DEFENCE NANOSYSTEMS FOR PLANTS AND ANIMALS

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Abstract

In nature, we can find a variety of systems by which plants and animals are defending against dangers coming from their surroundings. The aim of this contribution is to show three different types of nanostructures, which are one, two or three-dimensional and show their function in defense of objects in nature. The first type is nanoparticles. In specific types of plants, when they are growing they make primary nanoparticles which are made of silicon dioxide with a size from one till tens of nanometres. These structures then form a bigger globular formation. Moreover, these formations make up layers, which serve as a compact barrier against microorganisms. The second type of nanostructures has a prolonged shape with a diameter size of tens of nanometres and a length of hundreds of nanometres. It could be found on plant surfaces. These structures have wax characteristics which allow them to be superhydrophobic. From these kinds of surfaces even dirt can be washed away, as well as pathogens which stick to drops of water. The third type of nanostructure is a three-dimensional nanoformation in a conical shape, which can be found on the wings of cicada. The ratio of these formations is approximately 150 nanometres at the base and 30 nanometres at the top, with a height of 300 nanometres. These nanocones have the ability to disturb cell walls of bacteria which are sticking to it. The breaching of these cell walls is caused by physical-mechanical processes without using chemical substances.

Keywords: Natural objects, nanostructures, defense mechanisms

1. INTRODUCTION

Development of synthetic nanostructure in modern materials and technologies has been the matter of recent years. Nanostructure systems, which are found in natural objects have developed for decades incomparably longer. The ability to artificially copy these systems at conditions which are found in nature is still unreachable to us. Surface structures defense mechanisms that are used in nature are often created as hierarchical structures, based on nanoparticles present within long-form nanoscale or three-dimensional nanocones shapes.

In many plant types, we can find surface structures based on silicon dioxide. Made out layers serve as compact, impermeable barriers for the intersection of unwanted organisms. These layers are made of cells incrusted by nanoparticles made of silicon dioxide. They normally have a spherical shape and a size of about 20 nanometres with a tendency to aggregate in bigger globular shapes with a size of 100 nanometres. They interconnect each other by fine organic fibres [1]. Apart from silicon dioxide they also contain calcium, potassium, manganese, phosphor and very often aluminium [2]. Cells with incrustation - phytoliths have a characteristic shape depending on the type of plant. Phytoliths decompose very slowly in normal soil conditions and their lifetime can reach up to a thousand years [3]. Nanoparticles are formed in the process of photosynthesis from a solution of ortho-silicic acid, which plants absorb from the soil environment [4].

The most typical function of nanostructures found on the surface of a plant is to maintain hydrophobicity, which is for many plants essential. As it has the function of eliminating the water film on which unwanted organisms could dwell, e.g. spores of mould and bacteria. Structures which are from this point of view the most effective have a protracted stick shape or hollowed tubule. They are often placed on the micrometre-sized convex surface cells. With this, they ensure specific behaviour of their surfaces - hydrophobicity and superhydrophobicity or self-cleaning ability [5]. Mechanisms of these functions are based on water surface tension, hierarchical structure and chemical composition which arise on the bases of N-nonacosan as secondary metabolites in the process of photosynthesis [6].

The most frequent spatial nanostructures have the shape of a cone. They are placed geometrically compact next to each other to form a homogenous plane [7]. To the present day, the most studied function of nanocones is their specific ability to breach surface microorganisms, so to say to thin out their membrane till it erupts. The mechanism of this destruction process is based on surface tension and capillary forces in nanostructures. The studied nanocones are found e.g. in wings of cicadas or dragonflies [8,9].

2. EXPERIMENT

Three typical examples were selected to describe properties above mentioned:

- *Equisetum arvense* as a plant object, whose surface is a protected barrier (strengthened structure) made from nanoparticles of silicon dioxide,
- leafs of *Nelumbo nucifera* as an example of a superhydrophobic surface with self-cleaning abilities,
- wings of *Lyristes plebejus* with a surface covered with nanocones decomposing microorganisms.

For the evaluation of the size and distribution of the nanostructures, scanning electron microscope FE SEM Zeiss Ultra Plus was used. Before the use of SEM, samples were coated with a thin layer (nanometer units) of platinum. The evaluation of chemical composition EDX analysis was done with Oxford Aztec Energy detector. For precise chemical analysis, a method of ICP-OES (inductively coupled plasma emission spectrometry) was used. Hydrogen peroxide 30% p.a. was used to decompose the *Equisetum arvense*. For the evaluation of the effectiveness of the wing structure as a mean to eliminate gram negative bacteria *(Pseudomonas putida)*, the spectrofotometrical fluorescence method was used. The preparation of samples was different with each sample type as samples were quite different and targeted observation. For study and characterisation of silica nanoparticles in *Equisetum arvense*, observed parts were decomposed in a 30% solution of hydrogen peroxide p.a. The decomposition process took place in a closed glass container for a time of 6 months. Samples were observed visually and samples which were white after the end of the cycle were washed with distilled water and dried at laboratory temperature. Samples were analysed with SEM and EDX analysis. For the study and characterization of prolonged nanoshapes found on the leaves of *Nelumbo nucifera,* SEM analysis was used. The shape, distribution and the homogeneity of nanocones on the wings of *Lyristes plebejus* was analysed by SEM analysis as well as the number of microorganisms.

3. RESULTS AND DISCUSSION

Samples were prepared by decomposing *Equisetum arvense* in hydrogen peroxide and then evaluating by SEM and EDX analysis. Compact layer made of cells-phytoliths (guarded by a shell of silicon dioxide particles) with a size of 100 micrometres and a width of 20 micrometres **(Figure 1).** Individual cells fit to each other **(Figure 2)** and they are made of globular shapes which have a size of 60 nanometres. These shapes are made from oblong nanoparticles of silicon dioxide with a size from 8 to 10 nanometres [10]. After the decomposition of the sample in hydrogen peroxide it was identified that there is a different morphology of the inner and outer side of the crust. Even after a relatively long decomposition time, bigger or smaller amounts of accompanied ions were detected in the samples. Especially calcium, magnesium, and potassium which play an important role in the growth of the plant **(Figure 2)**. The presence of aluminium is associated with the plants ability to accept this ion in presence of silicon dioxide and it tends to make secondary aluminosilicate [11]. The concentration of these elements are tens to thousands ppm which tends to differ depending on the location of growth of the plant. Apart from essential elements and other mentioned elements, carbon waste was always identified from 5 to 20 at. % **(Figure 2)**. Carbon which remains in the sample cannot be removed during the process of hydrogen peroxide decomposition as it can by burning down or by usage of microwave reactor [12]. The amount of carbon waste depends on the length of decomposition. The amount of carbon in phytoliths relates with the ability of the plant to bond carbon dioxide to its structures and keep it for a longer time [13].

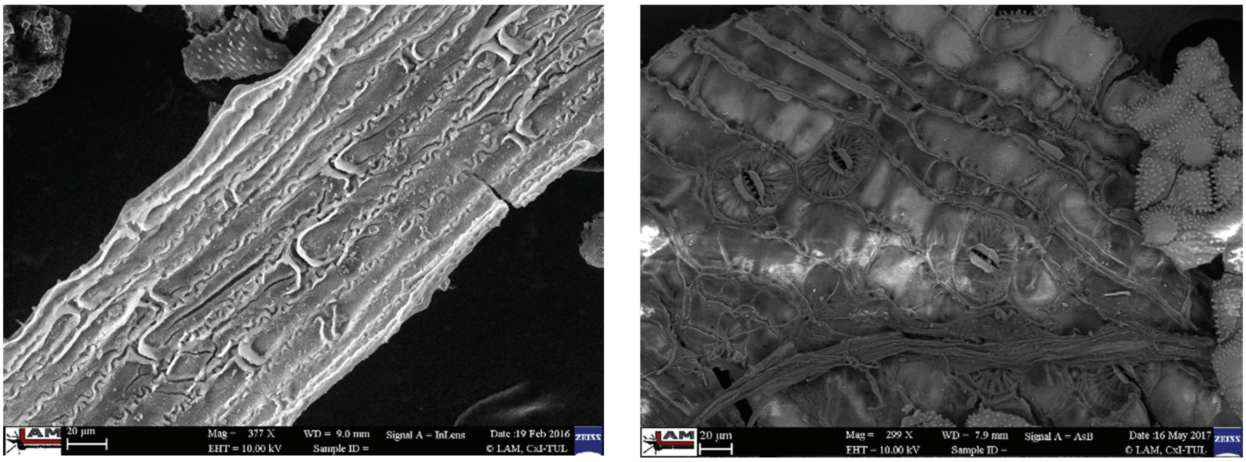

Figure 1 The image of the *Equisetum arvense* inner wall built from the compact silicon dioxide crust (left). The inner wall of the crust with visible circular shapes surrounding the vents. The morphologically different outer part of the crust can be seen in the image at the top right (right)

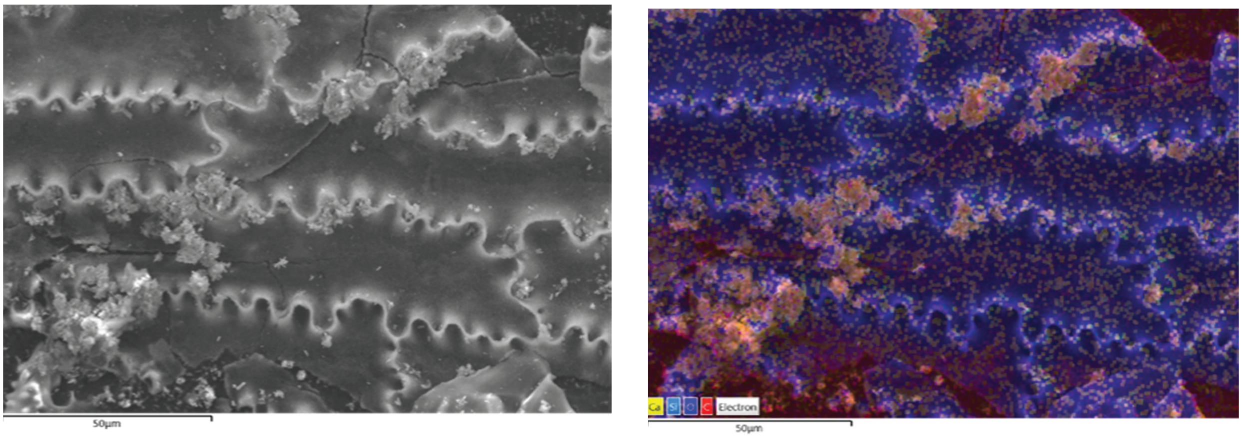

Figure 2 The image of the inner wall of the crust consisting of individual incrusted cells (left) and EDX analysis with apparent inhomogeneous calcium ion exclusion at the boundaries of the formations (right)

Leaf surface of *Nelumbo nucifera* was described based on images made by a scanning electron microscope. The leaf was dried out and its surface was coated with a layer of platinum up to 4 nanometres, which did not affect the geometry of the studied structure because plant nanostructures are bigger. Dehydration did not disrupt convex cells, therefore, its size is well observable **(Figure 3)**. At the base, the cell diameter is from 8 to 10 micrometres and at a peak of about 5 micrometres. The height is in range of 10 to 12 micrometres **(Figure 4)**. These cells are covered by hollowed tubes based on N-nonacosan [6]. The outer diameter of the tubes is from 110 to 130 nanometres, with an inner diameter of 50-60 nanometres, the length of it is approximately 600 nanometres. Based on simulated calculations, it was found that there are about six million of these tubes [14]. The tubes hold air between each other. Due to the surface structure of leaves and the physical reason like the surface tension of the water the contact area between a leaf and water droplet is minimal. Dirt which would stain the leaf is immediately bundled up with the drop of water and it’s taken away from the leaf. As with the study of the action of water on these types of surfaces we still have to consider that in nature these systems are dynamic.

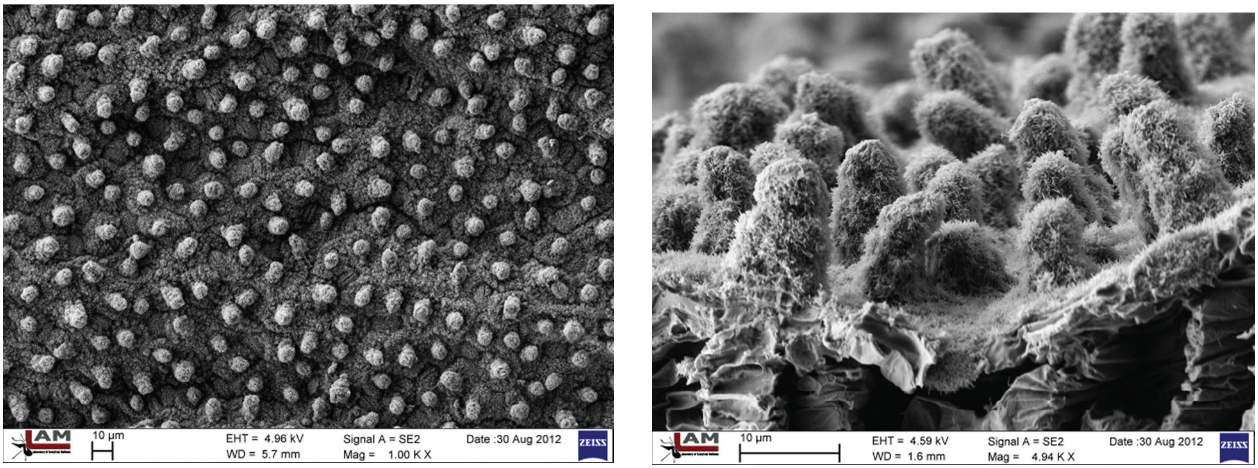

Figure 3 An overview image of *Nelumbo nucifera* leaf surface with the regularly spaced convex cells on its surface (left). An image of the convex cell arrangement (right)

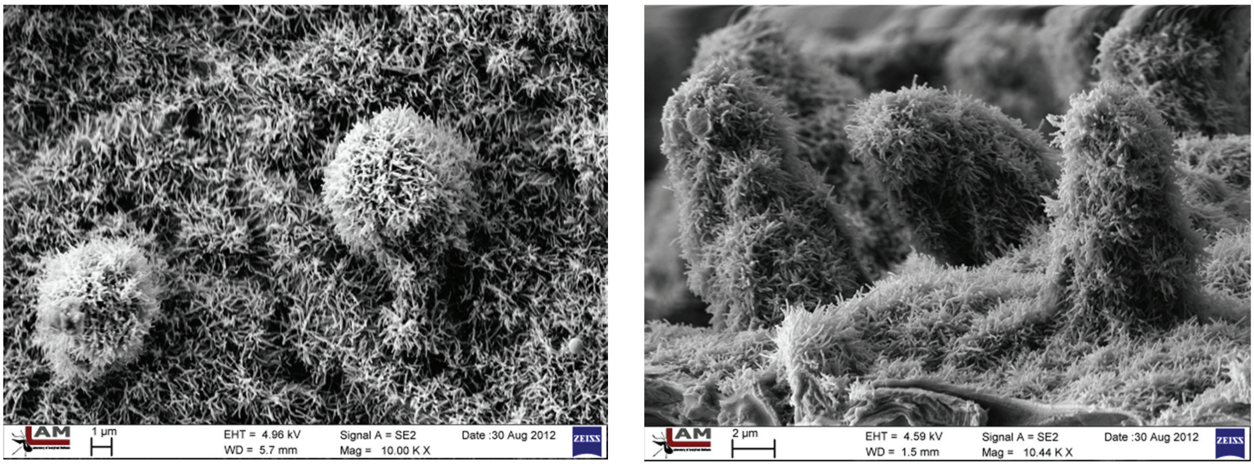

Figure 4 Detailed images of the surface of convex cells coated with wax nanotubes

Wings of *Lyristes plebejus* were evaluated based on images from the scanning electron microscope. Wings are transparent and their thickness is around 10 micrometres. From the point of nutrition and mechanical stability, they are reinforced by veins made of chitin [15]. The surface of the wings has long conical formations, which make a homogeneous area **(Figure 5)**. The dimensions of the cones are as follows: the diameter at the base of the cone is about 150 nanometres, the height is about 320 nanometres **(Figure 6)**. On the surface of the wing, despite its hydrophobic behaviour, immobilization of microorganisms can occur **(Figure 6)**. Microorganisms are sucked in by capillary forces between nanocones. Based on these processes, the membrane of the microorganisms between the cones is stretched and weakened until it eventually breaks. By this physical-mechanical way and without the use of chemicals, the microorganisms are disturbed and disposed of [7, 8, 9]. In the initial experiment, wings were subjected to an analysis of their ability to dispose of *Pseudomonas putida* (Gram-negative bacteria) as described above. Despite the published results, the ability of the "conical" structure to dispose of the microorganisms in the manner described above was not sufficiently proved. Intact and immobile organisms are visible on the scanning electron microscope image of the surface of the cicada wing **(Figure 6)**.

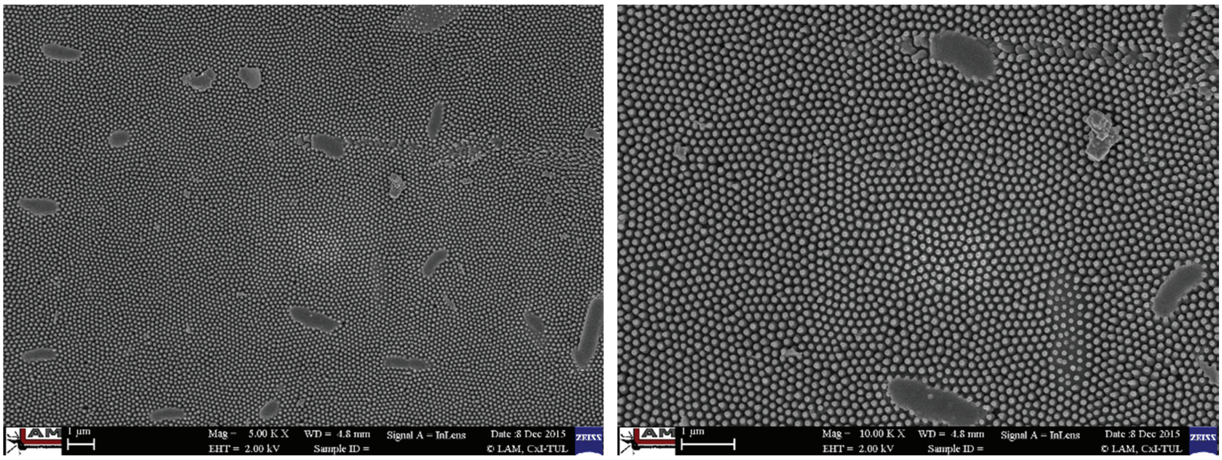

Figure 5 Overview images of the cicada wing surface

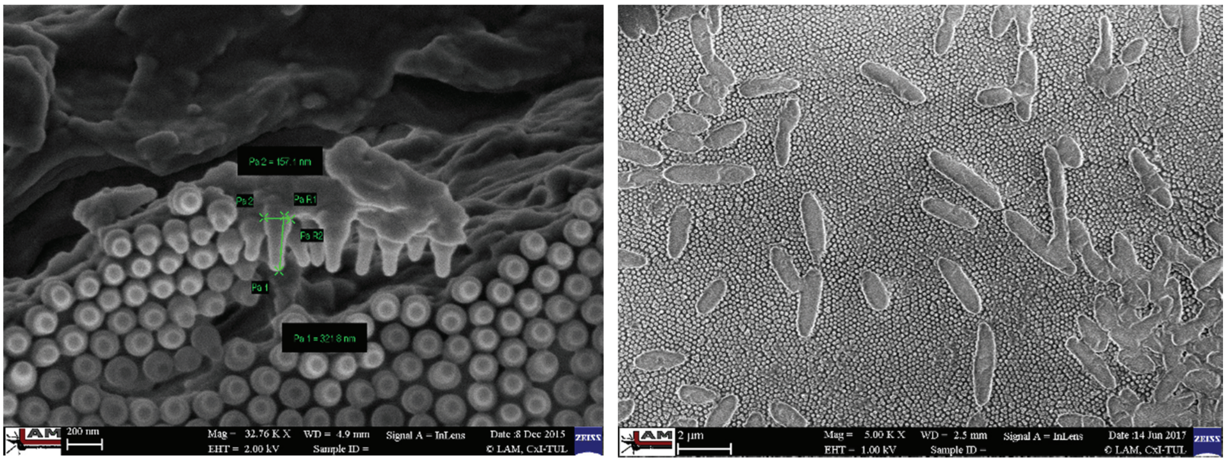

Figure 6 The detailed image of the shape, size, and arrangement of cones on the cicada wing surface (left). The presence of *Pseudomonas putida* on the cicada wing surface 24 hours after exposure (right)

4. CONCLUSION

Based on executed studies, it is obvious, that natural objects have many possibilities in which they protect themselves from the outside environment. For that, nanostructures are used in the form of 1D, 2D, and 3D, which are made based on biochemical processes and the process of photosynthesis. Despite the diversity of the described nanostructure function, they function on principles and mechanisms based on simple physical phenomena. As can be seen, natural systems exploit the potential of nanostructures in the same way as nanotechnology in biological processes. The importance of studying natural structures and their functions lies in trying to understand these mechanisms and transfer them into practical use. The above-mentioned structures, principles, and mechanisms represent a modern way to address existing and future problems associated with the environmental impacts of human activity.

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