"Some teams have already used the matrix to build entire organs from scratch - it may even repair brains"

TOMOHIRO INABA

Rebuild your body

Once thought to be mere scaffolding, we now know the matrix inside us can enable regeneration on a scale never seen before. **Andy Coghlan** reports

"IT STARTED as a little sore near my knee, probably a mosquito bite," says Elizabeth Loboa. But the antibiotic ointment wasn't working, and within two weeks what was one wound had become three. From the looks of the wound, her doctor suspected the superbug MRSA and prescribed powerful last-line oral antibiotics. It was at that point that the temptation just became too great. "Instead of taking them, I decided to test a treatment I'd been developing – on myself," she says.

Loboa wasn't just any patient. In her lab at North Carolina State University in Raleigh, the materials engineer had been cooking up a special kind of self-destructing super-bandage capable of healing infected wounds quickly, without scarring or standard antibiotics.

At the heart of Loboa's superplaster is a material that degrades until nothing is left but your own, newly regenerated, healthy cells. What's more, the same trick could one day be used to heal everything from shredded muscle and destroyed digestive tissue to shattered bone. Some researchers have already succeeded in using it to build entire organs from scratch, and it may one day play a role in repairing damaged brains. "It's pretty exciting stuff," says Suchitra Sumitran-Holgersson of Gothenberg University in Sweden. "We're trying to create a whole new human being."

So what is this superstuff? In the body, it is known as the extracellular matrix – the stuff that remains if you strip away the living cells from, say, a blood vessel, an organ or a bit of skin. This scaffolding gives the various parts of our body their detailed shape and solidity.

And that's all we used to think it did. "Everyone thought the matrix just holds things together," says Steven Badylak, a regenerative medicine researcher at the University of Pittsburgh in Pennsylvania who has been one of the early pioneers of matrix-based therapies. Regenerative medicine researchers had long tried to enlist it to regrow organs, believing only that it was a useful scaffold. In animal trials, for example, they would take a kidney and strip it of native cells using a mild detergent. Then they would use the remaining inert chassis as a template on which to deposit the presumed stars of the show: stem cells that recoat the matrix in live flesh.

But a few years ago, it became clear that the matrix does a lot more than it appears. "Now, we recognise its structure is secondary," says Badylak. "It's got loads of functional roles."

For one thing, the matrix is no biologically mute bystander. While it consists mainly of inanimate structural proteins such as collagen and elastin, it also contains proteins that coax the right cells to be in the right place at the right time. For example, hook-like molecules called fibronectins and integrins provide tailored molecular Velcro for specific cells.

Once these have summoned the right cells, the matrix has another trick up its sleeve: it can coax them to turn into bone, muscle or fat cells, according to the tension to which they are subjected once inside the matrix. In your body, this tension is simply a by-product of the everyday stresses of muscle movement. In the lab, it is done by manipulating the stiffness of the matrix. For example, high tension in the matrix's structure will persuade any incoming stem cells to become muscle or bone. Place them into a saggier matrix and they become fat cells (*New Scientist*, 13 February 2010, p 36).

Finally, having convinced them to develop into the right kind of cell, the matrix also has ways of nourishing them so that they continue to mature into larger structures. Its material contains potent growth factors that help blood vessels to form, which provide nourishing oxygen for the growing organs.

Exploiting these properties has revolutionised the way we grow organs. Earlier this year, Harald Ott of Massachusetts General Hospital in Boston built the world's first functioning artificial kidney, a wildly complex organ that stem cell researchers have always ›

assumed needed to be grown from scratch using numerous different cell types. Ott was astonished to find that although he fed only two types of cells into a decellularised kidney matrix – blood-like stem cells into the blood vessels, and endothelial cells into the labyrinthine plumbing that filters the blood – all the different kinds of cells formed in the sites where they were supposed to. The kidneys worked so well in rats that Ott is now using similar techniques to develop hearts, lungs and pancreases. His is not the only lab pursuing this goal. "We're working on livers, hearts, kidneys, oesophaguses, larynxes and small intestines," says Sumitran-Holgersson.

"The early results have been astounding. We've got guys mountain-biking who couldn't stand up before"

But organs are not the only things we want to regenerate. Badylak immediately realised that the matrix's location cues could help him solve a different problem – growing muscle. Damaged muscle can regrow to some extent, but if a severe injury destroys too much of one specific muscle group, scar tissue prevents it from growing back. The only alternative is transferring muscle from elsewhere in the body, but that doesn't work very well, says Badylak. Such an injury usually means amputation and a prosthesis.

But what if you could use the matrix to attract and grow muscle from a person's own cells? It would not be the first time: decellularised tracheas from cadavers have been successfully used in patients to create new fully working tracheas. So Badylak began a trial in which he used matrix taken from pig bladder to regrow big chunks of muscle in six people who had lost more than half of a muscle in road accidents or other trauma. Ron Strang, a 28-year-old US marine whose quadricep muscle had been destroyed by a roadside bomb in Afghanistan, volunteered. "Ron couldn't even get out of a chair without assistance," says Badylak.

After surgically clearing away all residual scar tissue, Badylak simply placed a strip of matrix into the exposed void, taking care to make it taut enough to signal to the body that it should become muscle, not fat.

The early results have been astounding. Six months later, the pig matrix is gone, replaced by a completely new, natural matrix from the volunteers' own bodies – with muscle to match.

"I go out hiking," Strang says, "and I'm able to ride a bike." He has also taken up football and basketball. All of the other volunteers also improved markedly. "We've got guys mountain-biking who couldn't stand up before," Badylak. His next goal is to restore at least 25 per cent of lost function to 80 further volunteers.

Building muscle is one thing. But what about rebuilding shattered bone? That's what Carmell Therapeutics in Pittsburgh is trying to do, except that the matrix the company is using is made from human blood. "Our material is effectively a highly concentrated blood clot," says Alan West, who runs the company, which is a spin-off from Carnegie Mellon University. The success of recent animal studies prompted him to begin a trial in human volunteers.

The dough-like substance carries high concentrations of growth factors known to promote bone repair. In a year-long trial in South Africa, this "bone putty" was applied to the broken shin bones, or tibiae, of 10 volunteers. While West says it is too early to report results, he hints that the accelerated healing the group has seen should help them win approval for larger trials, planned in Europe, and work on other bones.

Superbug battle

Still, there are limits to what a natural matrix can do. Loboa, for example, knew that the natural human extracellular matrix is not naturally antimicrobial. But she really got to thinking about this problem when a friend went into the hospital for an ankle replacement and the wound became infected with MRSA. "They ended up having to amputate the leg below the knee," she says. It was a wake-up call. "We're learning so much about how to regenerate so many different kinds of tissues," she says, "but how do you keep infection out in the age of drug-resistant superbugs?"

So Loboa began to work on a synthetic matrix that could do just that. It would need to turn slowly into the patient's own tissue without ever exposing the flesh beneath to microbes. "The idea is that it's a bandage that never needs to be changed," she says.

Normally, matrix is harvested from human or pig cadavers. To create her own version, Loboa began with polylactic acid, a biodegradable material often used in medical implants, and fashioned it into fibres designed to mimic the architecture of skin. "The novelty is what we can do with these fibres," she says. "We can make them solid, porous or hollow." She chose a porous structure, which could be impregnated with a cocktail of anti-inflammatory drugs and Silvadur, a substance containing small amounts of silver ions that are lethal to most drug-resistant bacteria, including MRSA. The material works in two phases: the first release overwhelms all present bugs, and then a second guard leaks out slowly to destroy any interlopers. The structure dictates how fast the silver ions and other drugs are released. Loboa tested the silver-seeping matrix in pigs: even 4-centimetre long wounds injected with MRSA or *E. coli* bacteria remained pristine.

And after seeing it work so many times in her porcine patients, it was this bandage that she applied to her own wound. And that's when the true implications hit home. "I put my scaffold on, and the sores were gone in three days," she says. Soon, the scaffold vanished too, leaving at first a dark scar that itself is now nearly gone. Loboa is about to submit her results for publication, with one omission. "I'm not including my leg data," she says with a smile.

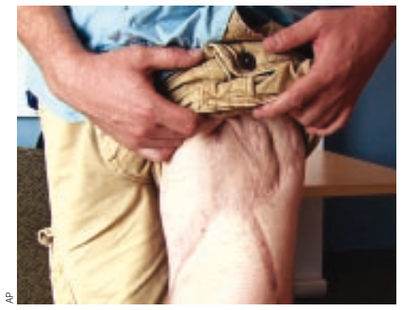
Synthetic matrix can also be used as a template to build body parts far stronger than those nature provides. One group that could benefit enormously would be the millions of people who undergo kidney dialysis every year.

Dialysis is rough on the body: you need to be hooked into machines that cleanse the blood three times a week, with a large arm vein punctured. To do a few days of the kidneys' work in a few hours, blood must be forced through the system at high speed. This heavy use often makes the veins collapse, so doctors have to continually reopen them. If all else fails, it is possible to graft veins from other parts of the body, but during the months it takes for a grafted vein to mature, a plastic catheterised vein must be inserted that often gets infected. "It's harrowing and painful," says Laura Niklason, a tissue engineer at Yale University.

So Niklason and her colleagues set to work on a making customised natural vein matrix parts that were stronger than the real thing. To do this, they first crafted a fast-decaying biodegradable polymer into a tube exactly the dimensions of a vein, but with a thicker vessel wall. Then they coated this tube with human smooth-muscle cells. Within days, the cells completely replaced the biodegradable tube with a matrix of natural collagen, identical to a patient's own – except thicker and better able to withstand the extra pressures of dialysis. After decellularisation, these tubes were then surgically implanted in the patient, and served as the vein for dialysis. Niklason has plumbed her vein matrices into the arms of 30 volunteers in Poland. After several months, they are going strong. Niklason has plans for 20 more implants in the US, and is so positive about the vein matrices that she is hoping they will find a use as coronary bypass arteries.

"The biodegradable implant was soon replaced by a matrix of natural collagen identical to the patient's"

RON STRANG/AP



After a bomb ruined Ron Strang's thigh muscles, implanted pig matrix helped regrow them

That said, artificial matrix still does not have the myriad properties of natural matrix. Sumitran-Holgersson, who has worked with artificial materials at Gothenberg University, says that despite the potential for synthetic implants, natural matrices will always be crucial for building organs. "It's definitely superior because it retains so many important factors to bind and differentiate the cells," she says.

For some applications, however, it has been possible to create hybrids that couple the best of both worlds. This provides a promising avenue to treat one of the worst consequences of Crohn's disease: peri-anal fistulas.

These abscesses eat a channel through the bowel which allows faecal matter to seep out through the skin or, in the worst cases, into a body cavity. "After 20 years with the disease, about 50 per cent of people will get these," says Eugene Boland, who runs Techshot in Greenville, Indiana, which designs custom medical devices. "And of those, 50 per cent will never heal." The prognosis is grim and untreatable, and includes diapers and constant painkillers.

Working with the University of Louisville in Kentucky, Boland designed a matrix-based plug for the fistula. He used polycaprolactone, a component that, like Loboa's fibres, can be tailored to provide extra niches for cells. He also added fibrinogen, the component of natural matrix that aids wound healing.

Brain rebound

To emplace them into two people with Crohn's, Boland coated the hybrid matrix with surface cells derived from their own fat, and implanted the plugs into fistulas that for years had resisted all treatment. It worked in both cases. "They had closed channels after just two weeks," he says. He hopes to treat up to 20 more people before proceeding to a full trial.

The matrix holds a dazzling array of future possibilities. Loboa, for example, is working on multilayered version that could simultaneously regenerate multiple kinds of tissue damaged in severe accidents. Badylak and others are even beginning to explore its potential for repairing brain damage. Greg Bix of Texas A&M University in College Station discovered a key component of the matrix that could promote brain repair all on its own: a signalling molecule released from brain matrix that has been damaged by stroke, called perlecan domain five (DV). It promotes the growth of new blood vessels. When Bix injected DV into mice and rats deliberately given strokes, the results were astounding. "In a fortnight," he says, "you couldn't tell they'd had strokes."

It will be a long time before these treatments become a reality, but Loboa is hopeful that her idea will generate new treatments for skin wounds in the not-too-distant future. And even if major artificial organs are a decade away, as Ott and some of the other researchers assert, matrix can still help heal more commonplace damage to the body, like the muscle, bone and tissue repairs targeted by Badylak and others.

Even simple skin wounds are becoming increasingly dangerous as antibiotic-resistant bacteria thrive in and out of hospitals, and that is where Loboa's change-free bandage could come into its own. "Right now, hospitals are scary places," Loboa says. n

Andy Coghlan is a biomedical news reporter for *New Scientist*