Hospital Acquired Infections With Multiresistant Microorganisms: UN Interagency Coordination Group on Antimicrobial Resistance Demands Immediate, Ambitious and Innovative Action

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Summary

The present review is mostly focused on decontamination of solid air interfaces in health-care units, such as hospital furniture, door-handles, computer keyboards, textiles etc., although solid liquid surfaces are also of great concern in hospitals such as faucets, showers, pipes and drains, where biofilms occur frequently. New methods, in addition to or as an alternative to appropriate use of disinfectants and antibiotics are required to reduce microbial associated infections and to reverse the dramatic increase in antimicrobial resistance. The requirements for such surfaces mandatory for prevention of hospital acquired infections and the emergence of multi-resistant microorganisms however are high. Many different chemical strategies and technologies for coatings intended to eradicate microorganisms from surfaces are described in the literature. None of these technologies meets the expectations for prevention of hospital acquired infections by formation of self-sanitizing surfaces except the in situ generated biocides on the basis of transition metal oxides embedded into surfaces. Surfaces decorated with metal oxide Lewis acids such as MoO₃, WO₃ and Zinc Molybdate show a broad-band and strong antimicrobial activity resulting in a reduction of the number of colony forming units by 6 – 7 log 10 within 1-3 hours. Their mechanism of action is based on the in-situ generation of H₃O⁺ ions through the reaction with moisture from the air, inspired by the body’s own defense mechanism imitating e.g. the acid coating of the skin. The resulting acidified surfaces have a pH of 4.5 and the H₃O⁺ ions are able to diffuse through the cell membrane of microorganisms where they can distort the pH-equilibrium and transport systems of the cell. In addition by these mechanism also free radicals e.g. oxygen radicals and hydroxyl radicals are formed which result in a synergistic mode of action including a positive zeta potential. This is reflected by an extraordinary fast eradication of microorganisms i.e. a reduction of 5 log 10 within 10 minutes.

Present Recommendations

Health care-associated infections, which are widely seen as preventable and often caused by unsatisfactory hospital conditions or human error, are costing health care facilities more than $30 billion annually and are, more importantly, claiming at least 800,000 lives per year in Europe — that’s 2200 lives each day. To combat the alarming 5% of all hospital admissions that result in readmission due to conditions acquired in health care facilities, all staff and contractors should be well educated on how to minimize the spread of fatal infections [1-3].
One problem remains the definition of “resistance” and how to measure resistance to a biocide [4]. This has yet to be addressed globally, although the measurement of resistance is becoming more relevant, with regulators both in Europe and in the United States demanding that manufacturers provide evidence that their biocidal products will not impact on bacterial resistance. Alongside in vitro evidence of potential antimicrobial cross-resistance following biocide exposure, our understanding of the mechanisms of bacterial resistance and, more recently, our understanding of the effect of biocides to induce mechanism(s) of resistance in bacteria has improved.

In order to block the spread of multi-resistant microorganisms in the hospital, cutting off the transmission route is a particularly important strategy along with actively treating the infective source based on the sensitivity of the particular strain, which is determined by characterising the strain for drug resistance. As we cannot rely on adequate eradication of microorganisms by disinfectants any highest emphasis is now put on adequate hand hygiene measures [5]. This however would require 50 - 80 times hand washing and disinfection. Over recent years, researchers have noted a steady rise in the number of serious infections caused by ethanol tolerant strains which appear to result in large part from adaptive and evolutionary changes in cell membrane composition [6]. The development of alcohol-tolerant strains has the potential to undermine the effectiveness of alcohol-based disinfectant standard precautions.

Considering the above information we have to reassess preventive measures for hospital acquired infections and to curb the dramatic increasing rate of emerging resistant microorganisms. It is obvious that we need a new, innovative approach to combat this problem which is jeopardising our clinical medicine.

Technologies for “self-sanitizing surfaces”

The formation of self-sanitizing surfaces - the correct term for surface which eradicates microorganisms from a surface without disinfectants - seems to be a promising technology providing a broad spectrum of activity, long lasting to permanent antimicrobial activity without induction of resistance.

The requirements for self-sanitizing surfaces are high

Broad antimicrobial activity, against Gram-positive, Gram-negative microorganisms, irrespective of their antibiotic susceptibility, fungi, legionella, Aspergillus spp., virus documented by the RODAC plate method.

- Fast eradication of microorganisms i.e., minimum 5 log 10 reduction within 1 hour: “self-sanitizing” surfaces must actively eradicate microorganisms on a surface within a reasonably short period of time. This should occur within less than 3 hours.
- Reduction of adherence, blockage of proliferation and biofilm formation is an additional important feature – however as a single feature it is in no way sufficient.
- Activity against a high inoculum size of 10^9 CFU on an area of 3 cm².
- No induction of resistance. This is of crucial importance
- Nontoxic, skin and soft tissue compatibility, no allergenicity, sbD (safe by design)
- Long lasting/permanent antimicrobial activity
- Water-, acid-, alkaline-, alcohol insoluble, UV Light stabile
- Activity in spite of soiling, cleanable with detergents
- Uncomplicated technical processability, heat stabile up to 400°C, non-corrosive
- Physical stability, activity irrespective of sweat, grease, blood, pus
- Authorisation by the European commission on biocidal products. no nanoparticles
- Favourable cost/benefit analysis

A number of technologies have been proposed:

Active drug eluting agents (e.g. ions or nanoparticles of silver, copper, zinc, or antibiotics, chloride, iodine, disinfectants). Active eluting agents must be incorporated into the metabolism of microorganisms and are eluted from the polymer of the surface: This shows crucial disadvantages:

The activity is limited to a short period of time. For silver this means duration of activity of 3 weeks [7]. Copper endowment of a surface has been investigated and preliminary results show a substantial reduction in the number of HAI which establishes the proof of concept. However adverse events have been observed [8]. Besides copper has a

- Poor antimicrobial activity. This activity is somewhat improved by alloys e.g. combination with zinc or tin but still does not meet the requirements.
- Copper is cytotoxic in use for implantable biomaterials.
- The surfaces are rapidly oxidizing and require
continuous cleaning.

- Copper cannot be used in cable insulations
- Copper cannot be used as transparent coatings of surfaces.
- Hospital surfaces are preferably white, the color of copper is not accepted by the majority of patients

Other drug eluting agents e.g. Polybiguanides, halogenated phenols, and polyethyleneimines have been immobilized on surfaces but show serious adverse effects on patients.

- PHMG: The use of Polyhexamethylene guanidine hydrochloride, resulted in severe/lethal pulmonary toxicity [9,10].
- Halogenated phenols (HP): are responsible for endocrine and neuronal persistent organic pollutant effects; it is a suspected carcinogen [11,12].
- Polyethyleneimine (PEI) is extremely cytotoxic to eukaryotes by two different mechanisms: the disruption of the cell membrane leading to necrotic cell death (immediate) and disruption of the mitochondrial membrane after internalization leading to apoptosis [13,14].

This approach is also referred to as biomimetic with respect to the activity of chitosan, a polysaccharide derived from exoskeleton of crustaceans or cell walls of fungi. Chitosan, an amino polysaccharide biopolymer, has a unique chemical structure with a linear polycation with a high charge density, reactive hydroxyl and amino groups as well as extensive hydrogen bonding. It displays good biocompatibility, physical stability and processability. Chitosan is known for its antimicrobial activity by penetration of the cell wall and interaction with DNA, inhibiting DNA transcription and ultimately protein synthesis [15,16]. However the application of chitosan is only possibly in the form of nanofibers or nanorods which however cannot be stable anchored on the surface of e.g. a polymer, glass, stainless steel. Nanoparticles of chitosan cannot be incorporated into polymers.

Nanotechnologies in general are subject to approval by the Biocidal product regulation (BPR) of the European Union by special requirements. They are time consuming and expensive. None of the nano-products passed the biocidal regulation up to this point in time.

Nano-coatings are generally not heat resistant, difficult to manufacture and expensive.

Anti-adhesive-hyperhydrophobic surfaces have been designed to reduce the adhesion force between bacteria and a solid surface to enable the easy removal of bacteria before a biofilm layer is formed. Attachment of bacteria or cells starts with an initial adsorption of proteins onto the material surface. Strategies to prevent protein attachment include superhydrophobic surfaces, often augmented by a hierarchical nanostructure as well as zwitterionic polymers. It has to be emphasized that microorganisms are deposited by the hand of the personnel. Such surfaces may suppress health care associated infections by blocking transmission paths involving surfaces, but they will not reduce the number of germs on the surface by killing them. For prevention of recontamination of the hands of the personnel, rapid eradication of microorganisms is mandatory [17].

Quaternary ammonium compounds (QAC) must be excluded from further considerations as these products induce cross resistance with antibiotics by induction of efflux pumps and may even enhance the growth of microorganisms (listeria monocytogenes) on surfaces [18]. QACs are toxic to a lot of aquatic organisms including fish, daphnids, algae, rotifer and microorganisms employed in wastewater treatment systems. Antibiotic resistance has emerged in microorganisms due to excessive use of QACs in household and industrial applications. The occurrence of QACs in the environment is correlated with anthropogenic activities, such as wastewater discharge from Wastewater Treatment Plants (WWTPs) or single source polluters. In addition a number of serious adverse events have been described after uptake e.g. coma, convulsions, hypotension and death, hemolysis, allergies, anaphylactic reactions, contact dermatitis. The mechanism of resistance transferred to antibiotics is due to induction of efflux pumps which covers also the majority of antibiotics [19].

In essence the elution of the antimicrobial compounds necessary for incorporation into the metabolism of microorganisms requires at least a moderate water solubility for biocide release and hence a hydrophilic surface. As the antimicrobial agents must be incorporated into the metabolism of microorganisms, one crucial consequence is the high risk of induction of resistance which is virtually inevitable by other mechanisms of activity.

Contact-active surfaces exhibit antimicrobial activity without releasing biocidal substances. Several mechanisms are believed to take place in contact-
active surfaces [20]. Nanostructures are frequently required, since effective air entrapment in the three-dimensional nanomorphology (nanopillars) renders them superhydrophobic and slippery. On inherently nanostructured hydrophilic aluminium, adhesion forces of bacteria were reduced by a factor of 4 down to 2-4 nN compared to the electropolished flat surface, resulting in an 88% reduction of colony-forming units (CFUs) for Staphylococcus aureus [21]. This effect was even more pronounced after applying a hydrophobic Teflon coating, yielding a 99.9% reduction under flow conditions. Nanostructured surfaces were also prepared using electrospun polystyrene nanofibers [22]. With oxygen plasma treatment, a superhydrophilic surface can be generated, which exhibits limited Escherichia coli attachment due to a negative zeta potential of -40 mV. After fluorination, a superhydrophobic surface can be obtained, which exhibited self-cleaning ability against bacteria, where the initially adhering bacteria were effectively removed with constant subsequent washing [21]. This however is not achievable under clinical conditions.

Mechanisms are dependent on:

• (i) A so-called spacer effect, where the biocidal group is attached to the surface through a polymer chain, allowing the biocide to reach the cytoplasmic membrane of the bacteria and to perforate them [22-24].
• (ii) Alternatively, positively charged QACs, e.g. 3-aminopropyl trimethoxysilane grafted to cellulose nanofibers, can detach phospholipids from the cell membrane and thereby kill the bacteria [25].

The problem which arises with “spacers” is that the activity of the spacer effect is obliterated by grease, proteins, sweat, pus, blood and does not sustain cleaning with tensides. As already indicated the consequences of the use of QACs are the induction of resistance genes in bacteria. The activity of chitosan has been investigated and a poor antimicrobial activity has been found. Last not least these compounds are available only as nanostructures which pose serious problems with acceptance as biocidal products. These technologies although attractive from a theoretical point of view lack practicability and are not applicable for self-sanitizing surfaces for prevention of hospital acquired infections.

It has been suggested to combine two functional principles to achieve synergistic effects, e.g. by embedding biocidal substances into anti-adhesive surfaces. Today, the majority of chemical modifications includes hydrogels or polyethylene glycol (PEG) to repel approaching microbes, metals (in particular, silver and copper), antimicrobial peptides (AMPs), quaternary ammonium compounds (QACs), and various nanoparticles [26,27]. Hydrophobic parts of a surface can act similarly to QACs by deforming the membrane through adhesion. The agents must be insoluble in water-, alcohol-, detergents, acid and alkaline, in addition they must show UV light stability. Antimicrobial agents adjacent to surfaces have the risk of abrasion with cleaning which is not achievable with the majority of the above mentioned compounds.

The topography of a surface can by itself significantly affect its hygienic status, either in a beneficial manner (reducing microbial retention) or otherwise (increasing retention). As such, modifications of surfaces to enhance antimicrobial properties should always take into account the effect of surface wear on subsequent fouling and cleanability. Therefore, efforts should be undertaken to characterize typical wear, assess interactions with the most likely microorganisms in that environment, and define the adverse effects of most appropriate and least damaging cleaning and sanitizing regimes.

**Anchoring antimicrobial agents by polymer brushes:** The rationale behind the use of polymer brushes is the observation that antimicrobial molecules lose much of their activity, once attached to a surface. When providing an anchor for the active molecule through a flexible covalently bound polymeric chain, the active molecule should still be able to reach the site of action at or within the bacterium, e.g. by penetrating its cell wall, but leaching is still suppressed [28]. Important parameters for polymer brush anchors are chain length and chain density. Polymer brushes have been shown to be effective for anchoring QACs or AMPs. Antimicrobial peptides (AMP) also known as ß defensins are comprised of 38 amino acids, formed by human epithelial cells upon contact with microorganisms. These amphophilic acid peptides are the responsible antimicrobial agents of the mucosal immunity and are part of the body’s defense mechanisms. The idea behind is attractive: Problems arise with the possible use of AMPs in clinical medicine. AMPs are not easy to be obtained. Embedding of AMPs in a polymer is again not feasible [29,30].

• AMPs must be incorporated into the metabolism of microorganisms. The activity is the disruption of the cell wall. In our investigations - in contrast to a
AMPs must be eluted from the surface in order to effectively penetrate bacterial membranes. Therefore the activity is limited to a few days if they are not constantly reproduced by epithelial cells [31].

- AMPs are not heat stabile.
- AMPs are not easy to be obtained. They could be obtained as Magainins from frog skin with very limited availability [32].
- Synthesis of AMPs is not solved as AMPs are lethal factors for microorganisms synthesizing antimicrobial peptides. Unpublished own investigations over 2 years disclosed that synthetic AMPs induce fast resistance against microorganisms – these microorganisms are in turn also insensitive to natural AMPs produced by the body.

**AMPs don’t withstand cleaning with detergents**

Surface-initiated atom transfer radical polymerization (ATRP) has been used to prepare copolymer brushes based on 2-(2-methoxyethoxy) ethyl methacrylate (MEO₂MA) and hydroxyl-terminated oligo(ethylene glycol) methacrylate (HOEGMA). These coatings were subsequently functionalized by a natural antibacterial peptide, magainin I, via an oriented chemical grafting on hydroxyl groups, which maintains the activity of the peptide. The antibacterial activity of the functionalized brushes was successfully tested against two different strains of gram-positive bacteria. No information is available regarding the duration of activity, resistance to soiling and cleaning. Polymer brushes don’t withstand cleaning with detergents or alcohol. There is no useful clinical application for the use QACs or antimicrobial peptides (AMP) for self-sanitizing surfaces for the prevention of hospital acquired infections.

Using surface-initiated atom transfer radical polymerization, QACs with charge densities of >1.5e10¹⁵, accessible quaternary amine units/cm² were anchored through poly-2-(dimethylamino) ethyl methacrylate chains. Interestingly, these surfaces were bioactive even though the polymer chains were too short to penetrate the cells with envelope thicknesses of 46 nm for Gram-negative *E. coli* and 45.55 nm for Gram-positive *Bacillus subtilis* [33]. This demonstrates that surface charge density can be more important than chain length. On the other hand, it was clearly shown that *N*-alkyl-pyridinium exhibited high antimicrobial activity when anchored through a 750 or 25 kDa poly-ethyleneimine (PEI) but showed no activity when using the 2 kDa analogue. Therefore, only long-chained, moderately hydrophobic, immobilized polycations exhibit microbicidal activity. Interestingly, polycationic polymer brushes are not subject to existing mechanisms of resistance such as multidrug- resistance pumps or multidrug tolerance protein-expressing cells, presumably since there are no analogue structures in nature [34].

Polycations on a surface are not heat resistant and can’t be extrusion molded. In addition the activity is obscured by many compounds e.g. grease, sweat, pus, blood proteins etc. which are abundantly coating hospital surfaces close to patients, besides the technology is complex and expensive. These compounds cannot be added to various coatings.

Existing antimicrobial modified surfaces suffer from a number of limitations, including the rapid release of the adsorbed antimicrobial agent in the first hours after implementation. This results in a relatively short duration of antibacterial activity. Furthermore, cytotoxicity has been reported for copper ions on mammalian cells which limit their application.

Current approaches to decrease microbial contamination on inanimate surfaces are either preventive or biocidal. The first category aims at preventing adhesion of the infectious agents on the surface through an anti-adhesive coating. These include poly (ethylene glycol), diamond-like carbon, self-cleaning surfaces (Lotus effect), and amphiphilic polymer coatings. Since the infectious agents are not eradicated, their presence poses still a risk for patients. A more reliable approach is the use of biocidal coatings on materials surfaces.

For some of the technologies the mechanism of antimicrobial activity is still under investigation and there is not enough information available on whether antimicrobial activity happens directly at the surface or whether small amounts of the active compounds are released into the test media where they will exert their antimicrobial activity, or whether both mechanisms are acting in parallel.

Nanoparticles are frequently constructed but face the problem of approval by regulatory authorities.

The spectrum of activity has to be very broad. Surfaces are contaminated with a variety of different microorganisms which affect each other limiting the spread and growth. If only one species is eradicated
the remaining microorganisms have the possibility to proliferate and spread uninhibited. Limited spectrum of activity is therefore unacceptable.

These findings underscore the importance of consistently being aware of the types of strains present in an individual clinic over time as well as monitoring the disinfecting regimens that are most effective against the specific strains. Neglecting this type of monitoring may lead to more serious consequences as bacteria acquire more drug-resistant genes or become otherwise tolerant to disinfectants used in hospital settings.

As all of the above described technologies did not meet the requirements for successful eradication of microorganisms from contaminated surfaces it was necessary to search for technologies without the above described limitations.

**In situ generated biocides by use of transition metal oxides**

**Antimicrobial activity using the concept of Brønsted-Lowry lewis acids with MoO₃**

Transition metal oxides are eager electron donors: electron transfer occurs to ambient water and results in the formation of various products detrimental to the viability of microbes either by formation of an acid pH, the formation of a variety of free radicals or the formation of a positive Zeta potential and paramagnetic ions [35-38]. The anti-microbial effect of acids is well known. The idea was to use acidic surfaces for antimicrobial properties imitating the body’s own defense mechanisms e.g. acid coating of the skin [39-41].

In situ generated biocides by transition metal oxides provide a number of favorable properties required for eradication of microorganisms from surfaces:

- Formation of an acid surface by formation of H⁺ ions from ambient water. Acidification of water molecules - imitating the acid coating of the skin results in an acid surface pH:. The surface pH can be investigated by a surface pH electrode (Sentex)

A pH between 4.6 and 4.2 is regularly achieved by transition metal oxides incorporated into a polymer or a coatings on the surface of samples. This corresponds to the pH of the acid coating of the skin [35].

- The resulting active substances in addition to acidified water molecules can also be free radicals e.g. oxygen radicals, hydroxyl radicals. There is a synergy on antimicrobial activity by the various mechanisms [39-41].
- A positive Zeta potential has been observed. This is the reason for the rapid bactericidal activity of surfaces [42].
- Paramagnetic Ions: documented by EPR spectra have the least clear described antimicrobial activity but contribute to the synergistic activity of the above described technologies [43].

Most important: No induction of resistance has been observed by these mechanisms of antimicrobial activity!

**Antimicrobial Surfaces for Health Care Applications using Transition - metal oxides**

It is known that MoO₃ reacts with water, e.g. from the ambient air to form H⁺ ions. Therefore, MoO₃ incorporation into polymers e.g. thermoplastic polyurethane, silicone and epoxy resin has been useful for the antimicrobial endowment of surfaces. A similar effective antimicrobial activity has been detected by incorporation of tungsten oxides onto polymers. However the oxygen saturated tungsten yellow oxide shows a low antimicrobial activity in contrast to the 5% oxygen deficient tungsten blue oxide.

The principle of the technology is shown in the equation: MoO₃ + 3H₂O = 2H₃O⁺ + MoO₄²⁻.

Figure 1 describes MoO₃ (yellow dots) applied to a surface for antimicrobial activity in thermoplastic polyurethane (TPU). The yellow dots indicate MoO₃ which is incorporated either into the polymer close to the surface or in a coating. Electrons emanating from the polymer transform ambient water into acid water molecules on the surface (H₃O⁻) but also into various free radicals (oxygen radicals \( \cdot O^2 \), hydroxyl radicals \( \cdot OH \)) which are formed on the surface.

![Figure 1](image-url)

**Figure 1:** Schematic illustration of the technology: MoO₃ (yellow) is embedded in a matrix (blue). At the surface of the compound, germicidal H⁺ ions will be formed from ambient humidity and various free radicals.
Hydratide Oxonium-Ions, \((H_3O^+)\)(OH\(_2\))\(_n\) (n=1,3) strip their hydrate water in contact with microorganisms, finally the water molecule. The naked protons penetrate the phospholipid bilayer of bacterial microorganisms by denaturation of the protein envelope. Protons destroy the fimbria preventing adherence of germs on surfaces. Protons also inhibit the activity of vital enzyme systems within the microorganisms which require a narrow pH environment. This is called protolysis (Coagulation necrosis).

Among the various metal oxide semiconductors molybdenum trioxide attracted attention due to its multifaceted functional properties and its application as catalyst in various selected oxidation reactions, sensors and photochromic and electrochromic systems.

The photooxidation of water to oxygen and protons in the presence of reducible additives by tungsten has been detected and demonstrated the photocatalytic activity of the powders. In this manner, WO\(_3\) is one of the interesting electrochromic inorganic materials. WO\(_3\) film exhibits a broad range of functional properties, such as small band gap energy (2.4-2.8 eV), deeper valence band (+3.1 eV), stable physicochemical properties, and strong photocorrosion stability in aqueous solution. The characteristics of WO\(_3\) film make them suitable for electrochromic layers e.g. in a smart window. Many studies pertaining to WO\(_3\) structures are mainly aimed at the formation of high active surface area in view of their use in electrochromic applications. Zinc molybdate (ZnMoO\(_4\)) crystals again are strong semiconductor inorganic solids and show extensive electronic properties in various scientific fields.

**Positive Zeta potential**

Zeta potential is a scientific term for the electrokinetic potential in colloidal dispersions. The usual units are volts (V) or millivolts (mV). From a theoretical viewpoint, the zeta potential is the electric potential in the interfacial double layer (DL) at the location of the slipping plane relative to a point in the bulk fluid away from the interface. The zeta potential can also be described as the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle [42].

Electronegative charged microorganisms are attracted by the positive surface charge. Upon contact the phospholipid bilayer of microorganisms is disrupted resulting in immediate death of microorganisms. This has been determined by laser scanning microscopy where the death of a large inoculum of microorganisms has been shown within less than 10 minutes. The technology even eliminates microorganisms embedded in a biofilm.

**Paramagnetic Ions:** The EPR spectra obtained indicate that there are considerable concentrations of unpaired spins (i.e., free radicals and also in some cases transition metal ions) present in all of the polymer samples tested. This was observed consistently with all samples, with only low levels of unpaired spins seen in the empty tubes. The nature and intensity of the signals vary with the polymer samples as would be expected from their different chemical composition and treatments. [43]

The yellow tinted mixed oxides Mo\(_x\)W\(_{1-x}\)O\(_3\) contain substantial amounts of Mo\(^{5+}\)-ions. The molar Mo\(^{5+}\)-concentration of samples are calcinated at 300°C. Jet milling in ambient air results in an oxidation of Mo\(^{5+}\) to Mo\(^{6+}\). The gradation of Mo\(^{5+}\)-concentrations in the three tested polyoxometalates is parallel to their antimicrobial activity. 3Mo:1W- and 1Mo:1W-samples are nearly identical. The endowment of dyes, lacquers, and plastic surfaces with transition metal oxides containing paramagnetic Mo\(^{5+}\)-ions is an efficient antimicrobial agent nontoxic and non-eluting. The same principle is applicable also to the oxygen deficient Tungsten W\(^{5+}\) blue oxide.

All these electrochemical properties/high energy potentials have been found to disrupt the phospholipid bilayer of microorganisms by electron transfer and high voltage surface charges documented by a positive zeta potential and electro paramagnetic properties documented by a high number of electro spins. This suggested that besides the H\(_3O^+\) there exist additional mechanisms of antimicrobial activity like paramagnetic Mo\(^{5+}\)-ions. Results of electro paramagnetic resonance (EPR) – spectroscopy supports this theory. From 77 K registered spins per Gram the molar concentration of paramagnetic Mo\(^{5+}\)-Ions was calculated.

Electron microscopic appearance of the effect of transition metal oxides on microorganisms (S. aureus and *E. coli*) is documented in figure 2.
Figure 2: Electron microscopic appearance of microorganisms on a surface endowed with transition metal oxides.

a) *S. aureus* on an unmodified surface of a PU tube (60 min), b) distorted cell of *S. aureus* on PU tube containing 5.0 wt.-MoO₃ 60 min after incubation,  
c) *E. coli*. on an unmodified surface of a PU tube (10 min),  
d) distorted cells of *E. coli*. on a PU tube containing 5.0 wt.-MoO₃ 10 min after incubation.

Water solubility:

MoO₃ has a low water solubility, which is even lower when it is incorporated into polymers. Tungsten and its suboxides are virtually water insoluble. Also Zinc Molybdate is water insoluble.

The incorporation of molybdenum trioxide into the tungsten crystal lattice in various concentrations renders Molybdenum oxides water insoluble. Elution experiments of a 100 cm² surface for 7 days in 1 l of deionized water disclosed > 0.0002 mg/l of Mo oxide and for Tungsten oxide below the level of detection. This also means a long lasting antimicrobial activity [44].

No toxicity was detected in extensive investigations [45,46]. Compounds containing Mo oxides and W oxides in various concentrations in the same crystal lattice are considered a new compound called polyoxometalates with additional properties.

Tungsten trioxide and polyoxometalates MoₓW₁₋ₓO₃ show similar antimicrobial activity compared to Molybdenum trioxide. This suggested that besides the H₃O⁺ there exist additional mechanisms of antimicrobial activity like paramagnetic Mo⁵⁺-ions and a positive Zeta potential. Results of electroparamagnetic resonance (EPR) – spectroscopy support these findings.

Uv light stability

Zinc Molybdate as well as tungsten blue oxide and polyoxometalates are UV light stable in contrast to molybdenum trioxide. This is important for endowment of melamine resin coated hospital furniture.

Experimental Investigations

Since the antimicrobial mechanism of in situ generated biocides is non-specific, there is a broad spectrum of activity including Gram positive and Gram negative microorganisms irrespective of their resistance against antibiotics, spores, fungi, Legionella, viral organisms e.g. influenza (H1N1, H5N1). As the activity is not based on incorporation into the metabolism of microorganisms, bacteria don’t develop resistance against this mechanism as is the case with antibiotics and organic biocides. In addition also microorganisms embedded in a biofilm are eradicated which is not achievable with antibiotics and disinfectants. Microorganisms in a biofilm are hibernating and don't take anything up from the environment.

In this work, MoO₃ was mixed by 2+/⁻0.1 (weight)% into thermoplastic polymers TPU, PP and PVC, liquid silicone and epoxy resin. The polymer samples were obtained on an extruder (Berstorff ZE25A), and the polymer was shaped into 10 × 10 cm² plates using a heated press.

The epoxy resin was a two-component Silicone system consisting of 60 parts resin and 40 parts hardener were obtained. The addition of 1 weight% urea provided improved hydrophilicity.

Determination of surface characteristics due to transition metal oxides

- Determination of surface pH: This has been achieved either by a Sentex surface electrode WTW or by the use of a pH paper. With both methods a pH of 4.5 ± 0.2 has been determined in a drop of distilled water at the surface of a sample e.g. melamine resin containing 2% of transition metal oxides e.g. Zinc molybdate or laquers containing 0.25% of Zinc molybdate. Also samples with 1% Molybdenum trioxide in Thermoplastic.
polyurethane showed a surface acidity of pH 4.5.

- Determination of free radicals e.g. hydrogen peroxide or $\text{-O}_2^{-}$: 0.05 ml of Potassium Iodide is applied to surfaces containing free radicals formed from ambient water under the influence of transition metal oxides e.g. zinc molybdate. Free radicals turn the transparent Potassium Iodide solution into a precipitant of yellow tinted crystals on the surface.

**Determination of antimicrobial activity**

The microbiological tests were carried out with $10^9$ CFU/ml (CFU = colony forming units) of three reference bacteria:

- **Staphylococcus aureus** ATCC 25923 (S.a., a typical germ on human skin, Gram-positive) also Enterococci e.g. Streptococcus faecium are within the spectrum of activity.
- **Pseudomonas aeruginosa** ATCC 15442 (P.a., a typical germ in air, soil and water, Gram-negative)
- **Escherichia coli** ATCC 25922 (E.c., a typical germ in excrements and a lead indicator for fecal contamination, Gram-negative)
- Carbapenem resistant Klebsiella spp. just as numerous other multi-resistant gram-negative microorganisms (e.g. Acinetobacter Baumanni, Serratia marcescens) are also covered by the spectrum of activity.
- Similar experiments have been performed with numerous fresh clinical isolates of *E. coli*, *E. hirae*, *Pseudomonas aeruginosa*. The determination of anti-microbial effectiveness was done by the drop-on method and the roll-out method, which are cost-effective and semi-quantitative tests resembling the “real life situation”.
- Also Legionella spp, Candida spp, are included in the spectrum of activity.
- Activity of transition metal oxides against a number of viral agents has been demonstrated (Influenza, Herpes virus, Respiratory syncytial virus, Epstein Barr Virus EBV)
- Polyoxometalates show strong activity against bacterial microorganisms and excellent activity against moulds. Also additional virus e.g. Hepatitis B and C are included in the spectrum of activity
- Activity against a variety of sweet water and sea water algae has been documented. Samples containing 2% of polyoxometalates are free of algae during a 2 years observation period after immersion in sea water and sweet water in contrast to controls which were coated with algae within 2 weeks.

Various products of transition metal oxides are available with similar antimicrobial activity and can be used according to the characteristic properties.

**I Molybdenum trioxide MoO$_3$**

Molybdenum trioxide is available as a light blue/gray powder with particle size of 2 - 5 µm.

**Advantage:** strong antimicrobial activity with addition of 2% to various polymers e.g. TPU for ECG lead wires which has been documented in numerous experiments. Thermal induced fracturing of the hydrates of molybdenum trioxide retains the orthorhombic crystal structure with particle sizes of 0.2 µm. The incorporation in various coatings e.g. liquid silicone results in a transparent antimicrobial addition to glass and stainless steel (Lambda half).

Acid surfaces formed by Brønsted-Lowry acids already prevent effectively adherence of microorganisms on surfaces, block proliferation and biofilm formation.

The powder is inexpensive and available in unlimited quantities.

**Disadvantage:** Molybdenum trioxide shows a water solubility of 0.003 mol/l at a pH value > 7.45. Elution experiments of a 100 cm² TPU surface containing 2% MoO$_3$ for 7 days in 1 l of deionized water disclosed a concentration of 0.0002 mg/l of Mo and tungsten below the level of detection. Molybdenum oxides are not UV light stable.

The safety data sheet shows carcinogenicity: A detailed evaluation of the data has shown that carcinogenicity is seen after inhalation of 100 mg/m³ of submicron particles over a period of 6 hours/day 5 days per week for 2 years in rats. 50% of rats were affected by pulmonary malignancy; no mice were affected by the same study protocol [46].

Molybdenum oxides incorporated into composite materials e.g. TPU, silicone, lacquers or various coatings are not present as submicron particles. In the rare case that molybdenum oxides are eluted from a polymer although the water solubility of molybdenum oxides, incorporated into composite materials is exceedingly low, molybdates and not molybdenum oxides are eluted, molybdates don’t show carcinogenicity.
This however is of relevance for master batch production. A dust free application of Molybdenum oxides into composite materials is required but is considered state of the art.

The toxicity of Molybdenum is remarkable low. It has to be emphasized that molybdenum – as well as zinc are essential trace elements in the body [47].

Molybdenum is a stabilising molecule in several enzymes important to animal and plant metabolism for elimination of sulfur from the body:

- Sulfite oxidase catalyses the oxidation of sulfite to sulfate, necessary for metabolism of sulfur containing amino acids. Sulfite oxidase deficiency or absence leads to neurological symptoms and early death.
- Xanthine oxidase catalyses oxidative hydroxylation of purines and pyridines including conversion of hypoxanthine to xanthine and xanthine to uric acid.
- Aldehyde oxidase oxidises purines, pyrimidines, pteridines and is involved in nicotinic acid metabolism.
- Low dietary molybdenum leads to low urinary and serum uric acid concentrations and excessive xanthine excretion.
- Molybdenum functions as an electron carrier in those enzymes that catalyse the reduction of nitrogen and nitrate.

Hazard statements for Molybdenum powder for incorporation into polymers of coatings: Causes eye irritation and may cause respiratory irritation.

Precautionary statement: Wear protective gloves/ protective clothing/ eye protection/ face protection.

Molybdenum trioxide is not UV light stable.

Molybdenum trioxide can be used for an antimicrobial activity of any surface which is not in constant water contact. The light blue color cannot be concealed by additional color pigments.

Submicron particles of Molybdenum can be produced by thermal fracturing [48]. This results in particle sizes of 0.2 µm. After addition to various coatings this results in a transparent highly active antimicrobial surface.

II Tungsten trioxide WO₃

Tungsten Trioxide has been investigated for in situ generated biocidal activity. Initial experiments with the oxygen saturated Tungsten yellow oxide disclosed limited antimicrobial activity. Further investigations disclosed that the oxygen deficient tungsten blue oxide WO₂.₇₅₋₂.₉₀ shows antimicrobial activity comparable to Molybdenum trioxide.

Tungsten trioxide is a dark blue powder and is available with 5 µm particle size.

Advantage: Tungsten blue oxide is completely water insoluble, the safety data sheet shows no adverse reactions except for necessary individual contact measures. Again this is only of relevance to the master batch producer as tungsten trioxide once incorporate into polymers is not released from polymers or coatings.

Tungsten blue oxide can be used for surfaces in permanent contact with water e.g. pipes faucets. Tungsten blue oxide is a suitable addition to kautschuk e.g. for side rails of escalators.

III Zinc Molybdate MoO₄Zn

The combination of Molybdenum trioxide with Zinc oxide in the same crystal structure in a triclinic orthorhombic crystal structure shows excellent antimicrobial activity plus additional features. For endowment of various polymers e.g. melamine resin, TPU, Silicone where a white color is mandatory, zinc molybdate is an excellent choice. Zinc molybdate is neither water nor alcohol soluble and stays firmly inside a polymer or paint. It is not toxic and can be used as a corrosion prevention agent, a flame retardant and smoke suppressant compound.

The strength of the antimicrobial activity is comparable with Molybdenum trioxide with activity against Gram positive and Gram negative microorganisms, legionella, spores and fungi. In addition also influenza virus is included in the spectrum of activity. Zinc molybdate is UV light stable, water, alcohol insoluble, non-toxic [46].

Zinc molybdate is available in 2 µm, 5 µm and 8 µm particles sizes. It has to be emphasized that regular crush milling of particles destroys the crystal structure of transition metal oxides required for its antimicrobial activity. Therefore other mechanisms have to be deployed:
Zinc Molybdate can be synthesized as 0.2 µm particles in fluids in unlimited quantities from Zinc oxide and ammonium dimolybdate assisted by ultrasound treatment [47].

Synthesis of submicron particles of Zinc Molybdate with correct crystal structure can also be achieved by the Atlas Syrris Synthesis reactor or the Pharmjet by Nanosaar.

There is also the possibility of fragmentation of 5 µm size particle by rod mill or by thermal fracturing [48].

Zinc Molybdate has a broad spectrum of applications e.g. in hospital furniture and any polymer where a white color is mandatory. Flame retardant/smoke suppressant properties make Zinc Molybdate suitable for application in airplanes but also in trains and public transportation.

Zinc Molybdate can also be used for heat exchange equipment in air conditioners.

Most uses are for paints (paints for NMR, angiography systems, C bows and CT scanners) Hospital furniture.

Coating is available for hospital interiors, restaurants, kitchen, and food production sites.

No carcinogenicity is described.

Skin tolerance test have been performed of lacquer samples with Zinc molybdate incorporated in a concentration 0.25 and 1%.

In 10 persons skin tolerance tests have been performed with a 5 x 5 cm lacquer sample of Zinc Molybdate 1% over a period of 24, 48, 72 and 120 hours on their forearm in a wet chamber. No adverse effects have been seen during the test period. No allergy was observed during one year 1 year following the initial observation.

Cytotoxicity has also be monitored using the 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide MTT or NBT assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Viable cells will reduce the MTS reagent to a colored formazan product.

Survival of MRC5 (immortalised mouse lung fibroblasts) cell line has been determined

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% MoO₃ in TPU</td>
<td>97% survival</td>
</tr>
<tr>
<td>2% MoO₃ in Epoxy</td>
<td>98% survival</td>
</tr>
</tbody>
</table>

NBT Test: No colorimetric differences between controls and verum have been observed.

Vero cells, osteoblasts, fibroblasts were grown well on these surfaces.

There are documents which describe the allowable exposition of people with MoO₃:


Limits of intake www.efsa.europa.eu/de/ndatopics/docs/ndatolerableuil.pdf [49].

Molybdenum: “This provides a UL [upper limit] of 0.01 mg/kg Bw/day, which also covers pregnant and lactating women, equivalent to 0.6 mg/person/day for adults”.

The concentrations of elution of transition metal oxides from surfaces in various coatings of polymers are 1000 fold below these levels.

IV Polyoxometalates, [H₂Mo₆W₆O₄₂]₁₀⁻

The observation of a moderate water solubility of Molybdenum trioxide prompted the development of polyoxometalates i.e. incorporation of the Molybdenum trioxide into the tungsten trioxide crystal lattice. Polyoxometalates are available by commercial producers e.g. in unlimited quantities [50].

Polyoxometalates can also be synthesized by the Pharmjet (additional investigative work on the basis of limited preliminary results).

Advantage:

- Water insolubility of molybdenum in the combined crystal lattice.
- Additional strength of antimicrobial activity against Gram positive and Gram negative microorganisms due to a strong Zeta potential.
- Additional antimicrobial activity against molds (Aspergillus spp).
- Activity against a number of virus (influenza, hepatitis B, C, Flavivirus, HIV, RSV, RNA Virus, (EBV), Herpes simplex as well as Norovirus) [51].
- Activity against algae, antifouling activity.
- These properties recommend the use of
polyoxomtallates as addition to filter systems in air conditioners and many other applications.

Additional remarks:

All the above mentioned additives as powder with particle sizes of 2 - 8 µm can be added to various polymers e.g. TPU, PE PP, HDPE, Polystyrol, Polyimine, silicone etc.. The surface has to be hydrophilic i.e. wettable with a contact angle of 30° or less. This can be achieved by the addition of hydrophilising agents (preferably Glycerin Stearat and others e.g. Codamol, Fleroxacin, Lubrophos etc. in a concentration of 1%) The optimum hydrophilising agent has to be investigated according the polymer in use.

Smaller particles (0.2 µm particles sizes) can be produced by thermal fracturing or by synthesis which preserves the crystal structure required for antimicrobial activity. Submicron particles embedded into transparent coatings are used for transparent antimicrobial activity on glass.

The crystal structure of the additives can be investigated by x ray diffraction studies, shown in figure 3.

Figure 3: XRD diagrams of the as-prepared MoO3 based gel and after annealing at various temperatures.

Experimental investigations:

The quantitative investigation for antimicrobial activity have been performed with two test systems (Figures 4-6).

a: JIS method
b: RODAC - push plate method

Figure 4: Investigation of lacquer samples by the JIS method (a) and the RODAC push plate method (b).

Figure 5: Investigation of a melamine resin samples by the JIS method (a) and the RODAC push plate method (b).

JIS method (Japanese industrial standard) testing is required by the biocidal product regulation (BPR) of the European Union. The surface is covered by a foil for 24 hours which prevents the evaporation of oxygen radicals from the surface. However this method is far from the real life situation, therefore the investigations have been performed according to the RODAC plate method.
RODAC (Replicate Organism Detection and Counting) push plate method (b): Microorganisms (ATCC reference strains) are stored in cryopellets at -25°C. In the morning prior to the investigations pellets are grown in Isosensitest broth for 6 - 8 hours. In the evening before the investigation microorganisms are transferred from liquid medium to blood agar plates with the addition of 5% defibrinated sheep blood at 37°C “overnight cultures”. After 8 - 12 hours colonies are harvested and suspended in Aqua distill at a final concentration of 10^9 CFU/ml. This method has been harmonized by ECHA and the Austrian ministry of environmental protection.

The number of colony forming units per ml is evaluated by a photometric method: an OD of 0.33 at 475 nm reveals a concentration of 10^9 CFU/ml of *Staphylococcus aureus*. Final concentrations were determined by several 1:10 dilutions until colonies were countable.

10 µl of the bacterial suspension containing 10^9 CFU/ml is applied to a 1 cm² surface of the material under investigation. The drops are dispersed over an area of 1 × 2.5 cm with a Drigalski spatula. The liquid dries on the surface within 15 minutes. From the initial inoculum (0 hour), after one hour and in three hourly intervals thereafter until 12 hours RODAC Plates i.e. Caso Agar with disinhibitor are pressed onto the contaminated areas. The push plates are incubated at 37°C for 24 hours. Then colonies are counted, the results are documented by photography as shown in figure 6.

![Figure 6: Antimicrobial activity investigation.](image)

Investigation of antimicrobial activity has also been performed after hand contamination of surfaces similar to a real life situation. It has been demonstrated that an assumed contamination with 10^9 CFU/ml (lawn) is eradicated within 30 minutes.

Polyoxometalates show also a reduction of the original inoculum size of 7 log 10 of Aspergillus spp. within less than 3 hours of and algae. Samples of TPU and PP containing 2% polyoxometalates in the polymer are 5 years after immersion in a sweet water pond containing an abundance of algae free of contamination in contrast to a control sample which was contaminated within 14 days.

Antifouling properties have been investigated also in see water. No growth of algae has been observed during an observation period of minimum 160 days.

The spectrum of activity of polyoxometalates also contains a number of virus e.g. Hepatitis B, Hepatitis C, RNA Virus, Herpesvirus and HIV [51].

**Technical application**

Transition metal oxides can be incorporated into various polymers and coatings e.g. liquid silicone, liquid poyurethane, silicium dioxide. The additives are heat tolerant up to 400°C. Therefore no problem exists with extrusion moulding.

It is essential that the surface is hydrophilic i.e. wettable with a contact angle of 30° or less. This is possible by the addition of various hydrophilising agents e.g. 1% glycerine stearate or Crodamol, Fleroxycin, Lubrophos. The hydrophilising agents have to be adapted to the polymer.

Antimicrobial cables have been produced by incorporation of 2% Molybdenum Trioxyd in thermoplastic polyurethane which is already sufficient hydrophilic on the surface. The investigation of antimicrobial activity has been performed by the roll on culture technique (Figure 7) [52].

5 cm pieces of cable containing 2% of molybdenum trioxide have been immersed into a suspension of *S. aureus* ATCC 25923 in a concentration of 10^9 CFU/ml for 6 hours. Thereafter the pieces have been rolled over an agar plate (Oxoid Agar containing 5% sheep blood) and then sample has been added to a sterile empty Sarstedt vial. This procedure has been repeated in 3 hourly intervals until 12 hours.

It has been shown that the initially contaminated samples did not contain any microorganisms after 3 hours and thereafter. In an additional experiment samples of cables produced in 2002 have been investigated with the same method. These 18 years old samples showed identical results compared to the original samples.
Transition metal oxides can be incorporated in various composite materials or coatings (liquid Silicone, liquid polyurethane, Silicium Dioxyd). These coatings containing 0.25% Zinc Molybdate in liquid silicone and liquid polyurethane (Coating I, Coating II) have been investigated with the push plate (RODAC plate) method compared with a negative control. The reduction of colony forming units constitutes 7 log 10 within 1 hour. As test organism *S. aureus* ATCC 25923 has been used (Figure 8).

Various technical application of Zinc Molybdate, MoO$_3$ and WO$_3$ in ultrathin transparent layers on glass, ceramic, stainless steel, enamel have been used by vapor deposition, (CVD), spray pyrolysis and Sol-Gel-techniques. Sol-Gel-techniques have been employed, which resulted in ultrathin films at room temperature. Investigations demonstrated excellent antimicrobial efficacy.

**Toxicologic investigations and biocompatibility**

Lacquer samples (100 cm$^2$ containing 0.25% Zinc-Molybdate) have been wiped with a textile 10000 times with water, alcohol, detergent. The textile has been investigated by the “spectro-turboquant-powder” method for content of transition metal oxides. No elution to the additive from the sample has been detected.

<table>
<thead>
<tr>
<th>Textile after 10 000 wipes</th>
<th>Control textile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg &lt; 0.01%</td>
<td>Mg &lt; 0.01%</td>
</tr>
<tr>
<td>Al &lt; 0.01%</td>
<td>Al &lt; 0.01%</td>
</tr>
<tr>
<td>Si 2.66%</td>
<td>Si 1.63%</td>
</tr>
<tr>
<td>P 0.01%</td>
<td>P 0.02%</td>
</tr>
<tr>
<td>S 0.27%</td>
<td>S 0.23%</td>
</tr>
<tr>
<td>Cl 0.45%</td>
<td>Cl 0.26%</td>
</tr>
<tr>
<td>K 0.03%</td>
<td>K 0.13%</td>
</tr>
<tr>
<td>Ca 0.14%</td>
<td>Ca 0.12%</td>
</tr>
<tr>
<td>Ti 0.05%</td>
<td>Ti 0.06%</td>
</tr>
<tr>
<td>V &lt; 0.01%</td>
<td>V &lt; 0.01%</td>
</tr>
<tr>
<td>Cr &lt; 0.01%</td>
<td>Cr &lt; 0.01%</td>
</tr>
<tr>
<td>Mn &lt; 0.01%</td>
<td>Mn &lt; 0.01%</td>
</tr>
<tr>
<td>Fe 0.02%</td>
<td>Fe 0.02%</td>
</tr>
<tr>
<td>Co &lt; 0.01%</td>
<td>Co &lt; 0.01%</td>
</tr>
<tr>
<td>Ni &lt; 0.01%</td>
<td>Ni &lt; 0.01%</td>
</tr>
<tr>
<td>Cu 0.03%</td>
<td>Cu 0.03%</td>
</tr>
<tr>
<td>Zn &lt; 0.01%</td>
<td>Zn &lt; 0.01%</td>
</tr>
<tr>
<td>Mo &lt; 0.01%</td>
<td>Mo &lt; 0.01%</td>
</tr>
</tbody>
</table>

**Solubility experiments of Mo and W in Epoxy resin and TPU**

One important requirement is the insolubility of Molybdenum (VI)-oxid as well as the Tungsten (VI)-oxides in water, alcohol and detergents. For Molybdenum oxide, a water solubility of 0.003 mol/l at a pH value >7.55 has been described [49]. Experiments documented that there is no decrease of the original pH value of water within 28 days after immersion of e.g. catheters or ECG cables containing 5% MoO$_3$ or WO$_3$ in the composite material (TPU). In alkaline pH (pH value >>9) a solubility exists with
elution of Molybdates and Wolframates.

The investigations regarding the solubility of Molybdenum trioxide and Tungsten trioxide have been performed with ICP-OES using two wavelength Mo (313.259 nm and 379.825 nm) and W (224.875 nm und 276.427 nm) on the basis of linear calibrations. Initially experiments have been planned for a 24 h/RT elution. As no measurable concentrations have been detected the elution time was extended to 1 week. There was the assumption that equilibrium between the additive and the solvent (water) would be established within this week.

In the majority of samples the concentrations have been below the limit of detection of 0.005 mg/l. In no instance the concentration was above the limit of detection. Investigations also disclosed that tungsten compounds are completely insoluble in water and alcohol values are consistently below 0.002 mg/l.

To solve the problem with water solubility of molybdenum oxides molybdenum trioxide has been incorporated into the tungsten crystal lattice revealing a new compound referred as polyoxometalates with additional antimicrobial properties. The unique properties besides the know activity against all bacterial microorganisms contained in the spectrum of molybdenum trioxide are the activity against Aspergillus spp and several viral pathogens. There is also a strong antifouling activity.

**Investigation of Cytotoxicity**

Repeated investigations of cytotoxicity disclosed the survival of the MRC 5 cells (mouse lung fibroblast cell line) 97% on catheters with 5% MoO₃ in TPU.

It has been documented that various cell lines e.g. verocells, osteoblasts, epithelial cell line also survive on surfaces containing 0.25% Zinc Molybdate as well as 5% Molybdenum oxide and are viable for minimum 2 months.

It has to be emphasized that molybdenum as well as Zinc are essential trace element sin the body. These transition metal oxides are stabilising enzyme systems for detoxification and elimination of sulfur which is contained in various amino acids e.g. Cystin, Cystein und Methionin from the body [47].

Skin tolerance has been investigated in 10 test persons. 10 × 10 cm metal plates with a lacquer containing 0.25% Zinc Molybdate has been applied during 120 hours at the forearm of 10 adult persons in a moist chamber. No adverse reactions no reddening, itching, scaling has been observed. There was no allergenicity during the next 6 months This is of no surprise as the normal pH of the skin is in a range of 4.6 - 5.0 similar to the pH value of the surface of the lacquer sample.

Extensive additional investigations have been performed with various composite materials containing 5% und 10% MoO₃ e.g. aging investigations over 9 months at 75°C. The antimicrobial activity has been unchanged. Also the elasticity, kinking stability, tensile strength was unchanged.

Dosis efficacy investigations have been performed. Good activity is seen at 0.5% of the additive for the majority of applications. If thin coatings (less than 100 µm) are used a concentration of the additive of 0.2% is sufficient. The best activity for example for addition of Molybdenum into thermoplastic polyurethane is 2%.

We can summarise that this new innovative technology provides a highly efficient endowment of surfaces with antimicrobial activity against a broad spectrum of Gram positive and Gram negative microorganism fungi and virus. The activity is independent on existing resistance against antibiotics and disinfectants; a reduction of an inoculum of 10⁹ CFU/ml of 7 log 10 is seen within less than 1 hour. The activity is lasting for documented more than 10 years. In contrast to disinfectants no induction of resistance is observed.

Transition metal oxides show an effective locally confined antimicrobial efficacy. The technology exhibits a very broad antimicrobial spectrum of activity against Gram positive, Gram negative microorganisms irrespective of their resistance against antibiotics, fungi including Aspergillus spp, many virus.

Metal oxides don´t induce resistance as these substances attack microorganisms from the outside and are not incorporated into the metabolism of microorganisms. Therefore no induction or selection of resistant microorganisms is observed. Transition metal oxides are not eluted from the surface of polymers. As a consequence the antimicrobial activity lasts a minimum of documented 10 years with regular cleaning cycles. The
technology is not toxic and biocompatible. In essence the additives are essential trace elements in the body. Skin tolerance has been investigated over 120 hours without adverse events. No allergenicity has been observed during the following 12 months. The additives are applied to various composite materials e.g. TPU, PE, PP. It is also possible to endow the additives into various coatings e.g. liquid silicone, liquid polyurethane and silicium dioxide. It has to be ensured that the surface is hydrophilic with a contact angle of 30° or less. With a particle size of < 0.2 µm also a transparent surface can be achieved. The technology is cost effective.

Investigations with artificial aging, exposure to an acid pH 2.0 or alkaline pH 9.5 for 5 weeks at 75°C, exposure to tap water over 6 months with continuous exchange of the eluent. Investigations of the antimicrobial activity after artificial ageing over 6 months, continuous elution in pat water over a 9 months period, elution experiments with 90% alcohol, acid pH 2.0, alkaline pH 9.5 at 75°C over 28 days did not show and decrease of antimicrobial activity.

Assessment of clinical application of various transition metal oxides

I Molybdenum trioxide MoO₃

Molybdenum trioxide is a light gray powder with particle sizes of 2 - 5 µm. Of crucial importance for the antimicrobial activity of Molybdenum trioxide is the correct crystal structure: The crystal structure is monoclinic and orthorhombic. This has to be confirmed by investigations by x-ray diffraction.

Advantage: Strong antimicrobial activity with addition of 2% in various composite materials, e.g. in TPU for ECG lead wires which show a high rate of contamination and biofilm formation. The efficacy. Molybdenum trioxide powder is cost efficient and available in unlimited quantities. Molybdenum is an essential trace element [47].

Disadvantage: Molybdenum is water soluble with 0.003 mol/l at a pH value > 7.45 at room temperature. Elution-experiments of a 100 cm² TPU surface endowed with 2% MoO₃ during 7 days in 1 l deionised water show an elution of > 0.0002 mg/l. Similar investigations with tungsten blue trioxide show concentrations below the level 0 detection. Molybdenum is not UV light stable.

The safety data sheet of Molybdenum trioxide shows carcinogenicity: Rates and mice have been exposed to 30 or 100 mg/m³ body weight of ultrafine powder by inhalation 6 hours a day, 5 days per week over 2 years. In 50% of the rats, pulmonary malignancy was observed. Mice in contrast did not show malignant transformations. It hast to be emphasized that carcinogenicity has been observed by inhalation of ultrafine powder which is not present if Molybdenum is incorporated into composite materials or coatings (liquid silicone or liquid polyurethane) as no elution has been observed from these polymers. It has to be emphasized, that during the manufacturing process inhalation of submicron particles is possible for employees. Precautions have to be established to prevent inhalation, these are in general state of the manufacturing process.

Fairhall et al. [46] exposed rats to inhalation of submicron particles of CaMoO₄ (125 mg Mo/m³) for 1 hr/d, 5 d/wk for 5 weeks. It was observed that 5/24 guinea pigs died following exposure but no other signs of toxicity were observed. Guinea pigs (51 animals, sex not reported), who were exposed to 250 mg MoO₃/m³ (164 mg Mo/m³) using the same exposure regime, experienced severe eye and nasal irritation, loss of appetite and weight, diarrhea, muscular incoordination, and loss of hair. Following the 10th exposure, 26/51 animals died.

Inhalation of 195 mg/m³ two 13-wk studies were conducted by NTP (1997) in which F-344/N rats and B6C3F₁ mice (10/sex/group) were exposed to molybdenum trioxide for 6.5 hr/d, 5 d/wk at concentrations of 0, 1, 3, 10, 30, or 100 mg/m³. All rats and mice survived to the end of the study. Significant increases in liver copper concentrations were observed in female mice exposed to 30 mg/m³ and in male and female mice exposed to 100 mg/m³ (males: 11.51 µg/g in the 100-mg/m³ exposure group versus 8.19 µg/g in controls; females: 6.51 and 6.98 µg/g in the 30-and 100-mg/m³ dose groups, respectively, versus 5.68 µg/g in controls). The increased copper concentrations were not regarded as being an adverse effect relevant for deriving a LOAEL and a NOAEL. No other clinical findings were observed in either rats or mice. Additionally, no significant differences in absolute or relative organ weights, sperm counts, or motility were noted in rats or mice.

In the NTP study (1997), rats (F344/N) and mice (B6C3F₁) (50/sex/ dose) were exposed for 6 hr/d, 5 d/wk at concentrations of 0, 10, 30, or 100 mg/m³ molybdenum trioxide for 2 yr experienced a significant exposure-dependent increase in blood Mo concentrations. Male and female rats exposed to 30 or 100 mg/m³ experienced
significantly increased incidences of chronic alveolar inflammation. Incidences of hyaline degeneration in the nasal respiratory epithelium in male rats exposed to 30 or 100 mg/m³ and in all exposed groups of females rats were significantly greater than those of the control groups. Incidences of hyaline degeneration in the nasal olfactory epithelium of all exposed groups of females were also statistically significant. For male mice, the incidences of histiocyte cellular infiltration in all exposed groups were significant. Incidences of hyaline degeneration of the respiratory epithelium of the nasal cavity in female mice at 100 mg/m³ were significantly greater than those in the controls (NTP 1997). Based on the 2-yr NTP study, the LOAEL is 10 mg/m³ for increased incidences of hyaline degeneration in the nasal respiratory epithelium and nasal olfactory epithelium in female rats.

II Wolfram Trioxyd WO₃

Tungsten trioxide has been investigated for its antimicrobial activity. Initially the investigations have been performed with the oxygen saturated tungsten yellow oxide or an unknown combination of the Tungsten yellow oxide with Tungsten blue oxide. Poor antimicrobial activity has been observed. Further investigations however disclosed that the 5% oxygen deficient tungsten blue oxide WO₂.₇₅-₂.₉₀ shows a strong antimicrobial activity which correlated with a strong electron release of electrons from a surface. Tungsten blue oxide is a dark blue powder with particle sizes of 5 µm. By ‘thermal fracturing’, the particle size can be reduced to 0.25 µm. By these means also transparent surfaces in combination with transparent coatings (liquid Silicon, liquid Polyurethane) can be constructed.

The advantage of tungsten blue oxide is the complete water insolubility. The safety data sheet does not disclose special precautions: Industrially Tungsten trioxide does not constitute an important health hazard. Operating facilities should be operated according to the related handling safety. The protection of persons and health management indicate no ingestion of the compound. Mist protective equipment is required to wear for prevention of inhalation. Hand protection and eye protection is not required: Popular work clothes are acceptable. Effects of overexposure: exposure is related chiefly to the dust arising out of the crushing and milling operations. Chronic inhalation of the dust may cause lung damage in humans. Carcinogenic assessment is not listed.

III Zinc Molydate MoO₄Zn

Molybdenum oxide as well as Tungsten blue oxide has a light blue (Molybdenum) or dark blue color which is not suitable e.g. for Hospital furniture. Molybdenum oxide in a zinc oxide crystal lattice has a white color and shows equal antimicrobial activity compared to molybdenum oxide. The spectrum of activity is also comparable. In addition a number of viral agents e.g. influenza virus are in the spectrum of activity. The crystal lattice has to be triclinic and orthorhombic. Zinc Molybdate is water and alcohol insoluble, UV light stable and has smoke suppressant and flame inhibitory properties. Zinc Molybdate is used as an anticorrosive agent. It can be added to various polymers e.g. melamine resin for hospital furniture but also for numerous coatings. Various pigments can be added to Zinc Molybdate to achieve every desired colour. Submicron particles in transparent coatings result in a transparent lamination of stainless steel and glass.

Zinc Molybate is available as 2 µm, 5 µm and 8 µm particle sizes. Submicron particles of Zinc Molybdate particles (0.2 µm) can be produced by synthesis. Various chemical engineering processes for synthesis are available.

The white color or the transparent coatings recommend Zinc Molybdate for a broad spectrum of applications. The flame retardant and smoke suppressant properties recommend Zinc Molybdate for the use in public transportation in airplanes, trains and cars. Zinc Molybdate can used for corrosion free application of heat exchangers in air conditioners etc.

The toxicity of Zinc Molybdate has been investigated intensively. No side effects have been described. No carcinogenicity is known.

IV Polyoxometalates, [H₂Mo₆W₆O₄2]¹⁰⁻

The fact that molybdenum shows minimal but measurable water solubility prompted further investigations. Molybdenum trioxide incorporated into the tungsten crystal lattice revealed a new compound referred as polyoxometalate with additional antimicrobial properties. The molecules in this crystal structure are completely insoluble in water and alcohol. The unique properties of this new compound are - besides the know activity against virtually all bacterial microorganisms contained in the spectrum of molybdenum trioxide - the activity against Aspergillus spp. In addition activity against algae and several virus e.g. hepatitis B, C.
Flavivirus, HIV, RSV, RNA Virus, Epstein–Barr virus (EBV), Herpes simplex has been observed [50]. The strong zeta potential is responsible for the rapid eradication of microorganisms. A reduction of 7 log 10 within 15 minutes has been documented by laser scanning microscopy. Polyoxometalates are eradicating also microorganisms present in biofilms. Documents in the literature show that surfaces endowed with submicron particles of Titanium or Vanadium doped Polyoxometalates show activity against Coronavirus [51]. Own investigations disclosed that polyoxometalates Mo:W 2:1 are highly active even without Titanium or Vanadium doping making the virus noninfectious. The high Zeta potential of these Polyoxometalates constitutes the vital element for the electrostatic interaction, and the electrostatic binding energy is strong enough to keep the complex stable.

Last not least a strong antifouling activity has been observed which benefits greatly from the complete lack of water solubility.

The application of the above described technology for self-sanitizing surfaces is nearly unlimited.

Surfaces are ubiquitously colonized by a variety of microorganisms e.g. bacteria, viruses, fungi. In the healthcare sector, there is a risk of hospital-acquired infections (nosocomial infection) which leads to increased morbidity and mortality amongst patients. Approx. one in ten patients will develop a nosocomial - hospital acquired infection, not linked to the initial disease. 1 on 100 patients will statistically die from it. Estimated by the European Science Foundation (ESF) indicates that 1.75 million patients develop a nosocomial infection every year – and 180,000 die from it. This number corresponds to 1 avoidable fatality every 3 minutes.

An important indication are self-sanitizing surfaces in hospitals e.g. hospital furniture, push buttons and control knows for ventilators and infusion pumps, ECG lead wires, oxygen monitors, textiles, light switches and door handles which are able to replace disinfectants. Also implantable biomaterials e.g. urologic catheters, endotracheal tubes and central venous catheters, antimicrobial suture material can benefit from the addition of transition metal oxides to the polymer [51,52].

A special application of this technology is dentistry e.g. dental implants and prostheses as well as the coating of equipment preventing contamination with Hepatitis B and C.

The technology is also suitable for antimicrobial paints and coatings for medical equipment e.g. CT scanners nuclear magnetic resonance imaging, X-ray equipment, endoscopes etc. The technology is useful for germfree surfaces in public transportation, airplanes, trans cockpits of cars e.g. in car sharing.

The technology is also used in food production and storage e.g. kitchens, restaurants where disinfectants can be saved.

Application in public transportation is highly desirable. Multi-resistant microorganisms prevalent in Asian countries are distributed worldwide by airplanes, distributed by tourists in trains, trolleys cruise ships and restaurants.

Patent protection

The technology of in situ generated biocides is worldwide patent protected. According to the intended use and the specific requirements one of the four additives (Molybdenum oxide, tungsten blue oxide, Zinc molybdate or Polyoxometalates) can be used. The transition metal oxides can be incorporated into various composite materials or coatings e.g. liquid silicone, liquid polyurethane or silicon dioxide or in transparent coatings of glass or stainless steel.

References

5. The world health organization: WHO Guidelines on Hand Hygiene in Health CareFirst Global Patient Safety Challenge Clean Care is Safer Care.


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