Curing osteoarthritis using button mushrooms

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According to the most recent data published by the World Health Organisation, it is estimated that between 2015 and 2050 the number of individuals aged sixty or over will have almost doubled, rising from 12% to 22%. This statistic hides a very real challenge because a progressively ageing population will have very real and practical consequences for daily life and the organisation of society in general. There was an urgent need to address the problem of an ageing population and to establish whether the health of this population was good or bad. For those individuals who fall into the latter category, there are some who suffer from osteoarthritis. This disease which affects the joints, affects 15% of the World's population. 18 million Europeans and one in three Belgians suffer from the disease. Today, it is possible to reduce pain or to compensate for the loss of mobility, but there is no real treatment for osteoarthritis. This fact underlines the importance of the thesis completed by Frédéric Oprenyeszk at the Bone and Cartilage Research Unit (UROC - Professor Yves Henrotin) of the University of Liege. The results obtained, which were published in PLOS ONE (1), show that chitosan, a vegetable compound naturally present in the stems of button mushrooms, is the key to regenerating the synovial fluid that is essential for healthy cartilage. In order to demonstrate this, diseased cells from patients who volunteered to take part in the study were used and placed in contact with a chitosan-enriched matrix.

This scientific advance is part of a broader fruitful collaboration between the academic world and industry(2). In order to fully understand the study conducted by Frédéric Oprenyeszk, of the Bone and Cartilage Research Unit (BCRU -Professor Yves Henrotin) it will be useful to take another look at osteoarthritis.

Suffering is not the only consequence of this pathology as the pain resulting from the condition is the expression of a progressive degeneration of the joints. This is why, as Professor Yves Henrotin, director of the Bone and Cartilage Research Unit explains, "with regard to degradation, it is estimated that, at 65 years of age and according to radiological criteria, around 70% of individuals show signs of osteoarthritis". This figure naturally increases with age. The condition has a very important socio-economic impact particularly in rich countries where ageing represents a real challenge for society. A recent study highlighted the fact that, in 2007, the total cost generated by this illness had reached 86.2 billion dollars. At a time when the benefits of an active retirement are constantly being evoked, osteoarthritis makes any such activity very difficult. In addition, research has shown that there is a link between osteoarthritis and the risk of developing other health problems such as diabetes, obesity and cardiovascular diseases. We now also know that inactivity is an added risk factor for fatalities among patients with cardiovascular diseases. It is therefore of paramount importance to ensure that there is a sufficient treatment for the condition. Unfortunately, there is no cure for the disease at the present time. We can only improve "algofunctional status, that is to say, reduce the pain and the effects of pain on movement. Currently only the symptoms are treated and not the structural and metabolic changes that occur during the course of the illness. Treatment of symptoms is mainly by means of pain killers and anti-inflammatories". These treatments have a major drawback: they can have fatal secondary effects(3). These medicines have an even greater effect given that osteoarthritis affects mainly elderly people who are already fragile. Due to weakened kidney function associated with ageing, elimination of toxic substances is slowed. The elderly are the biggest consumers of this type of medicine which they tend to use in the long-term to get relief from chronic pain such as that caused by osteoarthritis.
Hence the necessity of finding basic and healthy treatments which protect tissues or "change their metabolism in a positive way to stop them from deteriorating". Here, the word tissue particularly refers to cartilage which covers the joints but also the synovial membrane. It is the latter that undergoes an inflammatory reaction which is the root cause of the pain. The process by which a joint deteriorates can be summarised as follows: the joint and the extremities of the bone are covered by cartilage which in turn is essentially supplied with synovial fluid. This liquid is produced by the synovial membrane. When inflammation occurs, many factors are released into the joint and cause a deterioration in the synovial fluid which leads to degeneration of the cartilage and therefore the joint itself.

The objective of the study by Frédéric Oprenyeszk and the UROC team was to try to find a way to regenerate this "lubricant", to supply it with the elements necessary for its regeneration. To do this, it was necessary to develop a gel which could be substituted for the synovial fluid that is naturally present in the joints. This resulted in microscopic beads made from plant-sourced chitosan. Chitosan is a glycosaminoglycan rich in glucosamine. Its properties are remarkable: it is a buoyant and bioadhesive compound which means it can "adhere" to other tissues. There are two types of chitosan: it can be animal or plant-based. Animal-sourced chitosan is the most widely used commercially. It is taken from the shells of crustaceans such as prawns or squid(4) Plant-based chitosan is less widely used and is extracted from the stems of button mushrooms which makes this a very accessible source! It is no surprise that this method of obtaining chitosan is preferred by the Liege researchers. Professor Henrotin explains, "Firstly, it represents a new way of doing things. Secondly, it is hypoallergenic, plant-based chitosan does not
contain contaminants which can cause inflammatory or infectious reactions in the joint. Thirdly, it is a much more accessible and inexhaustible source. We are no longer confronted by the constraints linked to chitosan extraction from crustaceans which only allows for average levels of purity”. Significantly, one of the only producers of vegetable-sourced chitosan in the world, KitoZyme, is located in Liege (its biomedical activities have been transferred to Synolyne Pharma, a spin-off of the University of Liege). It was the perfect opportunity to create an interaction between the academic world and industry and it proved very successful.

When they come into contact with chitosan, diseased cells are treated

Central to the in vitro research carried out by Frédéric Oprenyeszk was a procedure patented by UROC: A matrix composed of alginate and enriched with chitosan in which diseases human cells known as chondrocytes, were encapsulated. The mixture of alginate and chitosan forms beads in which diseased cells were grown in culture for a period of 28 days either in chitosan or alginate beads. The purpose of this procedure was to demonstrate the added value of chitosan. Frédéric Oprenyeszk explains the procedure used in detail: “We began a process of cartilage digestion using enzymes that "break up" the cartilage and release the cells. These were then added to the chitosan alginate mixture and this mixture was then incorporated into a solution resulting in gelation which in turn resulted in the production of composites that were incorporated into chitosan alginate beads”. This is the patented procedure. "We are the first to succeed in creating chitosan beads into which cells can be introduced and brought into contact with the chitosan molecules".

Chitosan-alginate beads
While they were being grown in culture, the toxicity parameters were observed to verify whether the cells were still very much alive in the matrix. Three observation periods were observed: 3, 21 and 28 days. Histological studies were carried out as well as dosages of catabolic mediators (MMP-3, an enzyme involved in osteoarthritis which destroys cartilage), inflammatory markers, (IL-6, IL-8, PG E2) and anabolic mediators (hyaluronic acid and aggrecan).

The observation led to the conclusion that, in the presence of chitosan, there was a significant reduction in the inflammatory mediators IL-6, IL-8, PGE2 and the catabolic mediators MMP-3. Conversely, an increase in the anabolic mediators, hyaluronic acid and aggrecan, components of the matrix. "It is essential, in the context of physiopathology that the fact of adding chitosan makes it possible to have more components in the matrix and to inhibit the main inflammatory mediators that are responsible for the degradation of cartilage". Therefore IL-6 is as active with regard to the components as it is with regard to the synovial membrane resulting in degradation of the joint. The environment created in the beads is favourable to the cell and allows the cell to start glycosaminoglycan synthesis again.

The added value of chitosan in therefore unquestionable. In addition, apart from the use of this component, the method used is distinct from those that have generally been used to produce these kinds of beads. Placing diseased cells in contact with chitosan makes it possible to treat them. But that's not all. "We have demonstrated that the beads could be formidable cell transporters. We could well imagine placing medicinal cells into the beads to later inject them into the joints and engage in cell therapy by this means. It would therefore be necessary to see how stem cells which have been genetically modified to become medicaments behave under the same conditions. If the tests were seen to be positive, biotherapy treatments would become available".

For the moment, the first application will be by viscosupplementation. Professor Henrotin speaks of a "revolution "in this area. "Up to now we injected gels made of hyaluronic acid. But the problem is that this acid becomes absorbed and disappears very quickly in the joints. In a few days or even a few hours the individual is no longer able to perform. Once our gel is injected it will last longer because chitosan does not break down as quickly and will also protect the cartilage. So we will not only be able to reduce the pain but also improve the mechanical properties of the joint: less friction, less applied deleterious forces applied to the cartilage and less compression of the cartilage".

The next step will be the clinical trials which should begin soon at the start of 2016. A contract has just been signed with Artialis(6). "We hope that our first product will be on the market in2017".
(1) Chitosan enriched three-dimensional matrix reduces inflammatory and catabolic mediators production by human chondrocytes, Oprenyeszk Frédéric, Sanchez Christelle, Dubuc Jean-Emile, Maquet Véronique, Henrist Catherine, Compère Philippe, Henrotin Yves, in PLOS ONE, 2015, Ref. ORBI : $\text{http://hdl.handle.net/2268/182363}$

(2) Two patents have been issued for the joint research conducted by the respective teams of Professor Henrotin, and the firm KitoZyme, producers of vegetable-sourced chitosan. Consequently, in 2012, a spin-off, Synolyne, was created by Professor Henrotin to use these patents. Finally, in 2014, KitoZyme and Synolyne merged to create Synolyne Pharma, one of the world leaders in the medical transformation of plant-sourced chitosan.

(3) Paracetamol for example, can cause problems of the blood, gastro-intestinal and/or kidney complications, hepatic or tubular necroses. Generally speaking, the secondary effects of anti-inflammatories affect the digestive tract, the liver or kidneys.

(3) In 2003, there were around 65 producers of chitosan in the world. The great majority of them (93%) produce animal-sourced chitosan. This is a rapidly growing market because of the numerous applications of chitosan. For example, it seems to be a "fat binder", which is the reason why it is sold in the form of a pill for weight-loss. Chitosan can also act as fibre for digestion, as a preservative or even as an anti-bacterial agent. There is therefore no shortage of industrial possibilities for the product.

(4) Synolyne Pharma has its own GMP area (Good Manufacturing Practices), a necessary prelude to clinical trials. The company has the capability to produce chitosan, gels hydrogels and plant-based chitosan.

(6) First spin-off created by Professor Henrotin in 2010. It is specialised in clinical trials for treatment of diseases affecting bones and muscles etc.