Antibacterial coating

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How can steel manufacturers and biologists work together? Among other things, they can cover steel with an antibacterial coating. The Biocoat programme, which began in 2006, has recently been rewarded with several publications (1) and patent registrations. This has led to a technological innovation platform: Symbiose Biomaterials. Why? To expand beyond the steel sector and extend Biocoat’s assets to other media and new applications.

It was with many questions in mind that Professor Joseph Martial, director of the University of Liège's 'biology and molecular genetics' research unit at the time, went to a meeting in 2004 concerning the redeployment of Liège's steel industry. Up until then, biologists and steel manufacturers had never met and knew absolutely nothing about each other. But among his researchers, Professor Martial had a team in ULg's GIGA-R Biology and Molecular Genetics unit led by Cécile Van De Weerdt, now a project leader. And this is who Joseph Martial turned to after the meeting: "And what if we put proteins on steel?" And so began a good deal of spadework for Cécile Van De Weerdt. "I realised", she remembers, "that there were a lot of publications in the United States about molecular biomimetics. These studies observed how nature formed materials, structures adapted to particular environments. Of course, the goal was to succeed in imitating this way of doing things. If you take a closer look, you'll find that there are proteins that assemble inorganic material to create original structures. A good example of this know-how is mother-of-pearl, which is a composite endowed with exceptional properties as yet unequalled in synthetic composites. The proteins/materials association therefore made sense. Even more so considering that nature constructs materials using foundation stones that aren't toxic, during gentle procedures, i.e. at room temperature, most often in an aqueous solution, without emitting any pollutants... The dream of every industrialist, you could say."

The steel company Arcelor (not yet Mittal at the time) did indeed show an interest in these possibilities. Biologists and steel manufacturers then began to meet to get to know each other, with Catherine Archambeau, research engineer at Arcelor, taking responsibility for the project on the industrial side. The aim of these preliminary meetings was also to determine whether there was a real industrial need which the scientists could meet. Several possibilities came to light and several functionalities were researched, but it was antibacterial coating that was chosen. The Walloon Region subsequently supported the project, appreciating the fact that two of Wallonia’s strongest areas of activity, an old one (the steel industry) and an emerging one (biotechnologies), were joining forces. A PPP (public-private partnership), called Biocoat, was created in January 2006. Two other entities from the University of Liège then joined the programme: first of all, the laboratory of Professor Jérôme, and in particular Christophe Detrembleur (FNRS senior research associate, Center for Education and Research on Macromolecules); followed by that of Professor Anne-Sophie Duwez (Nanochemistry and Molecular Systems). Why? To create an innovative coating on steel with antibacterial properties by associating biomolecules and polymers, while implementing the greenest technologies possible and respecting the technical and economic constraints dictated by Arcelor. To put it plainly, the coating had to be made in a minimum number of steps from aqueous based solutions.
Nature provided the scientists with models on two levels: for the glue and the antibacterial substance. The choice of glue was quickly resolved owing to the great number of studies on this subject. Marine molluscs, especially the mussel, secrete a DOPA-based adhesive which allows it to stick to almost any kind of surface; Christophe Detrembleur's team took this active ingredient and inserted it into polymers. They tested different architectures until they found the best "glue", i.e. the anchoring layer that would allow them to bind the antibacterial coating to steel.
However, the most innovative aspect of the Biocoat programme is the use of biomolecules as an antibacterial agent. Cécile Van de Weerdt: "Our skin is involved in our immune system; it is equipped with a defence mechanism. This is even truer of batrachians, which often live in putrid environments. Their defence system is armed with ancestral molecules against which there is no known resistance. They are small, often positively-charged peptides because bacterial walls are negatively charged. They work by directly attacking the walls of the bacteria; these aren't targeted attacks unlike our antibiotics, which attack the construction of the wall in a far more targeted manner by attempting to block this mechanism. The enzyme that recognises the antibiotic just has to change slightly for the bacteria to bypass the problem and adapt to its attacker. This isn't possible with the batrachians' peptides. These molecules have yet another advantage. While small doses of these antibacterials are active against bacterial membranes, in larger doses, they are active against cell membranes such as human cells and, more specifically, cancerous cells. Antimicrobial peptides are therefore of great interest to researchers. New peptides are being entered into international databases every day."

**Silver nanoparticles**

However, before developing a bio-inspired coating - the great innovation in the Biocoat project - it was necessary to master all the techniques. This was easier to achieve with a more classic, better known but non-biological antibacterial: silver nanoparticles. "One of the major functionalities at the core of our project", Christophe Detrembleur explains, "aimed to provide industrial steel with permanent broad-spectrum antibacterial properties. Numerous methods can be used to provide surfaces with these types of properties but not many of them can be transposed to an industrial scale because it is too complicated and/or they use expensive compounds, or large quantities of toxic organic solvents. Developing a new concept of functional coating seemed important to us here. In concrete terms, the challenge was to develop an aqueous solution of a multifunctional polymer that combined bio-inspired adhesive and broad-spectrum antibacterial properties. In order to reduce the costs of the process, we envisaged applying the coating in thin, highly active layers..."
(several tens of nanometres) in a minimum number of steps. In 2009, we published a paper on such a highly efficient bio-inspired approach (J. Mater. Chem. 2009, 19, 4117-4125)."

So that's the end of the research? Not at all. First of all because the method developed required yet more successive applications of the active ingredients. Which, of course, isn't appealing to manufacturers. "Therefore, we wanted to simplify it", Christophe Detrembleur explains, "by using the same basic products but by changing the method of applying them. We cleverly pre-assembled the active ingredients (bio-inspired hydrosoluble polymers for the adhesive properties and antibacterial silver nanoparticles for antibacterial properties) in water in order to have a 'ready-to-use' solution, i.e. ready to be applied to the substrate by simply dipping or spraying it. The system turned out to be highly effective and a lot faster than the previous one." This research was published recently (Langmuir 2012, 28, 7233-7241).

Research that didn't stop there because the antibacterial substance wasn't organic and had no lasting characteristics: silver is expensive and polluting and as for the nano form, it raises questions if not fears. Not forgetting the real point of the matter: that the antibacterial properties of such assemblies aren't permanent. The silver nanoparticles actually migrate off the film covering the steel to go and kill the bacteria. Once all the nanoparticles have left the film, it no longer has any antibacterial properties. The following step in the research therefore consisted of using antibacterial peptides that wouldn't be rejected by the coating so that activity is maintained. "Grafting peptides wasn't easy", Christophe Detrembleur remembers. "They are sensitive molecules that mustn't be heated, and you can't use organic solvent you want. The goal was to work at room temperature in water." But mission accomplished here too (J. Mater. Chem. 2011, 21, 7901-7904).

**Ensuring activity in the long term**

The researchers from Liège have now reached the third stage: ensuring activity in the long term. The active ingredient applied to the surface
mustn't denature under the effect of light, for instance. Peptides are biomolecules and to be efficient, they must have a certain structure which, of course, they retain. But here too, the tests were positive. Even better, they showed that cutting steel sheets (which would be the norm when it comes to constructing objects (furniture, tables, fridges, etc.) from coated steel!) doesn't rip off the film and that the antimicrobial activity is maintained. Finally, it should be noted that contrary to the other solutions proposed, the researchers from Liège approached the antibacterial problem in a multifunctional way: to prevent the adhesion of bacteria on the surface, kill the bacteria or prevent the formation of biofilm (once on the surface, the bacteria produce a polysaccharide film that allows them to adhere even better). "We've now developed a 'ready-to-use' solution", Christophe Detrembleur points out, "that allows the functionalisation of substrates by biomolecules (antibiofilm enzymes, antibacterial peptides, etc.). We first studied these three functions separately; now, we have to unite them on the same surface film. The technology, the base and the platform are the same. Only the final layer of active film applied to the surface is different (Adv. Funct. Mater. 2012, DOI: 10.1002/adfm.201201106). Either an antibacterial molecule, or antibiofilm, or anti-adhesive. The three were validated separately; now we'd like to have the three functions together, on the same film."

The creation of Symbiose Biomaterials

The Biocoat programme has now ended. At least the first version of it. It has now been reoriented towards broader applications. Cécile Van De Weerdt explains, "Mussels stuck to all surfaces, including Teflon! We were therefore tempted to think that everything we developed with our biomimetic glue within the framework of the Biocoat project could be extended to other materials. The Walloon Region and the University thus decided to create a technological innovation platform called Symbiose Biomaterials, which will be hosted by MecaTech, the Walloon centre of competitive excellence in mechanical engineering. Its aim is to accelerate the marketing of developments resulting from the Biocoat research. Of course, other university laboratories, research centres and businesses will be associated with it." To go in which direction? Those in charge of the project have defined three possibilities. The first one is obviously related to everything to do with antimicrobial coatings for hospitals, butchers, cold rooms, etc. But this time, regardless of the surface, whether it be steel, glass or something else: we simply change the biomolecule but we keep the active ingredient; this is one of the interests of the basic choice of DOPA as glue. The second direction chosen is still coating, but specific to the medical implants sector. The advances in medicine can be seen in the increasing use of implants, or 'spare parts', in our bodies. Covering these implants with an antimicrobial coating will undoubtedly help to prevent rejections and infections. The third direction selected is undoubtedly the most innovative, and even the most promising. Biocoat has allowed the researchers from Liège to familiarise themselves with GEPI technology (Genetically Engineered Polupeptide for Inorganics). In other words, engineering and constructing small made-to-measure peptides, and using proteins to create an assembly controlled on a nanomolecular level. As its name indicates, GEPI is a polypeptide designed through genetic engineering that specifically recognises an inorganic material. Just what you need for nanodetection, sorting and the manipulation of nano objects. A patent was registered for a peptide capable of fishing out nanopowders from the middle of a pile. Or, another example, recovering rare earth elements from waste, whose value is continuously increasing. Or using them in filters to find a contaminant such as nanoparticles of TiO2 without filtering the water. Symbiose Biomaterials has an exciting future ahead of it!

(1) Among the most recent:

A green and bio-inspired process to afford durable antibiofilm properties to stainless steel; Faure, Emilie et al. In *Biofouling* 2012, 28, 719-728.

