Improving antimicrobial coatings

High throughput screening to identify effective concentrations of Nano-/MicroSilver
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Antimicrobial coatings should provide long lasting protection against microorganisms, throughout the lifetime of the coated product, without negatively affecting humans or the environment. A novel, high throughput testing technology can determine the optimal concentration of the antimicrobial agent under realistic conditions at much higher sensitivity than conventional methods. An example of a successful development is the use of metallic NanoSilver/MicroSilver as an antimicrobial agent in coatings.

A functionality which is especially relevant in hygienic sensitive areas like medical or food processing is the antimicrobial property of the surface of the product or the processing equipment. Either by binding biological active substances (biocides) on the surface or by incorporating such substances in the coating the proliferation of bacteria and fungi on the material can be reduced or avoided. From the large variety of organic or inorganic biocides, metallic silver is especially suited as an antimicrobial substance due to its high antimicrobial efficacy accompanied with a low or non-existent cytotoxicity. Additionally, metallic silver is approved for a large variety of applications in the medical or food area. The antimicrobial activity of metallic silver depends strongly on the surface area of the silver. Therefore nanoparticles of pure metallic silver have the advantage of giving a strong antimicrobial effect already at low concentrations.

Making Nano-/MicroSilver particles
By using an inert gas evaporation and condensation process, high purity silver (99.96 %) is evaporated in an argon atmosphere and subsequently condensed onto cooled surfaces. This process leads to agglomerated nanoparticles with a primary particle size of 80 to 150 nm. The particles are sintered together into a non-dusting, highly porous silver powder (Figure 1). The mean agglomerate size which can be obtained is between 5 and 10 µm depending on the process conditions. The specific surface area can be as high as 5 m²/g and is significantly higher than conventional, chemically produced silver powders due to the existence of an inner porosity of the particles which can be as high as 90%.
Vacuum evaporation on running liquids (VERL) is used for the preparation of dispersed nano-silver particles in non-aqueous, low vapour pressure liquids like various oils, resins, softeners or prepolymer. Metal nanosuspensions with particles - having a mean diameter between five and 20 nm and a specific surface area at 50 m²/g (Figure 2) - can be obtained. The particles do not sediment and are stable for convenient further processing. For each application a suitable liquid is needed which is preferably an already used ingredient of the coating formulation.

Homogeneous antimicrobial effect
These highly porous particles or nanodispersed particles can be mixed into liquid or powder coatings (e.g. 2-component polyurethane) or any other polymer by conventional dispersing techniques, including extrusion. It is advantageous that the highly porous structure becomes infiltrated by the liquid coating or the polymer as this leads to a high contact area between the metal and polymer and a good antimicrobial effect even at low metal concentrations. The high contact area enables the diffusion of silver ions through the polymer to the surface and leads to a homogeneous antimicrobial effect.

Agar diffusion testing has limitations
Agar diffusion testing has been the standard method for many years for in vitro assessment of antimicrobial loaded polymers. The activities of some antimicrobial agents like metallic silver are undetectable in agar diffusion testing and this makes new in vitro methods necessary. In this work a new microplate proliferation assay for in vitro antimicrobial testing of polymeric samples was used.
Typically, antimicrobial products show a macroscopic zone of inhibition when in contact with a bacterial lawn on a petri dish (agar diffusion test, Figure 3). The zone of inhibition is increasing with the concentration of the antimicrobial agent. The obvious disadvantage of such systems is that the environment is unnecessarily affected. Since only the product should behave in an antimicrobial manner there is no need to also generate a germ free environment. Anything more than this contributes to the emergence of a resistant germ flora in the environment and causes a continuous devaluation of valuable antimicrobial compounds. Testing with an agar-diffusion assay is only suitable for release systems. As a screening method it only generates releasing products, which to today’s standards are mostly out of date and do not live up to the requirements of a modern antimicrobial strategy.

Microplate assay monitors prevention capability of surfaces
The microplate assay offers considerable advantages because it monitors to what degree a given surface is able to prevent adherent cells from releasing daughter cells into their surroundings. It is this step which is crucial for microbial contamination and biofilm building. A surface which prevents adherent cells from proliferating shows perfect antimicrobial behaviour. In contrast to competitive methods the microplate assay is able to monitor this. It is precise, highly reproducible, scientifically acknowledged, successfully tested in a clinical background and the assay is already parallelized so that samples can be screened with a high throughput.
Different environmental conditions can be tested with fairly small samples. All measurements are made in direct comparison with an almost identical sample that is lacking the antimicrobial agent. Internal positive and negative controls are included for sample independent continuous process control. All samples are measured 8-fold. The assay is parallelized so that samples can be screened with a high throughput. The assay gives the temporal course of the proliferation of daughter cells on the surface as a function of contact time and the environment. Anything more than this contributes to the environment. Anything more than this contributes to the emergence of a resistant germ flora in the environment and causes a continuous devaluation of valuable antimicrobial compounds. Testing with an agar-diffusion assay is only suitable for release systems. As a screening method it only generates releasing products, which to today’s standards are mostly out of date and do not live up to the requirements of a modern antimicrobial strategy.

Flat line indicates bactericidal behaviour
In the extreme case that all germs on the sample surfaces are killed and therefore unable to proliferate over at least 48 hours a flat line is observed. That is indicative of bactericidal material behaviour. This exemplifies that samples can behave like self-disinfecting antimicrobial surfaces under the given conditions. It is noteworthy that the assay will also detect lower antimicrobial product activities that go
unnoticed and are overlooked by all competitive methods. For quality assurance it is a valuable feature of the dynamic proliferation assay, that it very suitable to monitor lot-specific variations of the production line. Due to its higher sample throughput, a superior QA/QM system can be set up which is well-prepared for a permanent production control. The method has been published [1] and is accredited according to DIN ISO 17025.

Bio-Gate assay method gave the most information

The antimicrobial efficacy of high-porosity silver in various 2-K polyurethane coatings was tested by using three different methods. These were: a) counting of colony forming units on agar plates (according to the Japanese standard) b) confocal laser microscopy using live-dead assay to distinguish between living and dead bacteria c) Bio-Gate assay according to the published method [1] (Figure 4). From all methods clearly the Bio-Gate assay is the only test which can give a quantitative result regarding the antimicrobial performance of different formulations of silver in a coating system or polymer. An application example is the development of an antimicrobial 2K PU system for a German manufacturer of automatic packaging machines and packaging lines. The coating is used on certain parts of the machine. By using the coating with its antimicrobial properties, product safety can be improved between cleaning and maintenance intervals by protecting against bacteria and other microorganisms, e. g. fungi (Figure 5).

The application shows an example where silver was used as an alternative to organic biocides for a hygiene-sensitive food processing area. Other fields of application for antimicrobial coatings include: automotive interiors, aircraft interiors, industrial machinery coatings and medical equipment and devices.

Ideally suited for many technical applications

Silver - known for its antimicrobial properties since ancient times - works against a broad spectrum of germs. Pure metallic silver in the form of microsilver or nanosilver can be added to a broad range of coating systems as an innovative antimicrobial agent (e. g. wet coatings, coil coatings, powder coatings). By using a new high throughput screening system the most effective concentrations of silver within the coating systems can be identified fast and cost effectively. Due to the metallic silver deposit a long term activity can be expected and makes this system ideally suited for many technical applications.

Reference

Results at a glance
- Microplate assays can monitor to what degree a given surface is able to prevent adherent cells from releasing daughter cells into their surroundings, a crucial step for microbial contamination and biofilm building.
- Different environmental conditions can be tested with fairly small samples.
- The technique can also indicate bactericidal material behaviour.
- When the antimicrobial efficacy of high-porosity silver with various 2-K polyurethane coatings was tested the Bio-Gate assay was the only test which can give a quantitative result regarding the antimicrobial performances.
- Silver can be used as an alternative to organic biocides for hygiene-sensitive areas such as in food processing. By using a new high throughput screening system the most effective concentrations of silver within the coating systems can be identified fast and cost effectively.

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Figure 1: Scanning electron microscope picture of high-porosity silver (mean primary particle size: 80-150 nm, agglomerates 5-10 µm)
Figure 2: TEM analysis of Ag particle with $\langle dp \rangle \approx 6$ nm in silicone oil
Figure 3: Three samples with increasing antimicrobial efficiency in an agar diffusion assay. The release of the antimicrobial agent into the surroundings leads to a zone of growth inhibition around the sample. Note that the diameter of the zone of growth inhibition will increase with larger concentrations of the antimicrobial agent.
Figure 4: Different methods to prove antimicrobial efficacy of high-porosity silver in a polyurethane coating (a) Agar plate test, b) Confocal laser microscopy, c) Bio-Gate assay). Testing in c) was done over a period of 48 hours.
Figure 5: Food packaging machines with antimicrobial coatings based on high-porosity silver. Biological samples have been taken of parts where people touch the surface frequently (e.g. handles). Biological activity of MicroSilver can be shown qualitatively by agar plate and quantitatively by the Bio-Gate assay.