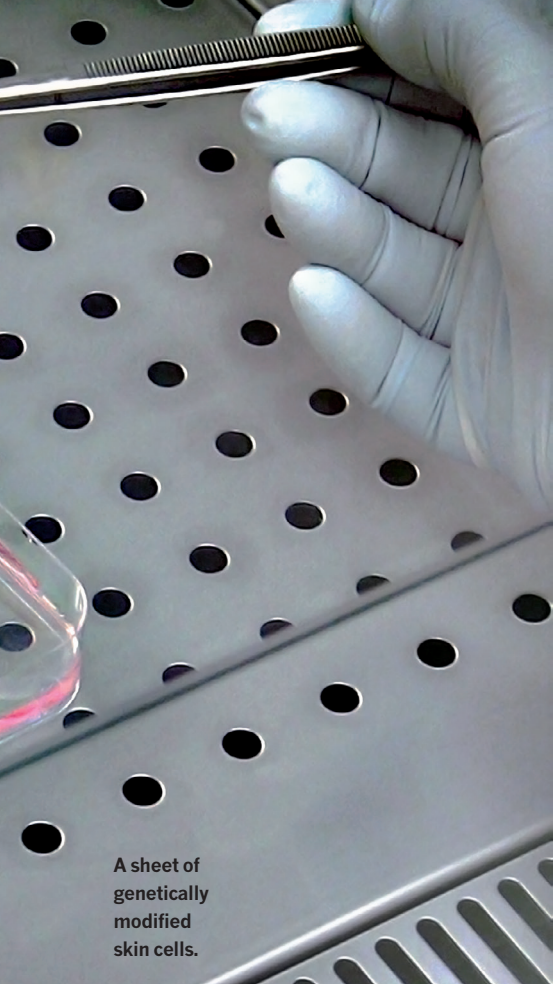
Luckily, this was just in time for Hassan. De Luca's team took a tiny unblistered skin sample from the child's groin, then carefully cultured the epidermal stem cells and modified them with a viral vector carrying a functional version of *LAMB3*. The next challenge was growing enough 12-centimetre-square sheets of modified cells for Ruhr University plastic surgeon Tobias Hirsch to wrap around the child's fragile body.

After two major operations to replace the skin on Hassan's limbs and torso, followed by some smaller procedures, around 80% of the entire epidermis had been replaced, making it the largest genetically modified graft performed to date. By the time the results were published in 2017, Hassan was like a different child, his raw blisters replaced with smooth, perfectly functional skin.



XIAOYANG WU/CYNTHIA LI

A skin graft with fluorescent staining.



A sheet of genetically modified skin cells.

RUHR-UNIVERSITY BOCHUM

As well as detailing Hassan's progress, the paper[1] reveals why the treatment was a success. The skin is made up of many different types of cells, some that are short-lived and others that are much more persistent. The researchers showed that long-term grafting was only possible if the genetically modified cells were holoclones — a relatively rare type of immortal cell that can self-renew indefinitely. By adjusting the culture conditions, De Luca and his team were able to encourage the growth of holoclones, greatly increasing the chance that the resulting grafts would work.

"After three years, his skin is stable with no blistering, and it should last a lifetime," says De Luca. "There are still some areas of blistering that weren't covered with the grafts, and there are other tissues like the mouth mucosa that we couldn't treat, but although we didn't completely cure the disease, we still fixed 80% of his skin."

FROM GRAFTS TO PATCHES

Over at the University of Chicago in Illinois, Xiaoyang Wu is generating genetically modified skin with a different purpose in mind. In 2017, he and his team showed that genetically modified skin grafts could be used as living 'drug patches' in mice[4], akin to plastic nicotine or hormone patches.

Using the gene-editing technique CRISPR–Cas9, the researchers modified epidermal stem cells with a version of the gene encoding GLP1 — a hormone that controls blood sugar levels and suppresses appetite — which could be switched on by the antibiotic doxycycline. They then grew the cells into small skin grafts and transplanted them onto the backs of mice.

The researchers found that the engineered skin grafts could successfully secrete GLP1 into the animals' blood in response to the drug, slowing weight gain and preventing diabetes in mice kept on a high-fat diet.

Wu's team has now used this technique to create similar patches of CRISPR-modified skin cells that produce a tweaked version of an enzyme called BChE, which breaks down cocaine[5]. Wu's version metabolizes the drug more than 4,000 times faster than the naturally occurring form, rapidly clearing it from the body and quickly killing the 'high'.

When tested in mice, the skin patch stopped the animals from becoming addicted to cocaine and prevented them from overdosing, pointing towards a potentially promising treatment for people with drug addictions. Wu and his team are also working on skin patches that could serve as long-term living biosensors — for example, engineering cells that change colour or fluoresce in response to blood glucose levels.

"Many researchers are focusing on gene therapy for internal organs like the liver, but the skin is much easier — we can culture the cells indefinitely and do the editing outside the body," Wu explains. "We can also very carefully choose the correct clones to grow up into patches, with no off-target effects or rogue genetic changes."

But human trials are likely to be some years off. "Right now we are still at the proof-of-concept stage," Wu says. "Once the technology is more established and we are confident in the procedure, we can think about moving into clinical trials to treat diseases."

Although De Luca finds this idea intriguing, he is more focused on making genetically modified skin replacement a viable treatment for the thousands of children born every year with genetic skin disorders. He is currently running two clinical trials for people with different forms of JEB, but is keen to expand into other forms of epidermolysis bullosa, which can be caused by a fault in any one of at least 18 different genes and affects around 1 in every 20,000 children born in the United States. And it's by focusing on the youngest patients, who have the most to gain from early intervention, that De Luca hopes to make the biggest difference.

"If we treat these children as soon as we can, we will prevent the formation of skin lesions rather than having to cure them — and, obviously, we need to grow less skin to cover them," he says. "If you asked me 30 years ago if it was realistic to replace the whole skin with transgenic epidermis, I would have said no, but we have done it. The final aim of my career is to make this gene therapy a real treatment for children — not a clinical trial or a demonstration of what we might do, but something that is used to treat everyone who needs it."

"After three years, his skin is stable with no blistering, and it should last a lifetime."

Three years on from his record-breaking skin replacement, Hassan is living testament to this possibility, regularly visiting the team in Modena for check-ups.

"When he was in hospital he weighed just 17 kilos and was dying, but now he is growing up," De Luca says proudly. "I last saw him two weeks ago and he is like a mascot for the institute — there is a big celebration every time we see him, and everyone who was involved in his treatment wants to give him a hug." ■

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1. Hirsch, T. *et al. Nature* **551**, 327–332 (2017).
2. Krueger, G. G. *et al. J. Invest. Dermatol.* **103**, 76S–84S (1994).
3. Mavilio, F. *et al. Nature Med.* **12**, 1397–1402 (2006).
4. Yue, J., Gou, X., Li, Y., Wicksteed, B. & Wu, X. *Cell Stem Cell* **21**, 256–263.e4 (2017).
5. Li, Y. *et al. Nature Biomed. Eng.* https://doi.org/10.1038/s41551-018-0293-z (2018).

Around 80% of Hassan's skin was replaced with genetically modified skin grafts.