ANTIMICROBIAL MATERIALS, COATINGS AND BIOMIMETIC SURFACES WITH MODIFIED MICROTOGRAPHY TO CONTROL MICROBIAL FOULING OF PRODUCT CONTACT SURFACES WITHIN FOOD PROCESSING EQUIPMENT: LEGISLATION, REQUIREMENTS, EFFECTIVENESS AND CHALLENGES

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Abstract

Pathogens and spoiling microorganisms which adhere to interior equipment surfaces may be transferred to food products during their processing. To improve the hygiene during food processing operations, either inorganic or organic antimicrobials are added to various materials of construction used in the manufacturing of food processing equipment.

In the USA, antimicrobials used in materials of construction must be registered with EPA under the Federal Insecticide, Fungicide, and Rodenticide Act, while in Europe biocidal products, included antimicrobial food contact materials, are subjected to Regulation (EU) No 528/2012. However, residual limits of antimicrobials released from food contact materials in food are still to be set via the existing European food contact material regulations. An optimal antimicrobial component for food contact applications should have the following properties: broad-spectrum antimicrobial activity towards undesirable microorganisms and without killing of beneficial microorganisms, fast-acting at low concentration, long-lasting effect or possibility to regenerate antimicrobial qualities upon loss of activity, no release of non-food grade substances, no development of resistance, and low cost. However, the majority of antimicrobial materials do not fulfil all these criteria, and quite often, in practical conditions, their hygienic effect is insignificant, due to the depletion of the antimicrobial substance within the material surface and because food residues, biofilms and scale deposits may exert a protective effect by prohibiting intimate contact between the microbes and antimicrobial surface, or by forming an obstacle for the passage of antimicrobials released from the surface.

Therefore, the European Hygienic Engineering & Design Group clearly states that materials modified with antimicrobials may not be considered as a substitute for hygienic design, and certainly not for proper cleaning and disinfection practices. As an alternative, biofilm anti-adhesive coatings are developed to reduce adhesion of microorganisms on the product contact surfaces of food processing equipment.

Key words: *Antimicrobial, Nano, Legislation, Hygiene requirements, Coating, Toxicity.*

1. Introduction

Pathogens and spoiling microorganisms which adhere to interior equipment surfaces may be transferred to food products during their processing. To improve the hygiene during food processing operations, either inorganic or organic antimicrobials are added to various materials of construction used in the manufacturing of food processing equipment. Both in the USA and Europe, these anti-microbial materials must comply with several food contact regulations. An optimal antimicrobial component for food contact applications must have broad-spectrum antimicrobial activity towards undesirable microorganisms and without killing of beneficial microorganisms, long-lasting effect or the possibility to regenerate antimicrobial qualities upon loss of activity, no release of non-food grade substances, no development of resistance, and must be fast-acting at low concentration and low cost. However, the majority of antimicrobial materials do not fulfill all these criteria, and quite often in practical conditions their hygienic effect is insignificant. As an alternative, coatings and bioinspired surfaces with modified microtopography are developed to reduce microbial fouling of product

contact surfaces within food processing equipment. This text gives an overview of the regulations and hygienic requirements that must be met, describes the effectiveness of several contemporary methodologies intended to control microbial fouling of food contact surfaces and the specific problems accompanied with the use of such materials of construction in the food environment.

2. Why should we use antimicrobials, antifouling coatings and surfaces with modified microtopography?

The mentioned methodologies should be used:

- to prevent the formation of biofilms, which are more difficult to remove by cleaning and disinfection.
- to prevent (cross-) contamination.
- to have beneficial effect on food safety when standard hygiene procedures are not employed correctly.
- to prevent biodegradation of materials of construction (microbial corrosion of metals, steels and alloys; and microbiological degradation of plastics, etc).

Antimicrobial materials and antifouling coatings are already widely used to reduce fouling and bacterial infection of implanted materials and devices (catheters, pacemaker leads, knee and hip implants).

2.1. Antimicrobial materials, coatings and surfaces

2.1.1 Legislation and regulations

United States

Biocides used as additives for materials must be registered with the Environmental Protection Agency (EPA) under the *Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)*. Treated articles are exempted from registration, with the exception of treated articles for medical purposes, which are regulated by the FDA. Explicit or implicit health benefit claims cannot be made about unregistered products. For products not claiming public health effects (e.g., "control of odour-causing bacteria"), no efficacy data are needed for the registration.

European Union (EU)

In the EU, we have the *Regulation on Biocidal products (EU) No. 528/2012*, that repeals and replaces the Biocidal Products Directive (98/8/EC). Regulation (EU) No. 528/2012 also covers food contact materials (including nanomaterials). The old Biocidal Products Directive 98/8/EC excluded food contact materials and only regulated treated articles or materials when the biocide is released to obtain an external effect. However, it does not regulate treated articles to which biocides are added for its own protection against biodegradation (internal effect).

The main principles of the *Regulation on Biocidal products (EU) No. 528/2012* are the following:

- Active substances contained in the biocidal articles must be approved for the relevant product-type.
- Active substances also must be mentioned in the list of Article 9(2) of Regulation (EU) No. 528/2012, for the relevant product-type and use.
- A distinction is made between "Articles with biocidal properties" and "Articles with biocidal function".
- Articles which contain biocides for their own protection are considered as having "biocidal properties"; while articles in which a particular or particular biocide(s) is/are integrated to exert an external antimicrobial effect are considered as having a "biocidal function".
- Labeling the article with the quotation "biocidal function" or "biocidal property" is required, and where applicable the term "nano" should be used.
- Precautions with respect to the application of the "biocide" are described. Approval for 1 EU country = approval for all countries in the EU.
- European Chemicals Agency (ECHA) must facilitate and coordinate the approval process, and provide scientific and technical backup to the EU commission and EU countries.
- Companies must provide efficacy data, and companies must share data.
- Costs of the assessment of active biocidal substances must be equally shared by applicants.

Limits are set by *Framework Regulation (EC) No. 1935/2004, Regulation EU No. 1183/2012 and Regulation EU No.10/2011*.

2.1.2 Definition of antimicrobial materials

Substances and food contact materials are antimicrobial materials, if they realize "a reduction of Colony Forming Units (CFU) > 2-log". The problem is a lack of internationally recognized methodologies for testing treated articles. Available test methods are defined in the ISO 22196, ASTM E2149-10 and ASTM E2180-07 standards. Further, the Economic Cooperation and Development (OECD) have developed a 'Guidance document on the evaluation of the efficacy of antimicrobial treated articles with claims for external effects'.

2.1.3 Application of antimicrobials

Antimicrobials are already largely used in cutting boards, knives, bowls, storage containers, countertops, kitchen utensils, refrigerators and conveyor belts. The antimicrobial compound may be incorporated throughout the materials or added as a coating on the surface of the material. However, the most common concept is to add the antimicrobial as a thin coating on the outside of the material.

Independent whether the antimicrobial compound is incorporated or applied as a coating, there are two different approaches to obtain an effect against microorganisms [1]:

- For polymers, the antimicrobial agent may be added before the polymerization process. Present throughout the material, the antimicrobial can migrate from the bulk to the surface of the plastic by migration. Once diffused to the surface, the antimicrobial can finally be released from that surface under humid conditions, with as advantage that microorganisms not in direct contact with the material can be inhibited. Also some surface-coating products exert disinfection action by allowing biocides to leach out of the polymer film on the surface. However, a leachable antimicrobial has as result that the antimicrobial activity of the material is time-limited, that it is conducive to development of bacterial resistance and that it may be transferred to food.

- The antimicrobial agent also can be immobilized to the surface, often by covalent binding, and the bacteriostatic or bactericidal activity is dependent on contact between the microorganisms and the material. The effect may be inhibition of growth, loss of viability, or prevention of attachment of microorganisms. Permanent, non-leaching materials are more desirable because they provide stability without releasing low molecular weight toxic products to the environment, don't cause problems of residual toxicity, prevent the transfer of the antimicrobial compound to the food, limit antimicrobial resistance development, and provide long-term effectiveness. However, immobilizing antimicrobial molecules may undermine their bacteriocidal activity and limit their access to microbes in the surrounding environment.

2.1.4 Requirements to be met by antimicrobials

If used, antimicrobial materials must meet the following requirements [1, 2]:

- Broad-spectrum activity against microorganisms; although selective activity especially towards pathogens, without killing of beneficial microorganisms.

- Active at low concentration and fast-acting.

- Select an antimicrobial in function of its conditions of service (e.g., wet versus dry conditions).

- Antimicrobial activity may not be too much inhibited in the presence of food

- Antimicrobials must resist the expected drastic environmental conditions encountered in the food and beverage industries, such as mechanical and chemical wear and tear.

- No resistance build-up over a period of time.

- Non-toxic for humans & food grade.

- Only released in very limited quantities into the food to eliminate any risk for consumers.

- Antimicrobials for contact with food must meet national or international legislation/regulations.

- Antimicrobial substances also must fulfil the regulations concerning maximal permitted intake.

- May not cause harm to the environment and wildlife.

- Must be compatible with the materials, plastics and additives in which they must be incorporated.

- May not change the properties and appearance of a material (e.g., plastic) (Ag^+-ions are highly reactive and, if not sufficiently stabilized, cause discoloration of plastics).

- Must be heat resistant when they have to be incorporated in plastic (they must retain activities after polymerization and extrusion).

- Good light stability.

- Long-lasting antimicrobial effect: controlled release of antimicrobial substances in sufficient concentration to prevent fast depletion. The user must avoid that the antimicrobial coating peel off.

- The cost of the antimicrobial compound must be low (e.g., triclosan is less expensive than Ag).

- Must be easy to apply or re-apply on a given surface (incorporation versus coating).

- Preferentially the antimicrobial qualities can be regenerated upon loss of activity (e.g., N-halamine).

**2.1.5 Inorganic antimicrobial agents**

In the past, organic antimicrobial coatings were applied to metals and steel to provide them with antibacterial properties. However, their antibacterial effect was not long lasting. Therefore inorganic antimicrobials were used as alternative. Inorganic antimicrobial compounds have as advantage that microorganism-reaction mechanisms are highly non-specific, making it rare for microorganisms to develop resistance. Although some microorganisms are capable of developing resistance to metals through natural selection or horizontal gene transfer. Inorganic antimicrobial compounds also have higher stability in harsh conditions (high temperatures, high pressure, cleaning chemicals, etc.), superior durability and longevity, lower toxicity and greater selectivity.

2.1.5.1 Silver-containing antimicrobial materials

Silver ions

Metallic (non-ionic, Ag°) silver has no antibacterial effect. The activity of silver relies on Ag^+-ions that diffuse from the substrate material and exert strong inhibitory effects (already at levels of 0.001 ppm) on a broad spectrum of microorganisms, such as bacteria, moulds, and viruses. It tends to be more active against

Gram-negative bacteria than against Gram-positive bacteria (Fig. 1). Higher Ag^+-ion concentrations are required for yeasts and moulds, because their cell wall is thicker. Dormant spores are not or less affected by Ag^+-ions due to the presence of different spore coat layers [1, 3].
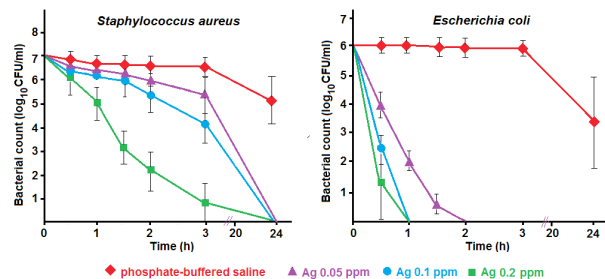
Figure 1. Effect of the silver ion solution on *Staphylococcus aureus* and *Escherichia coli* was investigated by conventional plate counting. The tested silver ion concentrations were 0.2 ppm, 0.1 ppm, and 0.05 ppm, and PBS was used as a control [3]

To avoid that treated articles are too fast depleted of Ag^+, silver is incorporated in a matrix of zeolite (e.g., Zeomic, Sinanen Zeomic Co., Ltd.) or zirconium phosphate (e.g., Alphasan®, Milliken Chemical), that act as ion-exchange resins, exchanging Ag^+-ions for cations in the environment [2]. Silver-exchanged zeolites, which are nontoxic and non-carcinogenic, have been used as coatings and in composites, plastic products, stainless steel and ceramics to confer long-lasting antimicrobial properties. The release of Ag^+-ions from zeolites to the environment is based on an ion-exchange process, where Ag^+-ions are exchanged to Na^+-ions or other cations that can be found in the environment. Strain-, species- and environment-specific differences in the inactivation rates of various bacteria exposed to silver zeolites has been reported. Environmental conditions may change with respect to temperatures, humidity, pH and ion strength. Some zeolite-based inorganic antimicrobials, such as AgION™ (AgION Technologies, Inc., AK steel) contain both Ag^+ and Zn^{2+}-ions within the same matrix. With regards to AgION™, it contains 2.5% (w/w) silver and 14% (w/w) zinc. Zirconium phosphate releases silver under humid conditions [4].

Silver-bearing stainless steel

The metal of choice for food preparation and handling is stainless steel (types 304 and 316) due to its mechanical strength, corrosion resistance, longevity and ease of fabrication. However, it has been shown that even with cleaning and sanitation procedures consistent with good manufacturing practices, microorganisms can remain in a viable state on stainless steel equipment surfaces. In addition, this alloy has been shown to be ineffective at reducing microbial load once it is contaminated.

Antibacterial stainless steel can be produced by adding an antimicrobial element to the alloy. Doping of stainless steel with Ag^+-ions can be achieved without loss of its physical properties. However, silver coatings on stainless steel are non-permanent, because the modified surface layer is very thin, and once worn off, the base material loses its antibacterial ability [5].

Chiang *et al.* [6] demonstrated that silver-alloyed 316 stainless steels could be used in areas where hygiene is a major requirement, but that leaching of silver, which is quite expensive, out of the stainless steel could be a problem. The impact of repeated cleaning and disinfection on the silver-bearing surface must be assessed.

Silver nanoparticles

A plenty of studies have demonstrated that nanosized silver particles exhibit antimicrobial properties. Nanoparticles of silver may be incorporated within polymeric matrices (e.g., antimicrobial Makrolon® polycarbonate - AM1825, AM24281 and AM2520, Bayer Material Science AG) or applied in coatings on materials. Also, the antibacterial effect of nano-silver is based on the release of silver ions, and nano-formulations of silver may cross biological barriers into the cell causing damage to the cell constituents. However, studies of silver nanoparticles revealed that silver nanoparticle aggregates are more toxic than asbestos [7]. Das Bundesinstitut für Risikobewertung [8] has recommended manufacturers to avoid the use of nanoscale silver or nanoscale silver compounds in foods and everyday products until the time that the data are comprehensive enough to allow a conclusive risk assessment which would ensure that products are safe for consumer health. In the EU and Australia, for a long time, nano-silver was treated just like silver, but as in the US now documentation is required on the safety of nano-silver products. At present, nanoscale silver has not yet been authorized to be used in plastic for direct food contact at EU level. The only authorized nanomaterial for direct food contact by the EU is nanoscale titanium nitride which was shown to be chemically inert and would not migrate. It was restricted to be used in PET bottles up to 20 mg/kg.

2.1.5.2 Copper-containing antimicrobial materials

Copper ions

The antibacterial and antifungal effects of copper ions have been well known for a long time. As early as in 1885, Milharde successfully developed the Bordeaux mixture (contains $CuSO_4$) to kill bacteria and fungi in vineyards. Microorganisms are extremely sensitive to the toxic effects of copper but copper ions have lower efficacy than silver.

For many organisms, copper is an essential trace element involved in numerous physiological and metabolic processes. Human tissue has low sensitivity to

copper, although toxicity in humans can occur at high concentrations. In general, exposure to copper is considered safe. The joint FAO/WHO expert Committee on Food Additives suggests a maximum daily intake value for copper of 0.05 mg/kg [9].

Metallic copper

Santo *et al*. [10] have studied the inactivation of bacteria when in contact with dry copper, and they found killing rates of within minutes (e.g., *Escherichia coli* cells were killed after 1 min contact with dry copper), much faster than it was on moist copper (Fig. 2). For bacteria in contact with moist metallic copper surfaces, there is a clear correlation between release of copper from copper surfaces in the medium and its accumulation in the cells. The antibacterial effect of wet copper is similar to that of copper ions in media, being chronic in nature as the consequence of increasing concentrations of copper ions in the bacterial cells with time. In contrary, contact with dry metallic copper rather causes acute effects on bacterial cells (copper shock). Wilks *et al*. [11, 12] studied the survival of *Escherichia coli* and *Listeria monocytogenes* on several moist coppers having 99 or 100 % copper content. In all cases a 7-log reduction of *Escherichia coli* and a 5-log reduction of *Listeria monocytogenes* were observed after respectively 75 - 90 min. and 60 min.
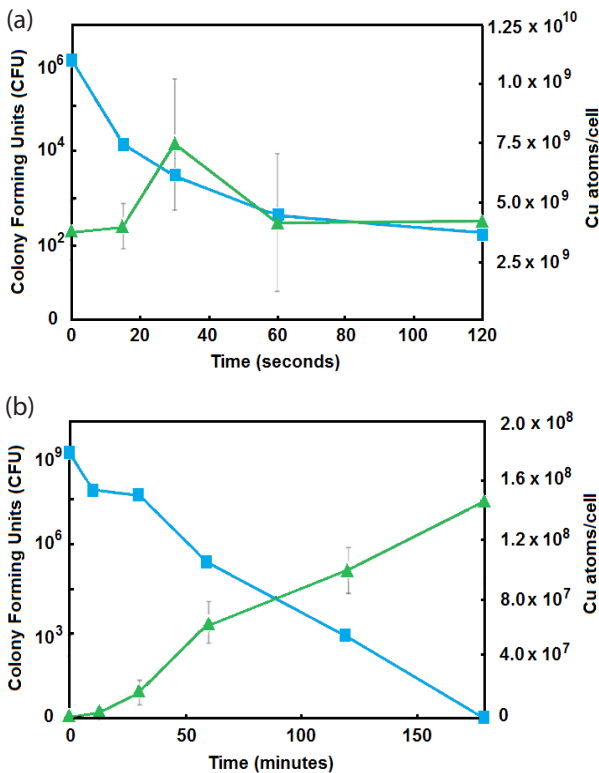
The best known applications of copper are vessels, traditionally used in many breweries and distilleries. Copper is largely applied in the non-product contact area, with as main application the tubes in evaporators installed in refrigerators and freezers, water pipes, etc. However, copper itself would not be a suitable alternative for stainless as food contact material, as it may cause unacceptable organoleptic effects. Moreover, it is quickly and severely affected by strong alkaline detergents, sodium hypochlorite (NaOCl), acidic and salty food. The rate of attack is slow enough that alkaline detergents can be used for the cleaning of copper vessels, but NaOCl renders copper surfaces less hygienic with time. As copper ions may leach from the copper metal, its surface roughness may increase. So even though it has such strong antibacterial properties, copper is a soft, not durable material that tarnishes easily. As copper prices are on the raise, cost considerations favor copper coatings over solid structural copper [13, 14].

Copper alloys

Wilks *et al*. [11] studied the survival of *Escherichia coli* on several moist copper-containing surfaces (Fig. 3). The inhibitory effect of the brasses with 78 or 95% copper content was less pronounced as compared with the coppers, requiring respectively 120 min. and 90 min. to obtain a 7-log reduction. The zinc content in the brasses seems not to have any impact on the survival rate. In the group of the bronzes, 7-log reductions are only obtained after 65 min, 90-100 min, 180 min and 270 min for bronzes having respectively 97%, 95%, 90% and 74% copper. Hence, the percentage copper required for significant biocidal effect must range between 55 and 100%. The greatest efficiency is seen in alloys with high copper content.

Brass (60 - 70% copper, 30 - 40% zinc) and bronze (80 - 95% copper, 5 - 20% tin) are more prone to corrosion by alkaline and acidic detergents, salty and acidic food than ferrous steels. Brass is also susceptible to
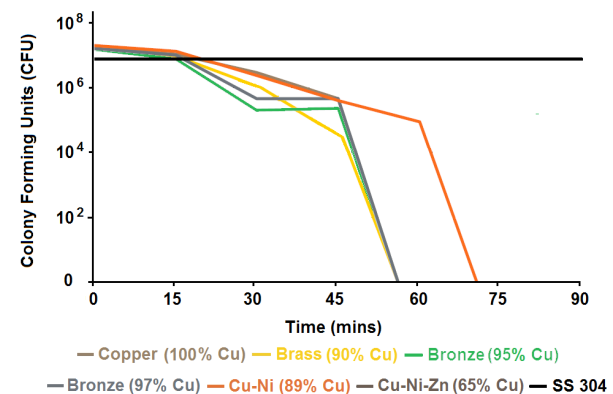

Figure 2. Number of survivors of *Escherichia coli* counted as Colony Forming Units (CFU) and copper uptake into *Escherichia coli* cells after exposure to dry (graphic above) and moist copper (graphic below) for the indicated time [10]

Figure 3. Number of survivors of *Listeria monocytogenes* counted as Colony Forming Units (CFU) on different copper alloys at 20 ⁰C [12]

de-zincification (in e.g., steam), and because cadmium and lead are co-elements to zinc, brass shall never be used in the food contact area. The use of bronze in the food contact zone should be avoided because it quickly becomes porous in contact with acidic foodstuffs, cleaning agents and steam [14].

Copper-bearing stainless steel

Besides bronze and braze, copper-alloyed stainless steels (e.g., 430Cu ferrite antibacterial stainless steel with 1.5% copper of Baosteel) have shown to possess antimicrobial properties. The basic principle of producing antibacterial stainless steels is adding a proper amount of antibacterial element (e.g., Cu) to alloy, and adopting a special heat treatment composed of solid solutioning and succedent ageing to form the active antibacterial phase in the matrix. Released copper ions can be absorbed on the bacterial cell membrane, where denaturation of proteins and oxidation of lipids may take place. The copper ions in the copper-bearing stainless steel are also strongly reductive, which means that they extract electrons from bacteria [9].

Notice however that in production environments, the efficacy of copper-alloyed stainless steels remains lower than that of silver-alloyed stainless steels, and also differs for various bacterial strains [2]. In the opinion of Robine *et al.* [15], copper-bearing stainless steels display poor and limited hygienic efficiency and they also put the long-lasting effect of biocide-based stainless steels on microbial ecology in doubt. Moreover, fouling may reduce the efficacy, as it provides a protective matrix for the bacterial cells to 'hide in' and copper ions released from the metallic surface may immediately be trapped by residues adsorbed on the surface.

Copper oxide nanoparticles

Copper oxide nanoparticles have been physically and chemically characterized and investigated with respect to potential antimicrobial applications. It was found that CuO nanoparticles in suspension, having particle sizes in the 20 to 95 nm range with a mean surface area of 15.7 m^2/g, show activity against a range of bacterial pathogens, including *MRSA* and *Escherichia coli* at minimum bactericidal concentrations ranging from 100 to 5000 µg/ml. As with silver, studies of CuO nanoparticles incorporated into polymers suggest that release of ions may be required for optimum killing. Azam *et al.* [16] have reported that the antimicrobial activity of CuO nanoparticles is lower than that of ZnO nanoparticles, but higher than that of Fe$_2$O$_3$ nanoparticles.

2.1.5.3 Zinc-containing antimicrobial materials

Zinc ions

Zinc has the ability to restrict bacterial growth, but the antimicrobial action of the Zn^{2+}-ion is less extensive than that of Ag$^+$. Gram-positive bacteria are the most acceptable bacterial group to Zn^{2+}, while Gram-negative aerobic bacteria are not inhibited even at the highest concentration. Like silver, to avoid that treated articles are too fast depleted of Zn, zinc is quite often incorporated in a zeolite which again acts as ion-exchange resin. Coleman *et al.* [17] reported for Zn^{2+}-ions incorporated in tober-mites, which behave like zeolites, a 5-log reduction in *Staphylococcus aureus* and *Pseudomas aeruginosa* cell counts, with *Staphylococcus aureus* being more sensitive to Zn^{2+} than *Pseudomonas aeruginosa*.
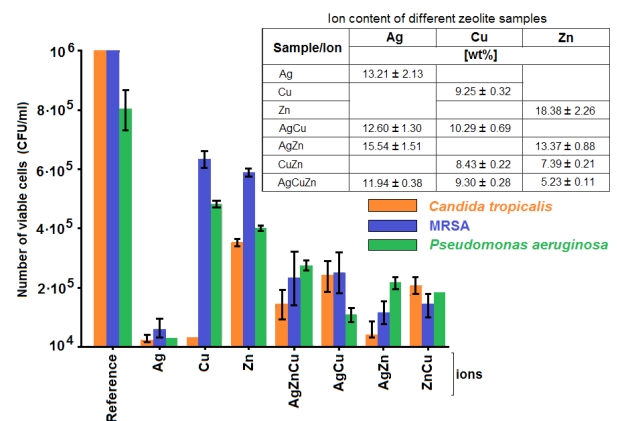
Figure 4. Suspension was inoculated onto the surface of a polyether polyurethane composite incorporated with ion-exchange zeolites, and the cells were allowed to grow on the surface for 24 h at 37 °C and 98% relative humidity [18]

Kaali *et al.* [18] observed the effect that zeolites containing Ag$^+$, Cu^{2+} and Zn^{2+} have on *Pseudomonas aeruginosa*, *Candida tropicalis* and *MRSA*. In Fig. 4, we can deduce the following order of activity: Ag$^+$ > AgZn > ZnCu > AgCu > AgZnCu > Zn^{2+} > Cu^{2+}. Zeolites containing Zn^{2+} or Cu^{2+} exert little antimicrobial effect on *Pseudomonas aeruginosa* as compared to zeolites containing both Zn^{2+} and Cu^{2+}. It can be assumed that each of these ions initiate different toxic mechanisms within the cell, and therefore duplex systems are probably more successful than single ionic systems. These authors also argued that different ions in binary and ternary zeolite systems may block each others' way, and therefore highly influence each others' release.

Zinc oxide nanoparticles

Nanocrystalline zinc oxide, which is safe under harsh processing conditions and can be fabricated at ambient temperatures, has more pronounced antimicrobial activities than micron sized zinc oxide particles. ZnO nanoparticles display photocatalytic and oxidizing capabilities against biological and chemical species. Their small size (less than 100 nm) and high surface-to-volume ratio allow for better interaction with bacteria. Hence, the antibacterial activity of ZnO nanoparticles

increases with reduction in particle size, and further with higher concentrations. However, crystalline structure and particle shape have little effect [19].

ZnO nanoparticles have been shown to have a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria, including against spores. Growth inhibition and in-activation of food-borne pathogens like *Escherichia coli O157:H7*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter jejuni* is observed in many studies. ZnO nanoparticles have selective toxicity to bacteria, with some microorganisms being more sensitive than others. In their study, Xie *et al.* [19] found that ZnO nanoparticles are more effective against *Campylobacter jejuni* than *Escherichia coli*, while the latter is more sensitive than *Salmonella*. An increasing antibacterial effect of ZnO nanoparticles (Fig. 5) is observed to the following microorganisms, and hence according to sensitivity: *Aspergillus niger* < *Candida albicans* < *Pseudomonas aeruginosa* (G⁻) < *Escherichia coli* (G⁻) < *Staphylococcus aureus* (G⁺) < *Bacillus subtilis* (G⁺) [16].

Azam *et al.* [16] demonstrated that ZnO nanoparticles have excellent bactericidal potential, while Fe_2O_3 nanoparticles exhibited the least bactericidal activity. The order of antibacterial activity was demonstrated to be the following: $ZnO > CuO > Fe_2O_3$.
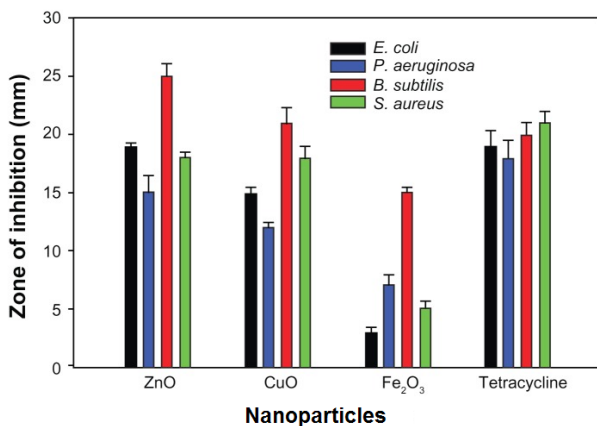
Figure 5. Bar graphs showing zone of inhibition introduced by different metal oxides against various microorganisms [16]

2.1.5.4 Titanium-containing antimicrobial materials

Titanium

Titanium has no antibacterial activity, but due to its resistance to crevice corrosion, impingement and pitting attack in salt water it was in the first place used in the construction of process equipment handling corrosive or delicate liquids such as dairy products, fruit juices and wine [20]. Its inertness is due to the phenomenon of passivation of the titanium surface by the spontaneous formation of a layer of titanium dioxide once exposed to air. This layer, which is very adherent to the metallic substrate, is scarcely removed at high temperatures even in contact with hypochlorites and bleach chemicals, and highly concentrated salt and acid solutions. Further the TiO_2 in that surface layer of titanium dioxide covering the titanium substrate is amorphous or crystalline in nature; from which crystalline titanium dioxide in particular shows three microstructures: rutile, anatase, brookite. An intriguing role is played by anatase, because of its photocatalytic and antibacterial properties. Anodization plus heating treatment of titanium results in the conversion of titanium oxide film from amorphous to crystalline and, in particular, the antibacterial anatase type of TiO_2. Titanium doesn't cause health problems, as it is generally considered to be poorly absorbed upon ingestion [13].

Titanium dioxide

Due to its brightness, high refractive index and resistance to discolouration, titanium dioxide is widely used as white pigment in paints, lacquers, enamels, coatings and plastics. For the same reasons, food grade TiO_2 is also approved in the EU as an additive (E171) in foods, in e.g. candies, chewing gum, products with white icing or powdered sugar toppings. Many products contain 0.01 to 1 mg Ti per serving.

Blake *et al.* [21] have demonstrated that the combined action of UV and titanium dioxide can be used for photocatalytic disinfection of food products, liquids and air. Photo-disinfection is the result of photocatalytic processes taking place on the TiO_2 surface, with anatase TiO_2 having inherent superior photocatalytic properties. Upon excitation by light with wavelength < 385 nm, the photon energy generates an electron-hole pair on the TiO_2 surface (Fig. 6). The hole in the valence band can react with H_2O or hydroxide-ions adsorbed on the surface to produce hydroxyl radicals (OH·), and the electron in the conduction band can reduce O_2 to produce superoxide ions ($O_2^{·-}$). Other reactive oxygen species (ROS) produced at the catalyst surface, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) have been reported. The principle reactive species are
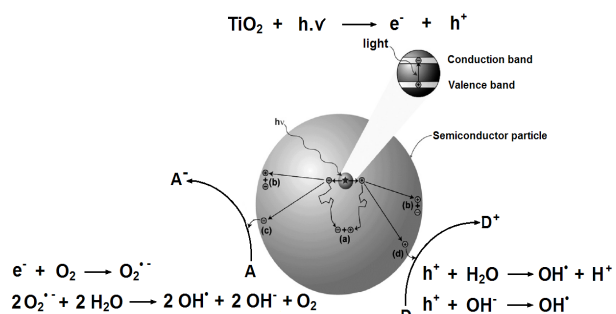

Figure 6. Photo-excitation processes in TiO_2 leading to redox behaviour: (a) electron and hole recombination in the bulk; (b) electron and hole recombination at the surface; (c) adsorbate reduction at the surface and (d) adsorbate oxidation at the surface [22]

the short lived hydroxyl radicals (OH·) which is a potent non-selective biocide with the ability to oxidize most organic compounds. When irradiated TiO_2 particles are in direct contact with or close to microbes, and the microbial surface is the primary target of the initial oxidative attack [22].

Specific problems that prohibit the use of titanium dioxide as antimicrobial on a large scale are: the need of UV light to be effective, restricting its use to open equipment surfaces, and the limited long-term bactericidal effect due to mechanical and chemical wear. Moreover, fouling and scale deposition may decrease the photocatalytic effect of titanium dioxide [11, 12, 22, 2].

Lu *et al.* [23] studied the photocatalytic inactivation of *Escherichia coli* Fig. 7 (on the left) shows the effect of the photocatalytic reaction by TiO_2 thin films on cell viability, while Figure 7 (on the right) shows the leakage of K^+-ions from *Escherichia coli* cells illuminated by UV light with λ < 385 nm.
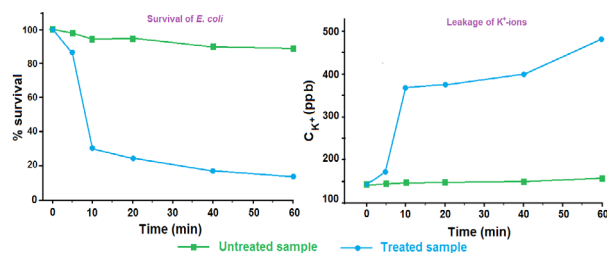
Figure 7. Effect of photocatalytic reaction by TiO$_2$ thin films on cell viability. The survival curves were obtained by colony counting of *Escherichia coli* cells illuminated in the absence (green) and presence (blue) of TiO$_2$ thin films at different time intervals (left figure). Leakage of K$^+$-ions from *Escherichia coli* cells illuminated by UV light in the presence (blue) and absence (green) of TiO$_2$ thin films at different time intervals (right figure). At point of time 0 min, *Escherichia coli* is intact; at point of time 10 min., the cell wall is decomposed and the cell membrane is exposed with loss of semi-permeability and leakage of K$^+$-ions as result, finally leading to only 30% survivors; at point of time 40 min., there are holes in the cytoplasmic membrane, with more leakage of K$^+$-ions and only 20% survivors; at point of time 60 min, there is a large hole in the cytoplasmic membrane with only 10% survivors [23]

Titanium dioxide nanoparticles

Vargas-Reus *et al.* [24] investigated the antimicrobial activity of nanoparticulate metals and metal oxides under anaerobic conditions against four Gram-negative species of bacteria: *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*. Table 1 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the four bacteria tested. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were in the range of < 100 µg/mL to 2500 µg/mL and < 100 µg/mL to > 2500 µg/mL, respectively. Based upon mean values, the activity of the 5 nanoparticles against the four species tested in descending order was Ag > ZnO > CuO > TiO$_2$ > WO$_3$. TiO$_2$ and WO$_3$ showed poor or no antimicrobial activity at the concentrations tested. TiO$_2$ and WO$_3$ showed poor or no activity. However, in the case of TiO$_2$, no ultraviolet light was used and it is well documented that in the presence of such ionizing radiation TiO$_2$ nanoparticles demonstrate bactericidal properties. The anatase form of nano TiO$_2$ and UV light excitation are required to ensure maximum antimicrobial activity.
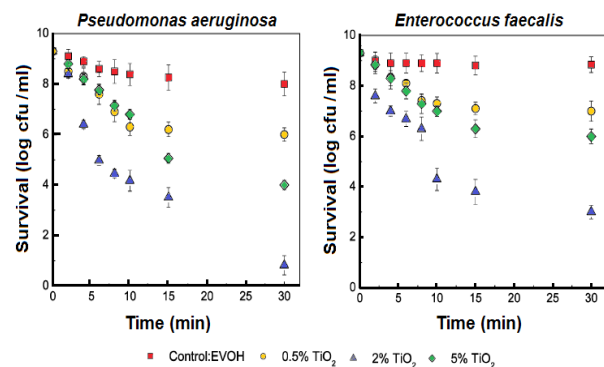
Figure 8. Process come-up time logarithmic red uction of the microorganism population suspended in Luria-Bertani medium. Survival curves for *Pseudomonas aeruginosa* and *Enterococcus faecalis* (figure left) as a function of the irradiation time for EVOH-TiO2 and control samples [25]

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values (in µg/ml) of the tested bacterial species with metal and metal oxide nanoparticles (n = 3 replicate determinations) [24]

Bacterial species	Ag		ZnO		CuO		TiO$_2$		WO$_3$	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Porphyromonas gingivalis	250	250	250	250	500	2500	2500	>2500	2500	2500
Prevotella intermedia	100	100	1000	1000	250	250	1000	>2500	2500	>2500
Fusobacterium nucleatum	100	100	250	500	250	250	1000	>2500	2500	>2500
Aggregatibacter actinomycetemcomitans	100	100	250	250	250	250	250	>2500	2500	>2500
Mean	137.5	137.5	437.5	500	312.5	812.5	1187.5	>2500	2500	>2500

Kubacka *et al.* [25] prepared ethylene-vinyl alcohol co-polymer, EVOH/TiO$_2$ nanocomposites, with different amounts of the inorganic TiO$_2$ component with anatase structure, primary particle size of ~ 9 nm, and BET area of 104 m^2/g. Because of the partial amphiphilic nature of EVOH copolymers, it is possible to include titanium nanoparticles in the final material with a good adhesion at interfaces between the two components. These nanocomposites present extraordinary antimicrobial properties against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Enterococcus faecalis* (Fig. 8).

Figure 8 gives an overview on the process come-up time logarithmic reduction of *Pseudomonas aeruginosa* and *Enterococcus faecalis*:

- No significant activity is detected in absence of UV light; however, when this was switched on *Pseudomonas aeruginosa* appeared to be more affected than *Enterococcus faecalis*. After 10 min of treatment, 3.0, 5.1 and 2.5 log-reductions were observed, respectively, for the 0.5, 2 and 5 wt% materials. These values increase up to 3.3, 8.5 and 5.3 by the end of the experiment (30 min.), in the characteristic tailing region displayed after an extended period of UV treatment. Once oxide aggregation is detected in the composite material, e.g. around 5 wt%, a certain loss of biocidal activity is detected. The joint analysis of all these points leads to the conclusion that the maximization of both the available TiO$_2$ surface area and dispersion homogeneity within the polymer matrix are key features in optimizing biokilling activity in our nanocomposite materials.

- With respect to *Enterococcus faecalis*, in the first 10 min we found a similar behavior to the one displayed in the case of *Pseudomonas aeruginosa*: e.g., 2.0, 5.0, and 2.3 log-reduction values for the 0.5, 2 and 5 wt% samples, respectively. The efficiency of the samples slows up toward the end of the experiment (characteristic tailing) but an optimum value of 6.3 log-reduction after 30 min. of treatment was obtained with the 2 wt% sample. Again, once oxide aggregation is detected in the composite material, e.g. around 5 wt%, a certain loss of biocidal activity is detected.

Once again, the biocidal activity of TiO$_2$ nanoparticles is rather poor.

2.1.5.5 Toxicity of nanoparticles

Nanoparticles and their properties

Many countries have developed programs to promote nanotechnology development. Nanomaterials are all materials with a structural component smaller than 100 nm in at least one dimension. Besides nanoparticles that have three dimensions in the 1 - 100 nm range, there are nanorods or nanotubes that have two dimensions in this range, and nanosheets or nano-membranes that have one dimension in this range. Nanoparticles of a specific material exhibit quite different physical (optical, electro-magnetic), chemical (catalytic with increased chemical reactivity as result) and mechanical properties as compared to their larger counterparts, mainly due to surface and quantum effects. Nanomaterials, as compared to similar larger-sized materials of the same chemical composition, have the potential to alter physicochemical and biological characteristics. Hence, a material that is considered nontoxic in bulk form might be extremely toxic at the nanoscale. It is virtually impossible to extrapolate the ad-verse effects of nanoparticles from their bulk properties [26].

Safety concerns

Not all nanoparticles on earth are man-made. Ash of volcanic eruptions, dust from desert storms and smoke generated during forest fires are nanoparticles commonly found in nature. Besides these natural nanoparticles, there are also man-made nanoparticles which are divided in two subcategories: anthropogenic nanoparticles (e.g. vehicle pollution, particles generated during welding, building demolition, etc.) and engineered nanoparticles (materials engineered at the nanoscale to have novel functionality or properties different from those of their larger counterparts of the same chemical composition).

Safety concerns about nanomaterials are mainly driven by the accumulated knowledge of the health effects of inhaled anthropogenic nanoparticles (Fig. 9), for example, ultrafine air pollutants which are responsible for the increase in cardiovascular, airways and pulmonary diseases, often resulting in death. Further, the adverse health effects of pathogenic fibers, such as asbestos and silicates are well known. Asbestos is responsible for a cancer of the lining of the lungs (mesothelioma). Due to their similar morphology, nanofibers and nanorods (single- and multiwalled carbon nanotubes) might - after inhalation - mimic the damaging effects of asbestos. Concerns have been raised that also nanorods (e.g., carbon nanotubes) might become a public health issue as well. Therefore, the health effects of the long-term use of nanomaterials and their interaction with other components of food (e.g., proteins, lipids, carbohydrates, and nucleic acids) are not sufficiently known. Hence, before nanomaterials are used in the food industry, risk assessments must be performed to identify and quantify their potential toxicity.

Nanomaterials in the body

Nanomaterials can be absorbed by inhalation of free nanomaterials present in the air, ingestion from food and water, and penetration through the skin (Fig. 10). Once nanoparticles enter the body, they can penetrate the airway or gut mucosal barriers and travel through the
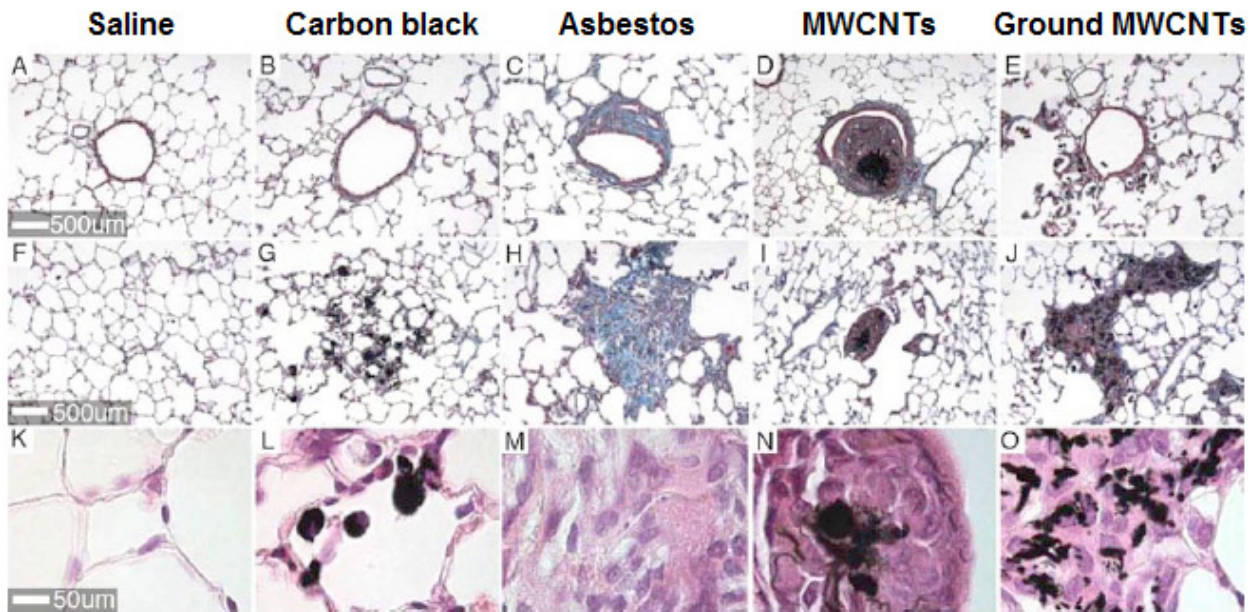
Figure 9. Lung lesions in rats after inhalation of nanomaterials: saline (A, F, K), carbon black (B, G, L), asbestos (C, H, M), multiwall carbon nanotubes (D, I, N), and grounded multiwall carbon nanotubes (F, J, O) [26]

circulatory systems and then deposit in various organs including the liver, kidney and brain, where they can remain for weeks and months. Biostable nanomaterials (e.g., nanoparticles made of metal, such as quantum dots and silver and TiO$_2$ nanoparticles; carbon-based nanoparticles such as fullerenes, carbon nanotubes, carbon soot; and silicon-based nanoparticles) have proven to exhibit long tissue retention times, in particular in the liver, spleen, and lymph nodes. Biopersistence, being the ability to penetrate into body tissues and to resist solubilization and normal clearance mechanisms (macrophage engulfment or renal/fecal excretion) are the main property of a nanoparticle influencing its longer term toxicity.

If the asbestos is considered to represent unity as a relative cytotoxicity index reference or figure of merit, the relative cytotoxicity index reference to asbestos can be measured by considering the relative cell viability (RCV) value for asbestos (table 2). Consequently, an index less than 1 will represent toxicities below asbestos while an index greater than 1 will represent a corresponding cytotoxicity greater than asbestos. The values represent relative cytotoxicities for the corresponding nanoparticulate materials referenced to chrysotile asbestos, in the concentration range of 5 - 10 µg/mL [7].
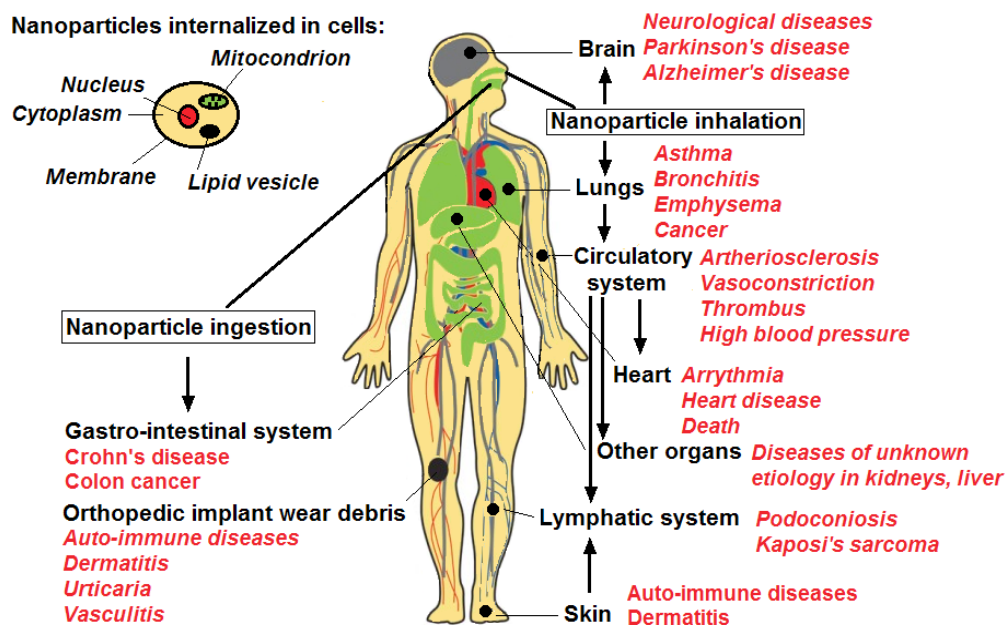
Figure 10. Diseases associated with nanoparticle exposure [26]

Table 2. Nanomaterials morphologies and relative Cytotoxicity Index [7]

Material	Component particle size	Relative cytotoxicity index (at 5 µg/ml)	Relative cytotoxicity index (at 10 µg/ml)
Ag (silver; 99.99%) (25 nm grade)	3-100 nm spherules; 30 nm (mean)	1.5	0.8
Ag (silver; 99.99%)	5-65 nm spherules; 30 nm (mean)	1.8	0.1
Al_2O_3 (γ- alumina)	4-115 nm spherules; 50 nm (mean)	0.7	0.4
Fe_2O_3 (γ- iron oxide)	5-140 nm spherules; 50 nm (mean)	0.9	0.1
ZrO_2 (zirconia)	7-120 nm spherules; 20 nm (mean)	0.7	0.6
TiO_2 (80% anastase)	5-100 nm spherules; 40 nm (mean)	0.4	0.2
TiO_2 (anastase)	5-40 nm spherules; 20 nm (mean)	0.4	0.1
TiO_2 (rutile)	Irregular, short fibres; 5-15 nm (diameter) ; 20-60 nm (length)	0.3	0.05
Si_3N_4 (silicon nitride)	10-150 nm spherules; 60 nm (mean)	0.4	0.06
Chrysotile Asbestos $Mg_3Si_2O_5(OH)_4$	15-40 nm fibre diameter; 20 nm (mean) 0.5 µm-15 µm length $\sim$ 5:1 - 500:1 aspect ratio	1.0	1.0
Carbon black powder	2-50 nm spherules; 20 nm (mean)	0.8	0.6
Single wall carbon nanotube powder (SWCNT)	Nanoropes/bundles 10-200 nm diameter	1.1	0.9
Multi wall carbon nanotube powder (MWCNT)	$\sim$50% nanotubes; 10 - 30 nm (diameter) ; 50 nm - 1 µm fibre (length) ; $\sim$50 nm fullerene diameters	1.1	0.8
Multi wall carbon nanotube powder (MWCNT)	$\sim$85% nanotubes; 3-5 nm (dia.); 15 nm (mean) ; 20 nm - 1 µm lengths	0.9	0.8

2.1.6 Organic antimicrobial agents

2.1.6.1 Triclosan

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) is widely incorporated in house-hold products like soaps, deodorants, lotions, shower gels, toothpastes and mouthwashes. Food contact materials: incorporated into or impregnated onto food contact plastics (e.g., in conveyor belts, cutting boards, shopsticks, food storage containers, etc.).

Triclosan has the following characteristics:

- Broad-spectrum antimicrobial effect against bacteria, molds and viruses at concentrations of 0.2 to 2%. Gram-positive bacteria are more sensitive than Gram-negatives.
- It must leach from the surface of the plastic to perform its antimicrobial function. Consequences: not long-lasting, and hence not permanent.
- Several studies report no or very limited effect (0 to 1-log), while one study reported a 5- to 6-log reduction in the surrounding media.
- Its effect is reduced in the presence of organic material (e.g., food residues).

- Its widespread use selects for bacteria resistant to both triclosan and antibiotics.
- It is persistent: detected in drinking water, sediments, fish, urine, blood and breast milk.
- It may give raise to the formation of highly toxic and persistent dioxins, due to the action of light and/or its decomposition when the plastic is burned in an incinerator.
- Potential endocrine disruption and subchronical toxicology effects.
- Max. migration limit (EU 90/128/EEC Directive, 1990) of 5 mg/kg of food.
- European Commission decided in 2010 to remove triclosan from the Provisional List of additives for use in plastic food contact materials.

2.1.6.2 N-halamine containing surfaces

N-halamine that dissociates in an aqueous environment to release free chlorine (less than 1 mg/L) in the form of hypochlorite or hypochlorous acid also may inactivate microorganisms on direct contact. In N-halamines (Fig. 11), one or more halogen atoms (Br or Cl,

but usually the latter) are covalently bonded to the nitrogen atoms of the compounds. The N-Cl or N-Br covalent bonds provide stability and slowly release free active halogen species into the environment. The N-Cl or N-Br covalent bonds may disrupt during contact with microorganisms, and oxidative halogen (Cl^+ or Br^+) may be directly transferred to their cell membrane. As such, oxidative halogens (Cl^+ or Br^+) may bind to the thiol groups or amino groups in proteins [1].
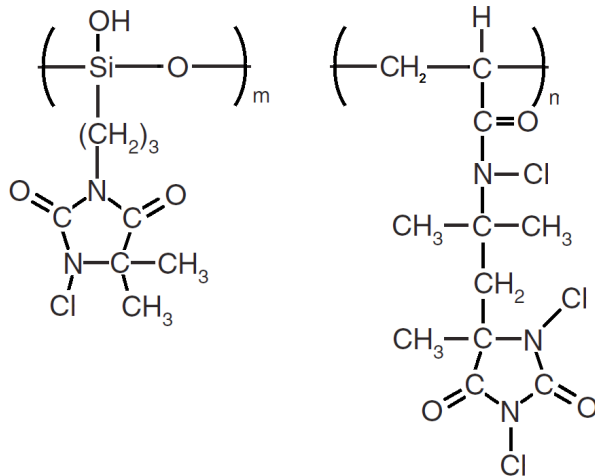
Figure 11. (a) poly [3-(1-N-chloro-5 5-dimethylhydantoinyl) propyl siloxane] [27], (b) poly[acryl-N-chloro-N-(1,1-dimethyl-2-(1,3-N,N-dichloro-5-methylhydantoinyl)) ethyl amide] [27]

N-halamines exhibit high activity to a broad spectrum of microorganisms such as Gram-negative and Gram-positive bacteria, fungi, yeasts and viruses within brief contact times (already within 5 minutes). The antimicrobial activity rates increase from amine- to amide- to imide-containing N-halamines. A 6-log reduction can be observed for many microorganisms but the presence of organic material has again proven to be detrimental for the antibacterial effect of N-halamines.

Liang *et al.* [27] studied the effect of the chlorinated homopolymer poly[3-(1-N-chloro-5,5-dimethyl-hydantoinylpropyl)-siloxane] (PHS) on *Escherichia coli 0157:H7* and *Staphylococcus aureus*. The biocidal efficacy data for chlorinated PHS on Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli O157:H7* are presented in respectively Tables 3 and 4. In both cases, a 6.7- and 7-log reduction was obtained.

2.1.6.3 Quaternary ammonium compounds immobilized on surfaces

Because of their low toxicity and high effectiveness as disinfectant, several approaches have been evaluated to immobilize quaternary ammonium compounds (QACs) on surfaces. The electrostatic/ hydrophobic interactions with the bacterial cell membrane/wall that undermine the integrity of that cell membrane/wall, are the main mechanism of QACs against microbial cells.

Table 3. Effect of polyhalamine siloxane on *Staphylococcus aureus* [27]

Sample coating	Inoculum concentration	Log reduction in a contact time (min)				
		0.5	1.0	5.0	10.0	30.0
Experiment 1						
Cotton control		0.37	0.44	0.44	0.37	0.52
PHS control (unchlorinated)	3.33×10^6 CFU	0.14	0.15	0.25	0.22	0.26
PHS (0.89% Cl^+)		6.52	6.52	6.52	6.52	6.52
Experiment 2						
Cotton control		0.51	0.56	0.57	0.60	0.68
PHS control (unchlorinated)	4.76×10^6 CFU	0.35	0.40	0.37	0.62	0.70
PHS (0.86% Cl^+)		6.68	6.68	6.68	6.68	6.68

Table 4. Effect of polyhalamine siloxane on *Escherichia coli O157:H7* [27]

| Sample coating	Inoculum concentration	Log reduction in a contact time (min)				
		0.5	1.0	5.0	10.0	30.0
Experiment 1						
Cotton control		0.18	0.20	0.33	0.30	0.42
PHS control (unchlorinated)	4.18×10^6 CFU	0.06	0.24	0.22	0.26	0.45
PHS (0.89% Cl^+)		6.62	6.62	6.62	6.62	6.62
Experiment 2						
Cotton control		0.06	0.10	0.24	0.22	0.13
PHS control (unchlorinated)	1.00×10^7 CFU	0.10	0.10	0.13	0.17	0.22
PHS (0.86% Cl^+)		3.80	7.00	7.00	7.00	7.00

Organosilane quaternary ammonium compounds

In a first approach, a quaternary ammonium salt is attached to siloxanes, giving organosilane quaternary ammonium compounds that can be bound to different types of materials. Due to their reactivity, silanes are frequently used as coupling agents and adhesion promoters. One company that has developed this technology is Ægis Environmental management (acquired by Microban Products Co., UK), that uses 3-trimethoxysilyl-propyloctadecyldimethyl ammonium chloride as monomer to produce a cross-linked polymeric antimicrobial siloxane coating (Fig. 12). The trimethoxysilyl functionality of this molecule has the ability to bond covalently to a variety of receptive surfaces, although there are also surfaces with which they cannot react covalently. In a wet finish process, the attachment of silane quaternary ammonium compounds to the surfaces proceeds by two mechanisms. Physisorption is the first mechanism; chemisorption is the second mechanism, in the course of which hydrolysis of the alkoxy groups and silyl ether bonding to receptive surfaces occurs. Then the other silanol functionalities left, allow the bound monomers to homopolymerize with each other to form a cross-linked polymer of extremely high molecular weight. So the silane quaternary ammonium molecules attach to the target surface and to each other, creating a polymeric siloxane coating. After the antimicrobial organosilane quaternary ammonium compounds have coated the surface in this manner, they become virtually irremovable, even on surfaces with which they cannot react covalently [28].

The octadecylammonium chloride portion of the molecule attached onto the surface provides the antimicrobial activity. The quaternized nitrogen provides a positive electrical charge by which micro-organisms carrying a negative charge are electrostatically attracted to the surface. The lipophilic octadecyl carbon chain, which fits well with the lipoprotein (fat-like) composition of microbial cell membrane, punctures the cellular

membrane of the offending microbe (Figure 13), after which the positive charge of the quaternized nitrogen finally electrocutes what is left of the microbe [28]. Figure 14 demonstrates the efficacy of several silicone rubber bound QACs against *Staphyloccoccus aureus 12600*, *Staphylococcus epidermidis HBH₂ 102*, *Escherichia coli O2K2* and *Pseudomonas aeruginosa AKI*.
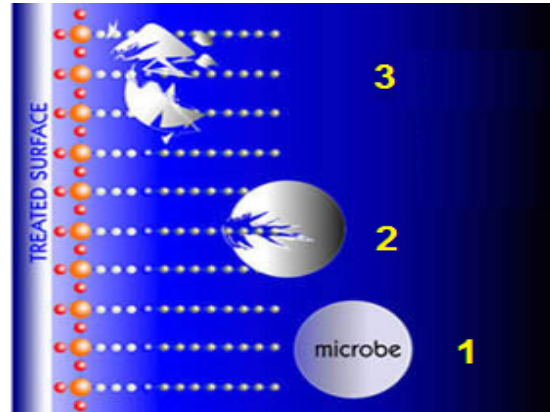
Figure 13. (1) The quaternized nitrogen provides a positive electrical charge by which microorganisms carrying a negative charge are electrostatically attracted to the surface. (2) The lipophilic octadecyl carbon chain, which fits well with the lipoprotein (fat-like) composition of microbial cell membrane, then acts like a "spike" that punctures the cellular membrane of the offending microbe, after which (3) the positive charge of the quaternized nitrogen finally electrocutes what is left of the microbe
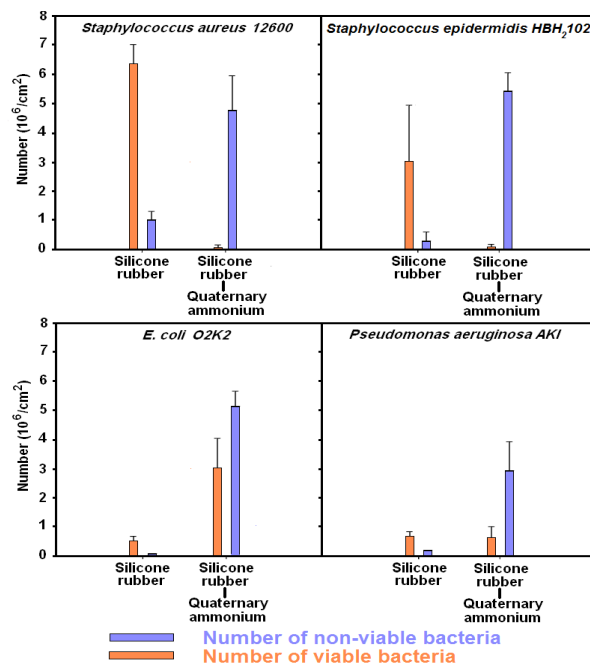

Figure 14. The numbers of adhering viable (orange bars) and non-viable (blue bars) bacteria on silicone rubber (SR) and quaternary ammonium silanized silicone rubber (QAS). Test microorganisms: *Staphylo-coccus aureus 12600*, *Staphylococcus epidermidis HBH₂ 102*, *Escherichia coli O2K2* and *Pseudomonas aeruginosa AK1*. Error bars represent the SD over 6 images collected in 2 experiments, with separately cultured bacteria and differently prepared coatings [29]

Figure 12. The trimethoxysilyl-functionalities of the 3-trimethoxysilyl-propyloctadecyldimethyl ammonium chloride molecules have the ability to bond covalently to a variety of receptive surfaces, after which the other silanol functionalities left may polymerize with each other to form a cross-linked polymeric antimicrobial siloxane coating

For the antimicrobial to be effective, it must come into direct contact with the microbes. Hence, food residues, biofilms, scale deposits and conditioning films will exert a protective effect by prohibiting intimate contact of the microbes with the antimicrobial surface. As long as the surface is kept clean with normal cleaning procedures, the surface will continue to inhibit any microbial growth.

N-alkylated polyethyleneimines

As another variation, hydrophobic plastic coatings with polycationic polyethyleneimine branches (N-hexyl, N-methyl-polyethyleneimine (Fig. 15) and N-dodecyl, N-methyl-polyethyleneimine) were developed, that also have demonstrated to kill microorganisms. As microorganisms are hydrophobic, the inter-action of the hydrophobic polycationic chains in the coating with the microbe cell envelope is favoured. These chains must be sufficiently long to reach the adhered bacteria despite a presumably rougher surface topography. The negatively charged microbial cells are further electrostatically attracted by providing these polymer chains with a positively charged moiety. These positive charges are also responsible for the repulsive interaction between adjacent polymer chains, causing these chains to stand erect on the surface. Hence, when the microorganisms are electrostatically attracted towards the treated surface, they are killed on contact because the microbial cell envelope is punctured by the erect fragments of the polycationic chains ("tentacles"). The polycationic imine-branches also act as quaternary ammonium groups. Where bacteria with compromised membranes may still be able to recover, this is not the case with N-alkylated polyethyleneimines. This will not be the case with immobilized N-alkyl-polyethyleneimine [22, 30].
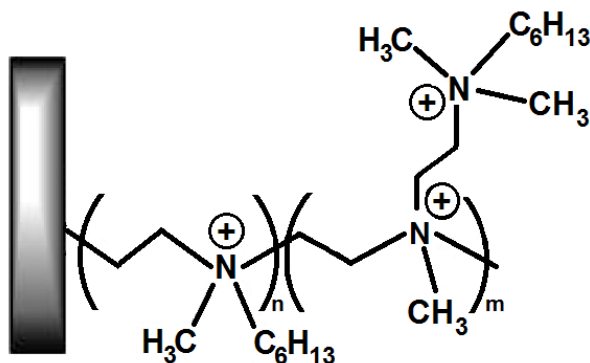

**Figure 15. Branched N-hexyl,
N-methyl-polyethyleneimine [22]**

Gottenbos et al. [29] studied the in vitro activity of covalently coupled quaternary ammonium silane coatings on silicone rubber, with as test organisms: Gram-positive *Staphylococcus aureus ATCC 12600* and *Staphylococcus epidermidis HBH2 102*, and Gram-negative

Escherichia coli O2K2 and *Pseudomonas aeruginosa AK1*. The viability of the adherent bacteria was determined using a live/dead fluorescent stain and a confocal laser scanning microscope. The numbers of viable and non-viable adhering bacteria on silicone rubber and quaternary ammonium-coated silicone rubber in vitro after deposition for 1h in the parallel plate flow chamber are presented in Figure 16. The coating reduced the viability of the adherent staphylococci from 90% to 0%, and of Gram-negative bacteria (*Escherichia coli O2K2* and *Pseudomonas aeruginosa AK1*) from 90% to 25%.

Onnis-Hayden *et al.* [31] studied the potential of N-hexylated polyethyleneimine covalently attached to sand to disinfect water. The efficacy of the filter disinfection process was evaluated with water spiked with *Escherichia coli* (Fig. 16).
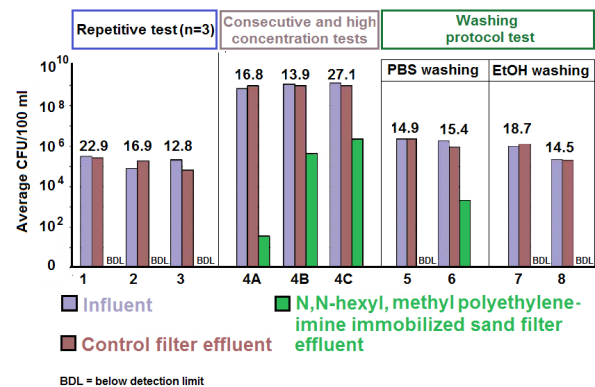
Figure 16. In test runs 1,2 and 3, conducted on different dates, phosphate-buffered saline (PBS) influent containing 10^9 *Escherichia coli* cells undergoes a 5-log reduction. When the influent *Escherichia coli* concentration was increased to 10^9 cfu/100ml, a bacterial count reduction of over 7-logs was achieved in the initial test (Test 4A). However, subsequent tests of the filter conducted on the same day showed decreased reduction. In test 4B, consecutive supply of PBS containing 10^9 *Escherichia coli* cells gives only a 3.5-log reduction. Consecutive supply of PBS containing 10^9 *Escherichia coli* cells (test 4C) gives only a 2,5-log reduction. The rather poor results in test runs 4B and 4C are due to bacterial debris and organic and colloidal particulate matter reducing the contact efficiency between immobilized N,N-hexyl, methyl polyethyleneimine and the suspended bacteria. In test 5 and 6, the antibacterial effectivity is restored when the filter is washed with PBS. In test 7 and 8, cleaning with respectively phosphate-buffered saline (PBS) and ethanol gives better results [31]

2.1.7 Surface coating with photosensitizers

Surface coating based on cellulose acetate containing photosensitizers, such as Toluidine Blue and Rose Bengal, immobilized within a polymer matrix, have demonstrated to possess the capability of destroying a wide variety of microbial cells when exposed to visible (white) light conditions. This technology to control microbiological contamination is also called photodynamic therapy (PDT), in which the excitation of the

photoactivateable or photosensitizing agent in the presence of light gives rise to the formation of cyto-toxic singlet oxygen and free-radicals. The result is that the sensitizer moves from an electronic ground state to a triplet state, that then interacts with microbial components to generate cytotoxic reactive oxygen species (ROS). One of the advantages of light-activated killing is that, in comparison with that experienced with more traditional chemical antimicrobial agents, resi-stance to the action of singlet oxygen is unlikely to become widespread,. A sensitizer ideally should absorb light at red to near-infrared wavelengths because these wavelengths are able to penetrate more. The most commonly tested sensitizers on bacteria have been tricyclic dyes (for example, methylene blue and eryth-rosine), tetrapyroles (for example, porphyrins),and fu-rocoumarins (for example, psoralen). Considerations in relation to the therapeutic use of light-activated killing of biofilms on host surfaces include (1) direct toxicity of the sensitizer, (2) indirect toxicity of the sensitizer in terms of "by-stander" damage to adjacent host cells, (3) penetration into the biofilm, (4) light exposure time required to kill bacteria within in vivo biofilms, and (5) wide spread relatively non specific bacterial killing. It has been shown that photosensitizers do not leach from the plastic matrix but they have the disadvantage that the reactive oxygen species (ROS) produced by the photosensitizer could, in the long term, degrade the matrix containing the photosensitizer [22].

Decraene *et al.* [32] studied the antimicrobial effect of light in combination with a cellulose acetate coating containing Toluidine Blue and Rose Bengal (Table 5).

Following illumination for 2 h, the Toluidine Blue and Rose Bengal coating was able to achieve a 99.6% re-duction in the viable count of a suspension containing

approximately $2 \cdot 10^6$ CFU/ml of *Staphylococcus aureus*. A 100% kill of a *methicillin-resistant* strain of *Staphylococcus aureus* was also achieved after 6 h of illumination. *Clostridium difficile* also proved to be susceptible to killing by the illuminated coating: a 100% kill of a suspension of the organism (consisting mainly of vegetative cells) containing $5.19 \cdot 10^6$ CFU/mL was achieved after 4 h of illumination. In contrast, the Gram-negative organism *Escherichia coli* appeared less susceptible and little killing was observed after 6 h of illumination. However, illumination for 16 h resulted in 100% kills of a suspension containing approximately $2 \cdot 10^6$ CFU/ml of the organism. *Candida albicans* also appeared to be less susceptible than *Staphylococcus aureus* to killing: an 88% reduction in the viable count of a suspension containing $1.99 \cdot 10^5$ CFU/ml was achieved after 16 h of illumination.

2.1.8 Microorganism-repellant coatings

The initial step in biofilm formation is a nonspecific, re-versible attachment of bacteria to substratum surfaces. Once permanently attached, bacteria start to syn-thesize insoluble exopolysaccharides (EPS) that encase the adherent bacteria in a three-dimensional matrix. With the accumulation of EPS and the reproduction of bacteria, colonies develop into mature biofilm. The EPS matrix not only helps the cell to adhere to a substratum and trap nutrients from the water due to charged and neutral polysaccharide groups, but the glycocalix ma-trix also protects bacteria from antimicrobial agents. Therefore, one of the most effective methods to pre-vent the formation of biofilm is to avoid or reduce the initial adhesion of bacteria to a surface.

Microorganism-repellant coatings aim to prohibit the adhesion of microorganisms and to delay the formation

Table 5. Viable counts on a cellulose acetate coating with Toluidine Blue and rose Bengal (at 25 µm), exposed to light from 25 W

| Organism | Light exposure time (h) | Viable count (CFU/ml) under the following condition | | | | Log_{10} reduction in viable count (L^+S^+ vs. L^-S^-) |
| | | not illuminated | | illuminated | | |
		S^-	S^+	S^-	S^+	
Staphylococcus aureus	2	1.84×10^6	1.99×10^6	1.58×10^6	7.71×10^3	2.4
Staphylococcus aureus	6	2.21×10^6	1.8×10^6	1.86×10^6	0	6.3
MRSA	6	2.69×10^6	2.81×10^6	3.02×10^6	0	6.4
Clostridium difficile	4	5.19×10^6	2.63×10^6	1.57×10^6	0	6.7
Candida albicans	16	1.99×10^5	2.18×10^5	2.33×10^5	2.39×10^4	0.9
Bacteriophage φX174	16	1.34×10^6	9.13×10^5	8.15×10^5	1.2×10^5	1.1
Escherichia coli	6	1.96×10^6	1.85×10^6	1.9×10^6	1.48×10^6	0.1
Escherichia coli	16	1.92×10^6	1.79×10^6	2.09×10^6	0	6.3

S^-: microbial suspension in contact with photosensitizer-free coatings.
S^+: microbial suspension in contact with photosensitizer-containing coatings.
L^-: not illuminated.
L^+: illuminated.

of biofilms on product contact surfaces. These biopassive polymer coatings (Fig. 17) were initially used to give minimal protein adsorption on the surface, as proteins promote the adhesion of bacteria. In contrary to bioactive polymers, biopassive surfaces do not actively interact or kill bacteria. A wide variety of polymers, including poly(ethylene glycol) (PEG) and zwitterionic structures have been examined as biopassive surfaces [33].

2.1.9 Coatings acting by steric repulsion and their "superhydrophilic" properties

Surfaces can be modified by covalent or non-covalent (physisorption) binding of hydrophilic polymers or block copolymers consisting of highly hydrated, flexible chains with low polymer–water interfacial energies in a high density, giving so called polymer brushes. These end-tethered polymer chains are forced to stretch away from a surface into the adjacent solution due to that high density of chains per unit surface area. By using polymer brushes that bind a lot of water, the brush-coating becomes highly hydrophilic. Microorganisms encountering the brush surface are repelled by steric hindrance due to the bound water in the brush (microbial cells are hydrophobic, and microbial attachment to the surface is not favoured due to the lack of hydrophobic binding sites) and the elasticity of the polymer chains [33, 34].
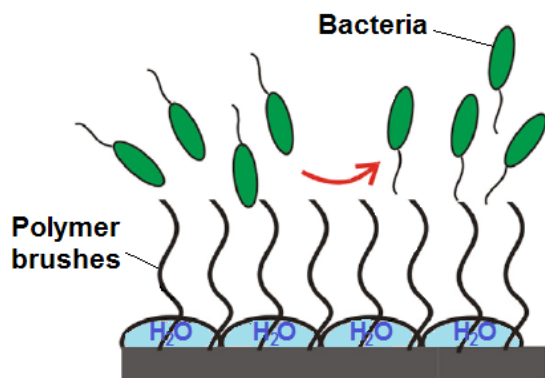

Figure 17. Passive protection of the surface by covalent immobilization or physisorption of hydrophilic well- hydrated polymers [33, 34]

PEG-brush coating

Polymer brushes are quite often poly(ethylene glycol) (= poly(ethyleneoxide)) systems, which are hydrophilic, meaning that water will be attracted into the brush layer and form a repellent layer close to the surface. These polymer brushes may inhibit the adhesion of microbes by up to a 3-log unit reduction in attached microbes. Sometimes low molecular weight proteins and peptides still can penetrate through the PEG (PEO)-layer, providing the bacteria an attachment platform [33, 34].

Nedjadnik *et al.* [34] studied brush-coatings made of a tri-block copolymer of polyethylene oxide (PEO) and

polypropylene oxide (PPO). They observed that the adhesion of *Staphylococci aureus* and *Staphylococcus epidermidis*, as compared to pristine silicone rubber, is reduced on PEO brush-coated silicone rubber (Fig. 18). Yet, Staphylococcal biofilms grew on both brush-coated and pristine silicone rubber, while the viability of biofilms on brush-coatings was higher than on pristine silicone rubber. However, *Staphylococcal* biofilms developed more slowly on brush-coatings and when exposed to high fluid shear, they almost fully detached from the brush-coating in contrast to detachment from pristine silicone rubber. Adhesion and growth of *Pseudomonas aeruginosa* were not significantly affected by the presence of the brush on silicone rubber, although here too the viability of biofilms on brush-coatings was higher. The slow growth of Staphylococci on polymer brush-coatings may allow more time for treatment with cleaning agents and disinfectants before a mature, more resistant biofilm can develop.
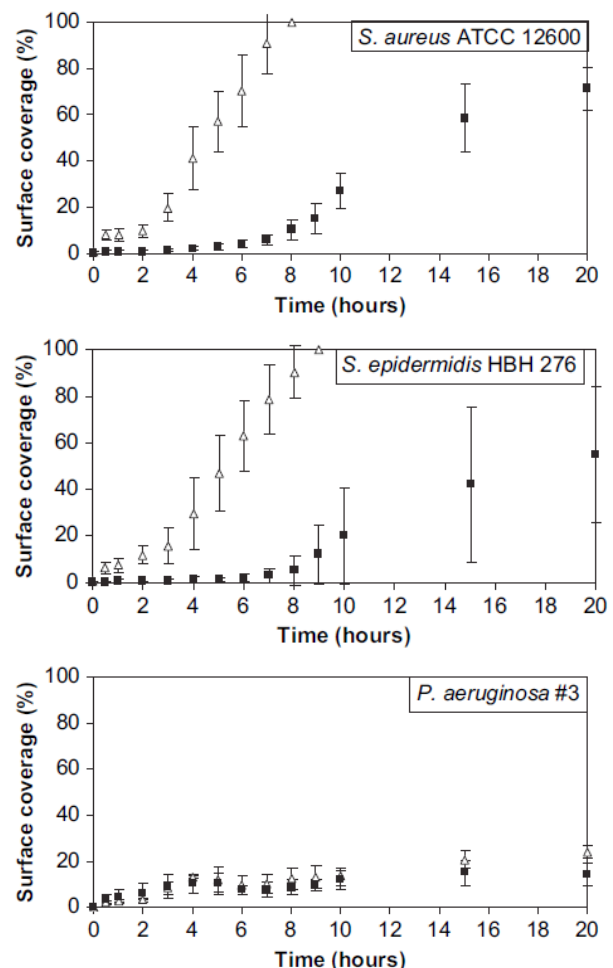
**Figure 18. Surface coverage by biofilms of *Staphylococci aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* as a function of time after initial adhesion and subsequent bacterial growth on pristine (Δ) and brush-coated (■) silicone rubber. Error bars denote the SD over four separate experiments with different bacterial cultures and silicone rubber sheets with and without a brush-coating [34]**

Poly(betaine) brush coating

Recently it has been shown that polymers with zwitterionic head groups can be applied as surface coatings which inhibit biofouling of the surface. They are also called zwitterionic polymer biomimetic surfaces, because they mimic lipid bilayers of biological membranes. Zwitterionic materials, such as poly(phosphobetaine), poly(sulfobetaine), poly(carboxybetaine) (Fig. 19) and other phospholipid polymers having a phosphoryl-choline group, contain both positive and negative charged units which can bind water molecules more strongly and stably via electrostatically induced hydration, as compared to PEG-materials which achieve surface hydration via hydrogen bonding. Due to the large amounts of water that are bound to the zwitterionic head groups, the materials become essentially hydrophilic. This leads to reversible interactions between incident microbes and the surface - discouraging adhesion of microbial cells [22].
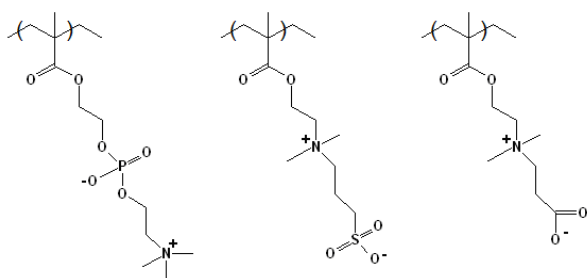
Figure 19. Poly(phosphobetaine), Poly(sulphobetaine) and Poly(carboxybetaine)

Hirota *et al.* [35] observed that, after 1 hour incubation, 2-methacryloyloxyethyl phosphoryl-choline (MPC) co-polymer coated surfaces repressed *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Candida albicans* attachment by at least 95% as compared to uncoated surfaces, and the dramatically reduced bacterial attachment was attributed to the strong hydration capacity of the 2-methacryloyl-oxyethyl phosphorylcholine-based surfaces.

2.1.10 Coatings acting by electrostatic repulsion

Electrostatic interactions can be attractive if the surfaces are oppositely charged. However, electro-static interactions are usually repulsive between bacteria and a substrate surface, because surfaces commonly tend to be negative due to conditioning by organic materials in the surrounding environment or its chemistry and bacteria are usually negatively charged due to cell constituents containing phosphate, carboxyl and acidic groups. At a separation distance of about 10 - 20 nm, the bacterial cell, although weakly held, is kept away from the substrate surface by increasing electrostatic repulsion forces due to an overlap of the electron clouds of both bacteria and surface. Hence, microbial adhesion is commonly impaired on negatively charged substrata

in comparison to positively charged ones. The level of electrostatic repulsion effect not only affects adhesion, but also subsequent biofilm formation [36].

Gottenbos *et al.* [36] studied the antimicrobial effects of both positively and negatively charged surfaces on adhering Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). In Table 6, the initial deposition rates (j_o), percentages of growing bacteria after 2 hours, generation times (g, min.) and desorption rate constants (k_{des}) of adhering bacteria are mentioned. Initial deposition rates were highest for *Staphylococcus aureus* and *Staphylococcus epidermidis,* and generally increased as the substrata became less negatively charged. Under conditions of electrostatic attraction, as on the positively charged PMMA/TMAEMA-Cl, initial adhesion rates were maximal for both Staphylococci.

During the growth phase, proliferating Staphylococci were present on all surfaces from 1 h after the introduction of growth medium. They grew on all substratum surfaces, although the addition of a negative and positive charge to PMMA decreased the relative number of growing Staphylococci by a factor of 4 and 2, respectively. Initial adhesion rates of *Pseudomonas aeruginosa AK1* were the lowest of all four strains, but also increased with decreasing negative charge and were the highest on the positively charged PMMA/TMAEMA-Cl. On the negatively charged surfaces, most of the *Escherichia coli* and *Pseudomonas aeruginosa* cells were proliferating within 30 min. The numbers of *Escherichia coli* increased slowly, because newly formed bacteria desorbed directly from the surfaces. For both *Escherichia coli* and *Pseudomonas aeruginosa*, there was no growth observed on the positively charged surface, and they had a very low desorption rate. For both, desorption was highest for the negatively charged surfaces.

As a conclusion, adhesion and surface growth may be oppositely affected by substratum charge. Positively charged surfaces may attract more bacteria, but this effect is readily counterbalanced by the absence of any growth, at least for the Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) used in the study of these authors. Further, a high ionic strength - which is often the case in the food industry - decreases the repulsive and attractive nature of the electrostatic forces, meaning that its role in bacterial adhesion may become negligible. Further, food proteins and proteins secreted by the bacteria will condition the surface and mask functional groups that reduce cell adhesion.

2.1.11 Coatings with modified surface energy

Hydrophilic and hydrophobic coatings

Hydrophilic surfaces are high surface energy materials, while hydrophobic surfaces are low surface energy
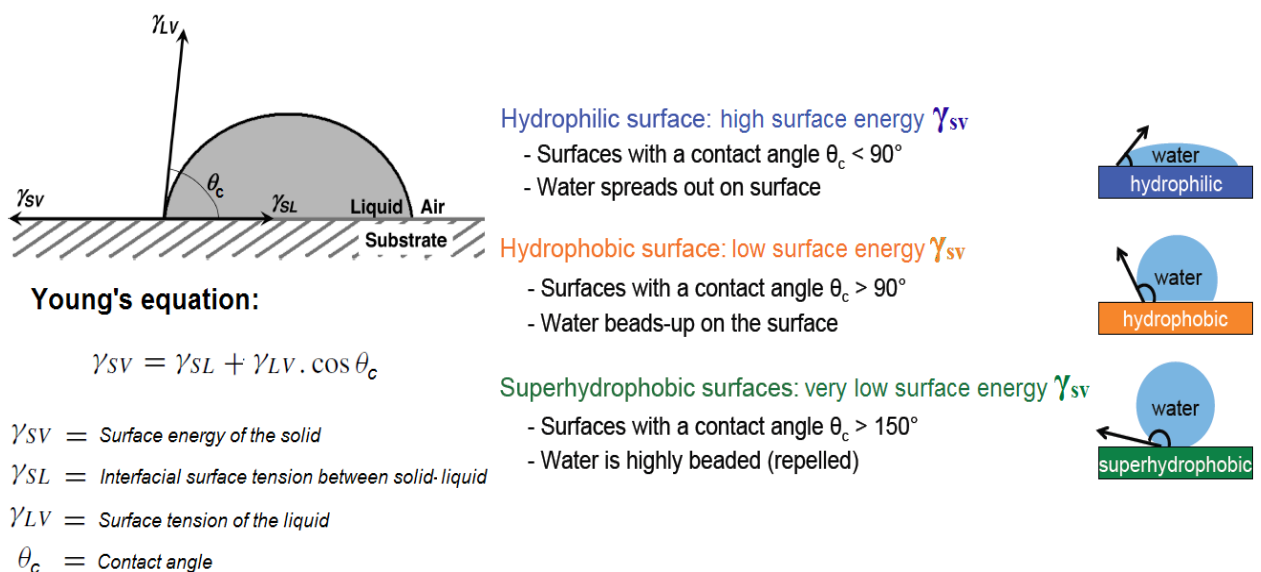
materials (Fig. 20). Hydrophilic surfaces (high surface energy) have been shown to be less quickly populated by free-swimming bacteria than hydrophobic surfaces (low surface energy). Microorganisms contain hydrophobic patches and these may be involved in the adhesion to the hydrophobic surface. However, some bacteria are more hydrophilic (*Staphylococcus aureus, Staphylococcus epidermidis*), and will more preferentially attach to hydrophilic surfaces. Moreover, different strains of the same bacterium may show differences in hydrophobicity/hydrophilicity.

For self-cleaning surfaces, either an exceptionally hydrophilic (contact angle θ ≤ 10°) or exceptionally hydrophobic (contact angle θ ≥ 140°) surface is required. Intermediate contact angles of 30 - 100° do not have easy clean features (Fig. 21). The water droplets slide across the surface, but the sliding action is inefficient at removing the microbes. These surface are significantly easier for microbes to stick to, and possibly form a biofilm [22].
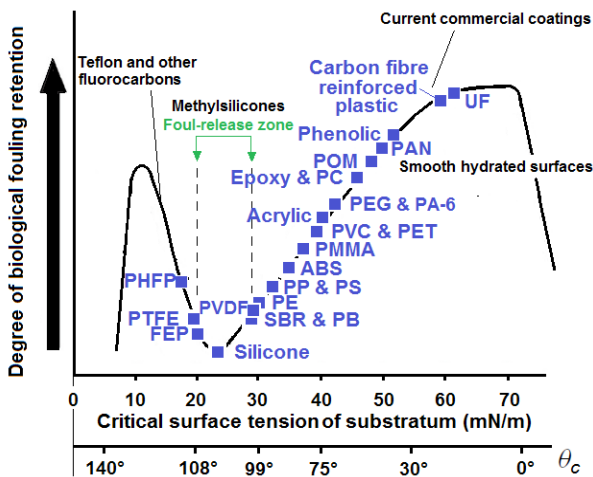
Table 6. Initial deposition rates (j_0), percentages of growing bacteria, generation times (g) and desorption rate constants (k_{des}) of Gram-positive and Gram-negative bacteria on PMMA/MMA (--), PMMA (-) and PMMMA/TMAEMA-Cl (+) polymer films with different charge [36]

Strain	Charge	j_0 (/cm²/s)	% growth	g (min)	k_{des} (/10⁵/s)
S. aureus ATCC 12600	– –	1600	8	41	8
	–	1780	34	32	15
	+	2700	13	39	23
S. epidermidis HBH₂ 102	– –	1900	7	48	22
	–	1360	26	50	12
	+	3630	15	48	17
E. coli O2K2	– –	240	91	24	70
	–	720	59	22	42
	+	1720	0	no growth	2
P. aeruginosa AK1	– –	350	75	32	12
	–	430	70	35	2
	+	660	0	no growth	1

Values measured for j_0, % growth, g, and k_{des} in duplicate experiments were similar within 20, 40, 5 and 25%, respectively.

γ_{LV}

γ_{SV} θ_c γ_{SL} Liquid \ Air

Substrate

Young's equation:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV}.\cos\theta_c$$

γ_{SV} = *Surface energy of the solid*

γ_{SL} = *Interfacial surface tension between solid· liquid*

γ_{LV} = *Surface tension of the liquid*

θ_c = *Contact angle*

Hydrophilic surface: high surface energy γ_{sv}
- Surfaces with a contact angle $\theta_c < 90°$
- Water spreads out on surface

Hydrophobic surface: low surface energy γ_{sv}
- Surfaces with a contact angle $\theta_c > 90°$
- Water beads-up on the surface

Superhydrophobic surfaces: very low surface energy γ_{sv}
- Surfaces with a contact angle $\theta_c > 150°$
- Water is highly beaded (repelled)

water
hydrophilic

water
hydrophobic

water
superhydrophobic

Figure 20. Hydrophilic, hydrophobic and superhydrophobic surfaces

Figure 21. As illustrated in the Baier-curve, surfaces with intermediate contact angles of 30-100° are more prone to microbial fouling, while superhydrophobic, hydrophilic and superhydrophilic surfaces show lower microbial fouling retention [37]

Superhydrophilic and superhydrophobic coatings/ materials

Microstructuring a surface - in other words adding in unevenness or asperities - amplifies the natural tendency of the surface: hydrophilic surfaces become "superhydrophilic" (contact angle $\theta \leq 10^0$) and hydrophobic surfaces become "superhydrophobic" (contact angle $\theta \geq 140^0$)

Superhydrophilicity can be created by irradiating TiO_2 coatings. The holes produced by photoexcitation provoke the apparition of oxygen vacancies which can be filled by adsorbed water to form surface hydroxide groups. The formation of these groups will increase the affinity of water toward the surface and result in complete wetting (water contact angle θ_c 0^0) after UV irradiation (typical application: photocatalytically self-cleaning glasses). Due to the photocatalytic effect, germs also can be destroyed. Superhydrophobic materials can be made in several ways - by coating a surface with a superhydrophobic material, by nanostructuring a surface, by applying nanoparticles to a surface or by a combination of these.

Superhydrophobic coatings are very low surface energy coatings such as special waxes.

Notice, however, that hydrophobic easy to clean materials reduce microbial contamination in the area treated, but does not address the problem of pathogenic microbes which are incident upon the surface. It merely moves them elsewhere, where they will have to be dealt with by other microbiocidal techniques.
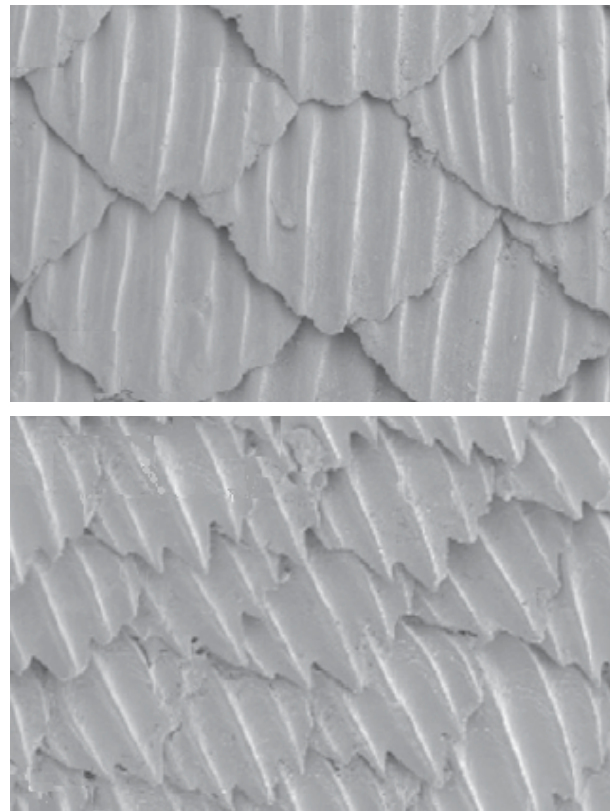
2.1.12 Bio-inspired surfaces with modified surface microtopography and chemistry

The surface chemistry and physical properties of a substratum are both crucial to prevent the recruitment of biofouling organisms. The idea to change surface topography and chemistry as tools to control fouling was taken from marine biology, because the natural surfaces of many marine organisms resist biofouling in these environments. These natural antifouling surfaces use a combination of chemical and physical structures to inhibit biofouling. Hence, surface modification techniques to tailor the surface energy via surface chemistry and surface topography have been developed to study the effects of changes in these surface properties on biofilm formation.

Natural antifouling surfaces

The skin of the approximately 900 species of Elasmobranchii, which include sharks, skates, and rays is embedded with placoid scales. These scales serve several functions including reduction of mechanical abrasion, reduced hydrodynamic resistance and most interestingly protection from ectoparasites. The skin of two members of the porpoise family, i.e., the bottlenose dolphin *Tursiops truncatus* and the killer whale *Orcinus orca* (Fig. 22) forms a system of ridges and grooves oriented transversely to the direction of flow. The natural wavelength of the ridges and grooves is 0.3 to 0.4 mm with a trough to crest wave height of about 10 μm. These topographic features and a mucosal coating secreted by epidermal cells contribute to the antifouling properties of these marine animals.

Figure 22. Scanning electron micrographs of natural textured surfaces: spinner shark skin (photo left), Galapagos shark skin (photo right) [38]

Bio-inspired surfaces with modified surface microtopography Sharlet AF

Sharklet Technologies has developed substrates with engineered surface microtopography under the name Sharklet AF (Fig. 23) that successfully delays bacterial biofilm formation. Sharklet AF uses topography similar to that of shark skin by including rectangular ribs in a diamond-like array. This engineered surface microtopography has nonrandom, clearly defined surface features (2 µm width and 3 µm height, with a spacing of 2 µm in between), typically tailored to the critical dimensions of the fouling organisms. It was at day 14 when bacteria were observed to form small, multilayer colonies within the recesses in order to extend over the protruding features and connect to other isolated colonies. The protruded features of the topographical surface provide a physical obstacle to deter the expansion of small clusters of bacteria present in the recesses [38].

*Superhydrophobic surfaces: Lotus-effect*

Nature uses rough surfaces on some plant leaves to produce a self-cleaning surface, known as the Lotus effect (Fig. 24). The Lotus effect is where water droplets on the surface of the Lotus-plant leaves have exceptionally high contact angles, typically greater than 140^0. Droplets roll and spin across the surface at very low tip angles (5^0 or less), picking up dirt, dust, bacteria and viruses from the leaf surfaces through its spinning action. Scanning electron microscopy shows a significant delay but not completely prevents biofilm formation.

| Day 0 | Day 2 | Day 7 | Day 14 | Day 21 |
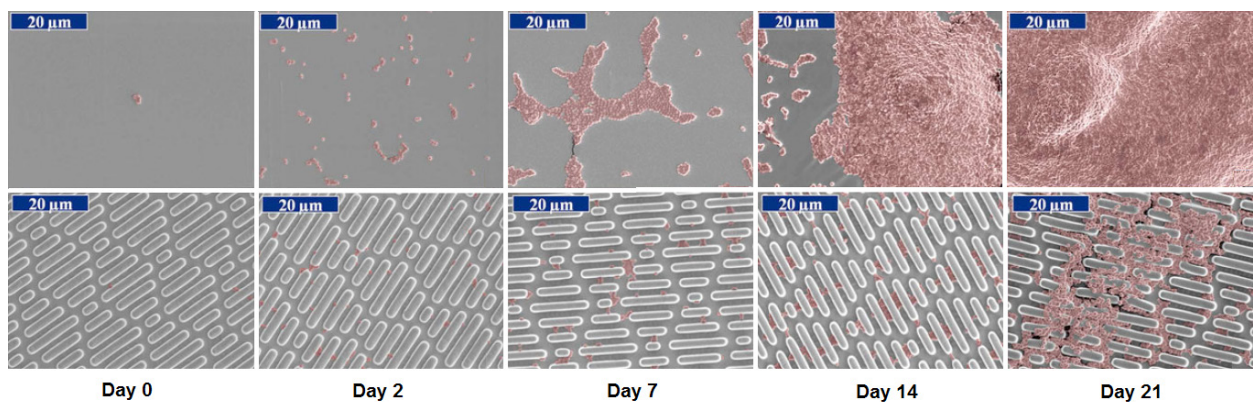
Figure 23. SEM images of the formation of a *Staphylococcus aureus* biofilm on a smooth surface (row above) and Sharklet AF™ surfaces (row below) at respectively day 0, 2, 7, 14 and 21 [38]


Figure 24. The leaves of the Lotus-plant are extremely hydrophobic with droplets rolling and spinning across the surface at very low tip angles (5° or less), picking up dirt, dust, bacteria and viruses from leaf surface
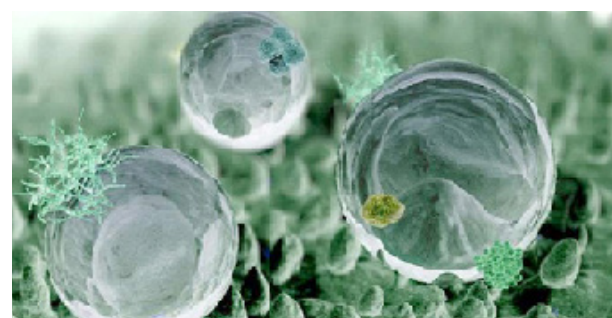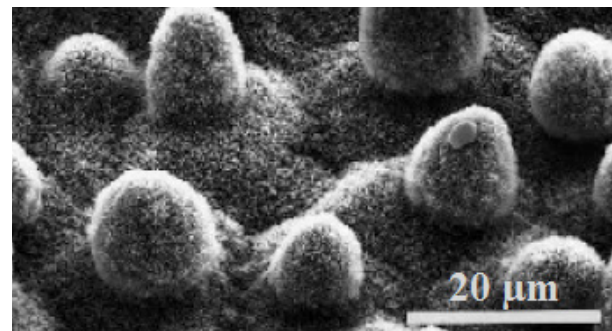
Figure 25. SEM photo of a Lotus-leave with papillae. The microstructural epidermal cells are covered with nanoscopic wax crystals (photo left). The Lotus-effect: contaminating particles adhere to the droplet and are removed when the droplet rolls off the surface (photo right)
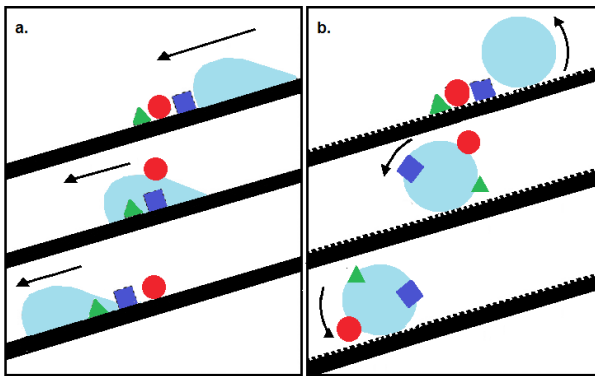
Figure 26. (a) Droplet running down on a flat hydrophobic surface: redistribution of adhering particles by a sliding drop of water on an declining solid surface; (b) Removal of adhering particles by a rolling droplet on a rough superhydrophobic surface [39]

The leaves of the Lotus consist (Fig. 25) of micro- and nanoscale papillae that are coated in a hydrophobic wax. This double structure makes the leaves superhydrophobic and water makes a contact angle of up to 170^0. A hierarchical structure in which the microscale asperities themselves have nanoscale roughness is found to give better superhydrophobic properties, allowing water droplets to remove adhering particles from the surface (Fig. 26). Several methods are available for artificial synthesis of the Lotus effect.

3. Conclusions

- In addition to routine cleaning and disinfection, other strategies to reduce microbial cross-contamination can be considered. Materials with antibacterial properties and coatings reducing adhesion have a beneficial effect on food safety when standard hygiene procedures are not employed correctly.

- However, in contrary to in vitro test results, effects in practical conditions are rather low (≤ 2 - 3 log).

- The antibacterial effect is further reduced once depletion of the antimicrobial component within the material surface occurs. Food residues, scale deposits, bio- and conditioning films may exert a protective effect by prohibiting intimate contact of the microbes with the antimicrobial surface, by forming an obstacle for the passage of the antimicrobials released from the surface and because dead microbial cells accumulating on antimicrobial surfaces may generate debris that neutralizes the antimicrobial effect and allows subsequent bacterial attachment.

- The hygienic effect of antimicrobial materials and coatings reducing adhesion is often insignificant. They rather delay but not exclude the formation of biofilms. They are supplement to, but not a substitute for cleaning procedures and standard infection control practices. Insufficient cleaning just impairs the effects of antimicrobial components. They also will never be a substitute for hygienic design.

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