

Nano3Bio: Tapping the potential of chitosans

Chitosans are an amazing class of functional biopolymers, perhaps the most versatile and most promising one. They can be used in medicine, in agriculture, in food industry, in cosmetics, in water and waste water purification, in paper and textile industries – and in biotechnology.

Traditional chitosan and new market requirements

Chitosan can be produced rather easily from chitin, one of the most abundant biopolymers that is wide-spread in nature, e.g. giving strength to insect shells, shrimp and crab carapaces. Thus, chitin and chitosans are renewable resources of almost unlimited availability. Waste material from the shrimp fisheries can be transformed into a valuable product of immense potential.

However, there is a catch, as with all things that sound too good to be true. Chitosan has been a ‘promising’ biopolymer for almost fifty years. But initial promises could not be kept. Results on bioactivities reported in the scientific literature did not lead to the development of products because the results were not reliably reproducible. Industry understandably was disappointed and lost interest. Today, two decades of fundamental research on structure-function relationships have led to the development of well-defined chitosans with known physico-chemical properties and reliable biological functionalities. These second generation chitosans are ready for the markets – to be used for the development of reliable applications and successful products. As a consequence, in 2014, the European Commission has registered chitosan hydrochloride as a ‘basic substance’ which can now be used e.g. in agricultural products without the need for lengthy and costly toxicity studies and registration processes.

But even the well-defined second generation chitosans are not perfectly suitable for all applications, in particular not in sensitive fields such as health care, pharmacology, and biomedicine. Here, the animal origin of chitosan is a hin-

drance to market entry, as is the – real or assumed – danger of allergen or even viral contamination. This is where the Nano3Bio project sets in, aiming at the development of biotechnological production processes for well-defined, third generation chitosans.

What chitosans can do for you

- ▶ Some chitosan sponges can stop bleeding, and some chitosan-based dressings can support scar-free healing even of chronic or large-scale wounds.
- ▶ Some chitosan nanoparticles can transport drugs across cellular barriers, including the blood-brain barrier, and DNA or RNA into cells.
- ▶ Some chitosans can stimulate the immune system, including that of animals, so that they can be used as a feed additive to reduce the use of antibiotics.
- ▶ Some chitosans can promote plant growth and induce disease resistance and stress tolerance in plants by strengthening the plant’s own defensive system.
- ▶ Some chitosans can form transparent films to be used e.g. as food packaging, keeping fruits and vegetable fresh and preventing spoilage.
- ▶ Some chitosans can stabilize creams and shampoos and at the same time, preserve them.
- ▶ Some chitosans can clean drinking water, filter wine, and remove proteins or heavy metals from the waste water of breweries or industry.
- ▶ Some chitosan-lined papers are more tear resistant and easier printable, and impregnating textiles with some chitosan can prevent body odour and mould stains.
- ▶ Some chitosans can be used as a biocompatible surface to cultivate human cells, e.g. as a 3D matrix in organ models.

Furthermore there are a multitude of other possible applications of this versatile class of biopolymers. Chitosans are completely non-toxic to plants, animals, and humans. They are non-allergenic and easily degraded in the environment.



New approach, advanced opportunities

The Nano3Bio project convenes an international team of researchers from universities, research centres, and companies around Europe and India, joining forces to make a dream come true: the biotechnological production of third generation chitosans.

1 **First generation chitosans** were rather poorly defined polymer mixtures of varying purity and varying composition. While these chitosans were suitable for technical applications such as water treatment that make use of the material properties of chitosans, they were mostly unfit for the development of successfully marketable products based on their bioactivities. These chitosans were dominating the market for decades, and they are still widespread today.

2 Today's **second generation chitosans** are well-defined in terms of their degrees of polymerisation and acetylation. They are thus more suitable for the development of reliable high value products due to known molecular structure-function relationships. These chitosans, and products based on them, are now increasingly appearing on the markets.

3 Future **third generation chitosans** will be even less polydisperse, or even monodisperse in the case of oligomers, with defined, non-random patterns of acetylation, clearly defined biological activities, and known cellular modes of action. These chitosans will create new market opportunities in the future.

Nano3Bio follows two approaches towards biotechnological production of third generation chitosans: An in vitro bio-refinery approach and an in vivo cell factory approach. Both approaches will make use of nature's own tools for chitosan production, namely enzymes such as chitin synthases and chitin deacetylases. Nano3Bio aims to identify such enzymes from widely different sources, heterologously expressing their genes in suitable production systems to yield purified recombinant enzymes. Bacterial, fungal, and algal chitin synthase genes will be used to synthesize chitin oligomers and polymers in vivo in microorganisms and micro-algae. The addition of suitable chitin deacetylase genes will lead to the production of partially

acetylated chitosan oligomers and, hopefully, also polymers. Alternatively Nano3Bio can use the recombinant chitin deacetylases in vitro on conventionally or biotechnologically produced chitin oligomers and polymers. In both cases, the project will obtain chitosans with known and defined, non-random patterns of acetylation. These will then be compared to their conventional counterparts, to benchmark their physico-chemical properties and biological functionalities against the best performing chitosans available today.

International capacity aggregated, breakthrough aimed

Towards this ambitious goal, leading experts from 22 universities, research institutes, and companies from Belgium, Denmark, France, Germany, India, the Netherlands, Spain, and Sweden have formed the Nano3Bio consortium, led by Prof. Bruno Moerschbacher from the University of Münster. Nano3Bio is the highlight of a sequence of European and international projects, which helped to build knowledge on chitosans for more than 15 years, first laying the path, then paving the way towards today's successful second generation chitosans. The Nano3Bio project intends to extend this success story towards third generation chitosans that will be of even higher quality, that are novel in terms of their structures and functions, and that shall open a door to new applications, new products, and new markets.



Learn more about Nano3Bio:

www.nano3bio.eu