Biomaterials research in Porto, Portugal

Photo: Fotolia/Christian Pedant

Inspired by Sea Creatures

Sea urchins have a clear advantage over humans, at least when it comes to their connective tissue and its state-shifting abilities. With the knowledge of how they do it, Ana Lopes Ribeiro, wants to design biomaterials for regenerative medicine.

The sea is full of strange and wonderful creatures. Take the echinoderms, for example. This phylum consists of a very diverse set of animals including sea urchins, sea stars and sea cucumbers. Although quite different at first glance, they share many similar physiological features.

Miracle tissue

One of the coolest is their connective tissues' ability to reversibly change its state from solid to liquid in a matter of seconds. That is, for example, how the sea urchin stiffens the base of its spines in defense when someone's foot gets too close; or how the sea star can one moment be firmly stuck to a rock and soften up and dangle its arm at the next; or how sea cucumbers can "pour" their entire bodies into a hole when escaping a predator and then stiffen back up when danger is gone. This special kind of connective tissue is popularly called catch collagen or, more academically, mutable collagenous tissue (MCT).

MCT isn't confined to echinoderms. It is present in humans as well – more precisely, in pregnant women. The connective tissue that ties the pelvis together is stiff before and during the first months of pregnancy, but gets much softer towards the end, allowing the opening of the pelvis during childbirth. It then slowly returns to its stiffer state. The process is very similar to the stiffening and softening that happens in sea urchins, stars and cucumbers, although many magnitudes of order slower.

MCT's main roles are locomotion, the energy-efficient maintenance of posture (e.g. being stuck to a rock for a long time) and defense. There is evidence that MCT is also responsible for the remarkable regenerative capabilities found in echinoderms.

MCT's amazing properties have caught the eye of materials scientists, who are trying to understand the mechanisms behind its transformation and use this knowledge to engineer new biomaterials. One of the groups involved in MCT research is the group headed by Mário Barbosa, based at the Institute of biomedical engineering (INEB) in Porto, Portugal.

Going fishing

Ana Lopes Ribeiro is the first author of the group's latest paper. She has chosen the field of biomaterials because it lies at the intersection between materials science and biology. "Research in the biomaterials field was very attractive for me because of the potential for practical application in medicine and its multi-disciplinary nature", she says. And what better place to undertake interdisciplinary biomedical research than at INEB, which has a mission to improve human life through engineering. She joined the lab of her supervisor Mário Barbosa, whose passion for the sea turned his interest towards sea urchin MCT.

Ana Lopes Ribeiro (right) and her colleagues explore amazing abilities of sea urchins (far right).

"My thesis advisor became fascinated by sea urchins while he was fishing from the rocky beaches of northern Portugal. Fish is not very abundant there, so he had plenty of time to look for sea life around him. He became intrigued by the burrows created by the teeth of sea urchins in the hard granite rocks that are typical of our coast. He decided to visit Daniela Candia Carnevali, an expert in sea urchins and other echinoderms and a professor at the University of Milano. They talked about starting a joint project and the unique properties of mutable collagenous tissue came up as a totally new approach in the field of biomaterials for tissue regeneration", Ana recollects.

Something to chew on

And that is how Ana's project in sea urchin mutable collagenous tissues was born. She is currently finishing her PhD at the INEB, as a part of the MIMESIS (Marine Invertebrate Models & Engineered Substrates for Innovative bio-Scaffolds) project.

"At the moment, my research is based on the fundamental mechanisms of MCT mutability and its biochemistry and biomechanics. I'm looking for new concepts for the design and engineering of dynamic biomaterials for tissue regeneration", says Ana.

In their latest *PLoS ONE* paper, Ana and her colleagues have identified new morphological specifics involved in mutability (*PLoS ONE* 6(9): e24822). They used the compass depressor ligament (CDL), isolated from the sea urchin *Paracentrorus lividus*, as a model MCT tissue. The CDL is a part of the sea urchin chewing system (the mechanisms around the mouth opening at the bottom of the sea urchin). The stiffening and softening of the CDL controls the movement of the mouth and its five calcified teeth – these teeth are responsible for the burrows that first fascinated Ana's boss and turned his interest towards sea urchins. The structure of the CDL is similar to other MCTs – it consists of parallel collagen fibres and some microfibres, which actively participate in the stiffening/softening mechanism.

The paper itself presents detailed, state-of-the-art microscopic analysis of the CDL ligament. They have studied the morphology of the three mechanical states of the CDL – stiff, standard and soft, and found that collagen fibres get much closer when the collagen stiffens, while the distance doesn't change between the standard and soft states. They have also identified the microstructure of the ligament in all three states and identified the relationships between MCT's main components – collagen fibres, fibrilin microfibrils and the proteoglycan-rich gooey matrix.

Fighting scars

Additionally, Ana and her colleagues analysed the morphology of the juxtaligamental cells, which are present in all MCTs, and correlated them with the ligament's stiffness. These cells are rich in granules, which probably contain the effectors that mediate the stiffening of the MCTs.

They divided the granules into two types according to their electron density – the darker, mature ones and the lighter ones. When the CDL was in its normal state, cells contained more of the mature granules, while after changing to either soft or stiff forms the mature granules became depleted. They concluded that there are probably two types of morphologically indistinguishable granules, containing either stiffening or loosening factors, which regulate the transition from normal to one of the other two states.

"Our results help to define the functional role of this specialised extracellur matrix, which is strikingly similar to mammalian collagen found in human tendons, ligaments, cornea, skin and blood vessels", Ana says. The main difference is that when collagen fibres break or cross-link in the human collagen tissues, the process can't be reversed. That is why tendons reach only a portion of their strength after injury and why skin collagen tends to lose its strength and elasticity with time.

In contrast, the bonds between collagen fibrils in the MCT are constantly being broken and re-established with the help of stiffening and loosening agents. Knowing exactly what these are will be the key in engineering biomaterial tissues based on collagen with tunable mechanical properties, which is the group's ultimate goal.

This artificial tissue could be widely used in human medicine. "Possible applications of these dynamic biomaterials could be regeneration of soft connective tissues or therapeutic de-stiffening of scar tissue, which tends to contract", Ana explains.

When a tendon (e.g. Achilles tendon) is torn, surgeons connect the broken collagen fibres but a side-effect is the formation of scar tissue, which makes the tendon weaker and less elastic. Doctors could apply the treatment based on MCT biomaterials to torn tendons and with modulation using stiffening and loosening effectors it could form a scaffold for a healthy, scar-free recovery.

Having a facial

The MCT biomaterial would also have limitless possibilities in the cosmetic industry – just imagine a treatment that could return elasticity to facial collagen in a matter of minutes! But there is still a lot of work to be done before these treatments hit the stores.

Multidisciplinary science, according to Ana, is what Portugal does well. It has good universities and research institutes, which are very well connected and often share equipment and infrastructure. The position of young scientists in common with the rest of Europe, is quite tough, "There are not many postdoc grants offered by the national foundation of science and technology. Also, the chances for a young scientist to get a permanent research position are quite slim – many laboratories don't have permanent positions for investigators at all."

But Ana has a dream: "My dream is to apply the knowledge from my PhD to develop tunable biomaterials inspired by MCTs, which would be used in regenerative medicine. These materials will be able to change their mechanical and structural properties in response to external manipulation. In the long run, we hope to build a biomaterial which would be a dynamic scaffold and would change according to the physiological needs and molecular signals of the surrounding tissue."

She believes that, with the strong team that they have and the joint efforts of both biologists and engineers, they will make it. Fingers crossed!

Irena Hreljac