From mice to medicine: harnessing the power of regeneration

Professor Malcolm Maden, of the University of Florida, is an expert in the science of tissue regeneration. He and his lab are exploring the molecules and mechanisms underlying the regrowth of limbs and organs in a pair of remarkable animals. In future, their findings could be used to heal a wide range of human injuries.

It is the stuff of science fiction: cut off one of your enemy's limbs and, hideously, it grows right back. While it may not be quite as instantaneous as we see in the movies – in fact it may take a month or two – one amazing amphibian can do just that. The axolotl is able to regenerate an entire limb, including all of its functional parts – bones, cartilage, joints, muscles, nerves, blood vessels and skin – in full working order. Not only that, but a remarkable mammal, the African spiny mouse, can even grow back large sections of tissue without any scarring. The implications of such abilities for medical science are therefore far from hideous.

These two humble animals are the basis of research in Professor Maden's lab. His team are working to understand the signalling pathways and processes underlying successful regeneration in both the axolotl and spiny mouse, with the aim of stimulating these pathways to promote similar healing processes in patients. Applications of their research could range from scar-free skin reconstruction in burns victims, to repairing the heart after a heart attack or the lungs following chronic lung disease.

FOREVER YOUNG

The axolotl is a type of salamander, found only in a small part of Mexico and critically endangered in the wild, but kept alive in labs across the world. Salamanders, like all amphibians, start life as an aquatic tadpole and metamorphose into their adult form. The axolotl, however, is unique in that it remains at the larval stage throughout its life.

Research has shown that axolotls, other salamanders, and the closely-related newts can regenerate damaged tissue in organs as diverse as the brain, spinal cord, retina, lungs, skin, limbs, intestines and nerves. Professor Maden's research focuses on how signalling molecules in axolotls are able to tell when and how much of a limb has been amputated, and stimulate precise regrowth of the missing portion.

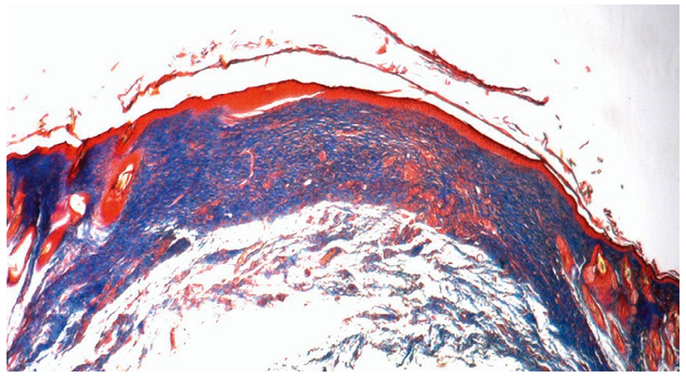
The spiny mouse is able to regenerate perfect new skin, including hairs, sweat glands and different muscle layers

Since the 1940s it has been thought that nerves in the remaining part of an amputated limb produce a chemical signal which stimulates cells beneath the surface of the wound to multiply, forming a mass of cells known as a 'blastema'. However, it was not until last year that one of Professor Maden's colleagues, James Monaghan, of Boston's Northeastern University, identified a potential candidate for the mystery signalling molecule, 'neuregulin-1'.

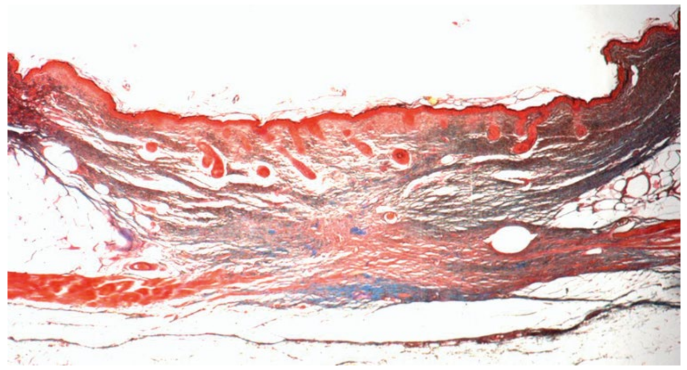
Professor Maden's lab are now working – in a project funded by the US National Science Foundation – to determine the role of neuregulin-1 and other candidate molecules, and to further characterise how cellular signals control the process of limb regeneration in axolotls. Using genetic techniques that explore the impact of deactivating genes in turn, they hope to identify the components of the pathway that causes cells to proliferate, so that ultimately, they can stimulate similar pathways in human tissue.

LIMB FROM LIMB

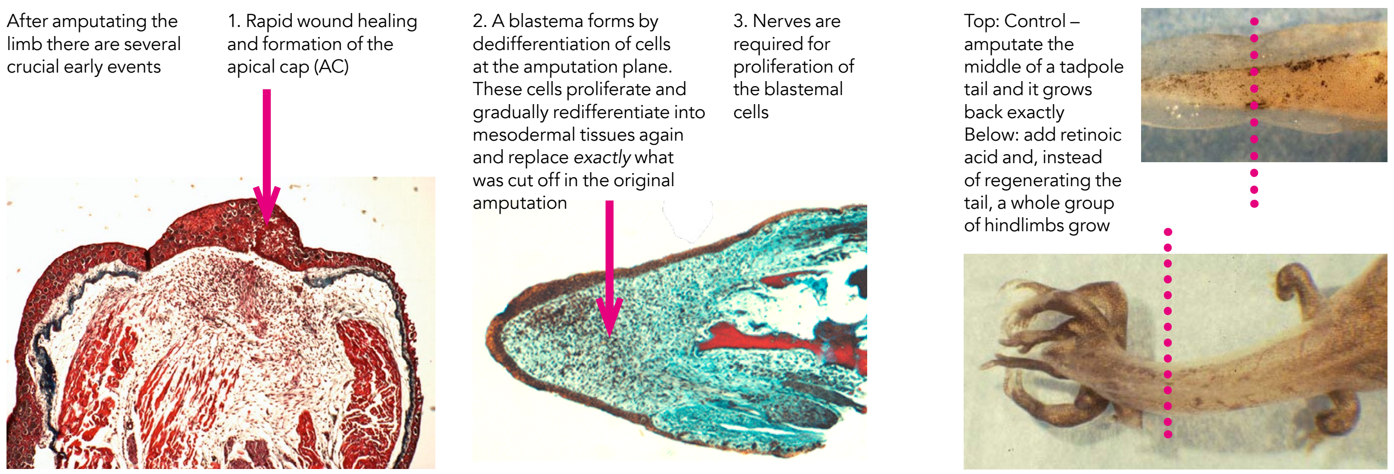
The team are also investigating the intriguing question of how the axolotl's body knows how much of a limb it has lost and therefore how much to regenerate. Back in the 1980s, Professor Maden found that applying retinoic acid, a derivative of vitamin A, interferes with this process. He therefore hypothesised that retinoic acid forms part of the signalling pathway controlling regeneration. An axolotl that is missing a hand will normally regenerate just a hand; however, if the stump is treated with retinoic acid, an entire limb will regrow in place of the missing hand! Similarly, in frog



Lab mouse (Mus) wound 17 days after wounding showing a collagenous scar covered by a uniform wound epithelium



Similar wound in spiny mouse (Acomys) after 17 days showing hair follicles regrowing from the wound epithelium and a far less collagenous wound bed with regenerating muscle below it



tadpoles, if the end of the tail is amputated and treated with retinoic acid, an entire new rear end will grow, giving a very bizarre-looking tadpole.

To investigate the role of retinoic acid, the Maden lab developed a transgenic axolotl, containing a gene for a fluorescent green pigment, which is only active in the presence of retinoic acid. They found that when a limb is amputated, the axolotl fluoresces green – indicating that retinoic acid is present – in two very specific places: the outer layer of tissue covering the blastema at the wound site, and the inner edge of the blastema where cells enter from nerves in the intact tissue. This explains why applying retinoic acid across the whole area confuses the system of regeneration into producing an entire new limb. A second key area of Professor Maden's current research is to identify the molecules with which retinoic acid interacts, to try and characterise the signalling pathway by which the nature of a regenerated limb is specified.

A MIRACULOUS MOUSE

The field of regenerative biology received a big impetus in 2012 when it was discovered, by one of Professor Maden's team, that not only 'lower' vertebrates, such as amphibians, can regenerate tissue, but also at least one mammal.

Ashley Seifert, now at the University of Kentucky, discovered the extraordinary African spiny mouse in Kenya. As protection against their main predators, snakes, spiny mice have weak skin which rips off sacrificially in the mouth of a snake, allowing the rest of the mouse to escape (albeit with a large wound). Instead of healing the wound with abnormal scar tissue, as most mammals do, the spiny mouse is able to regenerate perfect new skin, including hairs, sweat glands and different muscle layers – until you would never know it had been injured.

Professor Maden's colleagues have shown that the process of regeneration in the spiny mouse is remarkably similar to that in the axolotl, even down to the production of a blastema. His research group are now examining the mechanisms behind this perfect healing process. They think part of the answer lies in the mouse's immune system, which is dampened – preventing scar formation. Again, this reduction of the immune system is very similar to observations seen in axolotls.

Further research into the spiny mouse at the University of Florida has shown it can also regenerate skeletal muscle, cartilage, and even heart tissue following a heart attack. The implications for this, if the mechanism can be replicated in humans, are of course enor-mouse! It may sound like science fiction, but Professor Maden's carefully verified, yet innovative and ground-breaking science, is proven scientific fact.

Axolotls are able to tell when and how much of a limb has been amputated, and stimulate precise regrowth of the missing portion ”

Q&A

The axolotl is an incredibly unique animal in so many ways. Do you think its ability to regenerate is somehow linked to the fact that it retains its juvenile form into adulthood?

No, the ability of the axolotl to regenerate many organs is not related to its retention of its juvenile form for two reasons: 1) adult newts can regenerate as many organs as axolotls and they have metamorphosed into adults; 2) you can intentionally metamorphose axolotls by giving them thyroid powder and we showed that after metamorphosis they still regenerate as effectively as before metamorphosis. Once a regenerator always a regenerator!

How did you discover that retinoic acid has such significant effects upon regeneration?

The effects of vitamin A were first described by an Indian researcher, I. A. Niazi, in 1978 using frog tadpoles. He was interested in trying to delay metamorphosis to see if that would promote limb regeneration (frogs lose the ability to regenerate their limbs after metamorphosis). He produced bizarre looking extra limbs which were not well analysed and I looked at concentration effects, used different pure retinoids including retinoic acid to establish potency and analysed the structures in detail to show that increasing concentrations of retinoic acid could gradually change the positional information of cells in a gradually more proximal (towards the shoulder) direction.

Why do you think the signalling pathways involved in regeneration are so similar in such evolutionarily distinct animals as the axolotl and spiny mouse?

Because to regenerate structures such as a limb, evolution is unlikely to have evolved a completely new set of signals and signalling molecules. Instead it is more economical to use the signalling molecules that were used in the first place during development. Because all limbs from fish (fins) to mammals develop in essentially the same way then they are going to regenerate in essentially the same way as they will re-use their developmental pathways to regenerate. What needs to be discovered is the differences in the initial response to damage/amputation between a regenerator and a non-regenerator as I think the secret to regeneration lies here – how to kick start the process. The spiny mouse and axolotl can do this kick starting and the normal lab mouse and humans cannot.

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Do you find your research is controversial? Have you had interactions with animal rights activists, for example? How do you manage that?

No, I have not had any interactions, good or bad, with animal rights activists. Axolotls have a reputation as being cute as you can see from the large number of websites, You Tube videos etc. and people are just so amazed that they can regenerate their limbs that is does not seem to arouse hostile emotions. I also think that being able to regenerate would so obviously be of benefit to humans that most people can immediately see positive value in this research. Of course, axolotls are amphibians and very distant from humans which may mean that they do not arouse such emotional feelings as cats or dogs.

What steps need to be taken before your research can start to be applied to human treatments?

We need to find and identify the kick start molecules (see #3) which get spiny mice regenerating and are absent in the lab mouse so that we can administer them to the lab mouse and get them regenerating too. Then we'll begin a programme of applying this molecular knowledge and identified compounds to humans.

Detail

RESEARCH OBJECTIVES

Dr Maden's research utilises the ability of particular animals to regenerate organs and limbs. He and his research team investigate the science behind regeneration, aiming to adapt what they learn to try and stimulate regeneration in relevant human tissues.

FUNDING

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COLLABORATORS

• Edward Scott (University of Florida)
• Ashley Seifert (University of Kentucky)
• James Monaghan (Northeastern University, Boston)
• Ketan Patel (University of Reading, UK)
• David Fuller (University of Florida)
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BIO

Professor Maden received his PhD in Genetics from the University of Birmingham in 1976. Following this, he did a post-doc at the University of Sussex focusing on the nervous control of axolotl limb regeneration. Between 1989 and 2008, he worked at both King's College London and the MRC Centre for Developmental Neurobiology.

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