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(54) **Title:** ANTIBACTERIAL FILMS OBTAINED BY SPUTTERING, AND METHOD FOR CONFERRING ANTIBACTERIAL PROPERTIES TO A SUBSTRATE

(57) **Abstract:** A method for conferring antibacterial property to a substrate, characterised in that it comprises the coating of said substrate with a film comprising a glass, ceramic, or glass-ceramic material, and a metal having antibacterial properties, by radio-frequency co-sputtering.



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Antibacterial films obtained by sputtering, and method for conferring antibacterial properties to a substrate

The present invention relates to a method for conferring antibacterial properties to a substrate, and to substrates provided with antibacterial coating.

Infections due to micro organisms constitute a serious problem for human health, therefore the development of antibacterial materials and coatings is the object of a widespread research. Such coatings are useful for surgical and hospital equipments in general, as well as for all the equipments used in public places, where contamination and infection due to common use surfaces may constitute a problem, such as, for example, in schools, pre-schools, public transport, and food industry, particularly relative to the packaging and handling of products.

A number of antibacterial materials have been identified, among which natural and inorganic substances, such as tea extracts, chitosan, copper, zinc, and silver. Among these materials, silver or silver ions are well known due to their powerful antibacterial activity.

Silver in the ionic or metallic state has been widely studied for the above-mentioned applications. Although the discussion on the precise action mechanism is still open, the antibacterial properties thereof are well documented and generally connected to the ionic silver release. A number of techniques to manufacture an antibacterial surface on different materials have been developed. The sol-gel technique allows obtaining thin films with antibacterial properties obtained by employing tetraethoxysilane-based solutions; see reference texts [1, 2, 3]. The method requires the mixing, stirring, gel-forming, and drying steps, and is time-consuming; furthermore, it is difficult to control and adjust the silver ions to metallic silver ratio. The gel solidification requires high temperatures, which make the coatings impossible on many polymeric materials.

JP 2005263767 discloses antibacterial films obtained using a composite aqueous solution comprising an alkaline silicate solution and an aqueous silicate solution containing crystalline particles of silicon dioxide and copper. By applying the antibacterial solution to the

surface of a substrate and following the solidification, the crystalline particles of silicon dioxide precipitate on the surface to make the surface hydrophilic and provided with antibacterial properties.

A number of articles relate to the formation of silica particles through the Stöber method, followed by a coating of silver/silver nanoparticles with the formation of an antibacterial composite; see reference texts [4, 5]. However, these composites are not mechanically stable, and require to be melted in order to produce thin films or coatings, following which it is difficult to control the silver/silver ion ratio. Again, the high temperatures involved make the use of these methods difficult to coat some polymers.

The reference text [6] discloses the formation of nanoparticles on the silica surface, thus obtaining structures which suffer from a reduced mechanical stability.

In the reference texts [7, 8], antibacterial coatings are described which are obtained by using conventional melting techniques of silver-doped glasses. These techniques require high temperature treatments of the whole substrate to be coated, therefore they have a reduced applicability. The chemical vapour deposition has been used to deposit titanium dioxide films containing silver ions, (see reference texts [9, 10]), as well as inorganic films containing silver ions.

The drawback of the known techniques for the deposition of antibacterial films consists in that such techniques do not allow obtaining coatings provided with a suitable thermal and mechanical stability; furthermore, the time-related release of silver ions is efficient for a short period of time, following which the antibacterial properties decrease or cease.

Object of the present invention is to provide a method that allows obtaining a coating suitable to any substrates, provided with wear and aging mechanical and thermal resistance, while obtaining antibacterial properties which are active for an extended period of time.

For such purposes, it is the object of the invention a method to confer antibacterial properties to a substrate, characterised in that it comprises the coating of said substrate with a

film comprising a glass, ceramic, or glass-ceramic material, and a metal having antibacterial properties, by radio-frequency co-sputtering.

By using the radio-frequency co-sputtering technique (rf) employing a silica-based glass and a metal with antibacterial properties as targets, the preparation and deposition of silica-based thin films containing high concentrations of the metal and the metallic ion having antibacterial properties are made possible. The technique allows obtaining a coating with mechanical and thermal resistance properties, and due to the stability of the silica films, the antibacterial ion release also has a time-stable behaviour. Furthermore, by regulating the procedural parameters during the co-sputtering, the concentration of the antibacterial metal ions can be controlled in order to confer suitable antibacterial properties and, thus, also a suitable duration of the effectiveness time.

The co-sputtering technique using silver and silica as targets is described in detail in the reference text [9]. The main object of this publication was to obtain films containing metallic nanoparticles of silver in silica films for applications in the photonics and sensor systems field. Through such method, the application of an annealing thermal treatment stage is possible, during which the silver ions diffusion and the aggregation thereof is caused, in order to form metallic silver nanoparticles.

The method according to the invention may optionally contemplate a high temperature thermal treatment stage that, anyhow, may generally reduce the antibacterial effectiveness, if it is not carried out in the proper manner. Since the annealing causes the antibacterial agent formation in the metallic form, at the expense of the ionic form, the ionic form availability is consequently reduced. However, an appropriate annealing can be advantageous to the aim of modifying the release rate of the ions of the metal having antibacterial properties, and modifying the antibacterial properties and the effectiveness duration of the film. Preferred thermal treatment conditions range between 400°C and 800°C, and for periods of time ranging between 30 and 90 minutes.

The metal having antibacterial properties can be selected particularly from copper, zinc, and silver, silver being the preferred metal. The usable siliceous materials comprise glass,

ceramic, and glass-ceramic materials, including materials having bioactive properties.

The method according to the invention can be applied to the antibacterial film formation on different materials, including polymers, glasses, ceramic materials, metals, and alloys. Generally, the applications include any substrates, surfaces, or devices in relation to which it is advantageous to achieve antibacterial properties, while providing a coating surface which is mechanically and thermally stable for extended periods of time.

Further characteristics and the advantages of the method according to the invention result from the following exemplary embodiment.

Example

Samples preparation

For the samples preparation, the rf-co-sputtering technique disclosed in the reference text [9] is used, without application of a thermal treatment. Silica or silicon was used as a substrate.

A conventional sputtering chamber in an inert atmosphere was used, where the metal-glass target was obtained by gluing small silver plates (10 mm diameter) on a glass surface with a conductive silver-based glue. The deposition parameters of the rf-sputtering method can be varied in order to optimize their influence on the silver concentration in the coating film.

To the aim of verifying the antibacterial properties of the obtained samples, and to assess the inhibition zone, a qualitative microbiological test was carried out according to the NCCLS standards (according to the reference text [10]).

A bacterial broth was prepared by melting a *Staphylococcus aureus* (ATCC 29213) freeze-dried disc in 5 ml Brain Heart medium, and it was incubated at 35°C overnight; then, 10 µl suspension were extracted, which were spread on a blood-agar plate to allow bacterial

growth and CFU (Colonies Forming Units) formation.

After incubation under the same above-mentioned conditions, a Mc Farland 0.5 suspension (containing approximately $1 - 2 \times 10^8$ CFU/ml) was prepared by inoculating some colonies which were grown on the plate in physiologic solution. The Mc Farland index is a measurement of the solution cloudiness (a cloudy solution has a higher bacterial content than a clear solution), and an optical assessment was carried out (Phoenix Spec BD Mc Farland).

In order to carry out the inhibition zone assessment, an aliquot of the described suspension was evenly spread on a Mueller Hinton agar plate; the silver-containing samples were deposited on the agar surface and incubated at 35°C for 24 hours. The antibacterial behaviour was assessed by observing the inhibition halo, that is, a zone where the bacteria had not grown, all around the samples after incubation.

The inhibition zone assessment tests demonstrate the effective antibacterial properties of the samples: silver ions coming from the glass surface produce a significant and reproducible halo of about 6mm all around the sample.

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CLAIMS

1. A method for conferring antibacterial properties to a substrate, characterised in that it comprises the coating of said substrate with a film comprising a glass, ceramic, or glass-ceramic material, and a metal having antibacterial properties, by radio-frequencies co-sputtering.
2. The method according to claim 1, characterised in that the metal is selected among silver, zinc, and copper.
3. The method according to claims 1 and 2, characterised in that it comprises an annealing thermal treatment of the coated substrate.
4. The method according to claim 3, characterised in that said thermal method is carried out at a temperature ranging between 400°C and 800°C.
5. A substrate coated with a film having antibacterial properties, obtained by a method according to any claim 1 to 4.