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(54) Title: MULTIFUNCTIONAL COATING WITH ANTIMICROBIAL ACTIVITY AND CELL ADHESION REGULATING SURFACE CHARACTERISTICS AS WELL AS A METHOD FOR PREPARING THE SAME

(57) Abstract: The present invention provides a multifunctional coating having antimicrobial activity and the capability to regulate cell adhesion, comprising, deposited on a substrate surface, i) an optional anchoring layer, ii) a Zn-release layer deposited on the anchoring layer or directly on the substrate surface and iii) a functional barrier layer deposited on the Zn-release layer, wherein the functional barrier layer is capable to slow down the diffusion of Zn from the Zn-release layer through the barrier layer and wherein the surface of the functional barrier layer regulates cell adhesion. In a more specific embodiment of the invention, the polymeric film of the Zn-release layer has been formed by plasma assisted polymerisation of zinc-organic precursors, e.g. Zn (acac)₂, bis (pentafluoro-phenyl) -zinc, and zinc-hexafluoro acetylacetonate.



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**Multifunctional coating with antimicrobial activity
and cell adhesion regulating surface characteristics
as well as a method for preparing the same**

The most common cause of hospital acquired infection today is the entry of bacteria such as *Staphylococcus aureus* into fresh wounds during an operation or during the first 3-4 days following an operation and before the wound has closed thus offering no further pathways of entry. Infections occur as a result of transfer of airborne and surface attached spores (on surgical equipment and/or the implant itself) and bacteria into the wound.

Materials such as steel, Teflon, and titanium and their composites are generally used for implantations since they have been proven to be (a) biocompatible and (b) "slow" fouling. While this "slow fouling" ability is advantageous for reducing bacterial infection, at the same time it prevents the incorporation of long lived implants into the tissue (e.g. dental implants, cardiovascular stents) thus enhancing rejection of the implant into the body.

Therefore, it is very desirable to improve the surface characteristics of such implant materials in order to control the adhesion of cells from the host tissue thus facilitating and promoting integration of implants into the host body. In this case either a cell adhesion promoting top layer, or a cell adhesion reducing top layer are desirable depending on the application or location of the implant in the body. For this purpose, several methods have been proposed and a common approach involves the deposition of an organic polymer film on the substrate surface. A number of polymers, such as

polyallylamine or pentafluorophenyl methacrylate, are known to enhance cell adhesion properties. Plasma polymerised polyethylene glycol films and related materials have been shown to exhibit reduced cell adhesion properties. Both types of layers can be provided on implant surfaces by means of e.g. plasma assisted deposition. Moreover, even the "slow fouling" properties of the implant materials mentioned above are not fully satisfying and an enhancement of the antimicrobial properties of such implant materials is desirable.

In an attempt to simultaneously address the problem of both antimicrobial activity and improved cell adhesion, Schröder et al. (Materials Science Forum Vols. 638-642 (2010), 700-705) developed a titanium-based implant material wherein copper, which is a known antimicrobial material, has been introduced into the Ti implant by means of ion implantation techniques. Subsequently, the Ti surface has been coated with a polyallylamine film by means of plasma assisted deposition/polymerisation in order to improve the cell adhesion characteristics of the implant surface as well. Said approach suffers from the drawbacks that additional equipment as well as laborious and time consuming additional process steps are required for the incorporation of the antimicrobial copper dopant into the Ti material itself. Also, these steps involve rather high temperatures and are only applicable to metallic implants.

In US 2009/0187258 A1 medical implants which are able to induce fibrosis within the patient's body are described and as one suitable implant material among others zinc or a zinc alloy is disclosed. The implants can be surface-modified, e.g. for improving bioresorption. None of the examples actually uses zinc as an implant material and there is no specific disclosure with respect to this option.

In view of this prior art, an object of the present invention is to provide an improved multifunctional coating with excellent antimicrobial activity and cell adhesion regulating, in particular cell adhesion promoting or reducing, surface characteristics which can be produced in a fast, convenient and cost efficient manner. A related object of the invention is to provide an improved method for preparing such a multifunctional coating.

Said objects are solved by the coating of claim 1 and the method of claim 12. Further aspects and specific embodiments of the invention are the subject of additional claims.

The multifunctional coating having antimicrobial activity and the capability to regulate cell adhesion according to claim 1 comprises, deposited on a substrate surface,

- i) an optional anchoring layer,
- ii) a Zn-release layer deposited on the anchoring layer or directly on the substrate surface and
- iii) a functional barrier layer deposited on the Zn-release layer, wherein the functional barrier layer is capable to slow down the diffusion of Zn from the Zn-release layer through the barrier layer and wherein the surface of the functional barrier layer regulates cell adhesion.

The substrate surface to be coated with the multifunctional coating of the present invention is not critical and may comprise or consist of a metallic, semi-metallic, polymeric, or ceramic material or any other material. Specifically, the substrate material is any biocompatible or bioresorbable material used for medical devices, in particular implants and surgical equipment. More specifically, the substrate is selected from the group of metals, in particular steel, Ti

and their alloys and composites and organic polymers, e.g. Teflon, polyurethane, polyethylene, polypropylene.

The optional anchoring layer of the multifunctional coating according to the present invention may be any layer which is capable to bond to the respective substrate surface as well as to the Zn-release layer. It improves the stability of the multilayer on the substrate material, in particular in aqueous medium and in body fluids.

In one preferred embodiment, it consists of a plasma polymerised hexamethyldisiloxane film which can bond to an implant surface via Si-O bridges on the implant side and via Si-C- and Si-O bridges on the multilayer side. Techniques for preparing such an anchoring layer are known in the prior art (Cf.: Plasma polymerized hexamethyldisiloxane in adhesion applications, S. Pihan, T. Tzukruk, R. Förch, Surface & Coatings Technology 2009, 203, 1856-1862; Adhesion Improvement of plasma polymerized maleic anhydride films on gold using HMDSO/O₂ adhesion layers, A.N. Chifen, A.T.A. Jenkins, W. Knoll, R. Förch, Plasma Processes & Polymers 2007, 4, 815-822; Recent and expected roles of plasma polymerized films for biomedical applications, Renate Förch, Anye N. Chifen, Angelique Bousquet, Hwei Ling Khor, Li-Qiang Chu, Zhihong Zhang, Ivy Osey-Mensah, Eva-Katrin Sinner, Wolfgang Knoll, Chemical Vapour Deposition 2007, 13, 280-294).

Alternatively, an anchoring layer can be formed by plasma treatment of the substrate surface with gases, e.g. argon, oxygen, nitrogen.

The antimicrobial activity of the present coating is based on the release of Zn (in the form of Zn²⁺ ions or Zn-clusters) from the Zn-release layer. Typically, said Zn-release layer

comprises a Zn containing polymer coating deposited on the anchoring layer or directly on the implant surface, preferably by plasma assisted polymerisation. The use of zinc in the coating of the present invention is especially advantageous due to its well-known antimicrobial activity together with its ability to be metabolised by the human body and is thus considered to be of lower toxicity than, for example silver. This latter property gives zinc a particular advantage over other metals such as silver, which currently represents the state of the art and is present in a variety of commercial products in wound care, medical devices and implants. As demonstrated in the examples, the Zn in the polymeric film comprises nanoparticles of ZnO and/or Zn as well as complexed zinc ions. Typically, the nanoparticles have diameters in the range of 1 to 50 nm.

The polymeric Zn-release layer can be conveniently synthesised from organo-zinc monomers of low molecular weight that are suitable for plasma deposition processes. Preferably, the monomeric precursors are selected from the group of zinc-organic compounds, e.g. Zn(acac)₂ (zinc acetylacetonate), bis(pentafluorophenyl)-zinc, zinc-hexafluoro acetylacetonate and similar monomers. Typically, the Zn-release layer contains up to 10 atom-% Zn (XPS surface analysis) and has a thickness in the range of 1 to 500 nm.

The functional barrier layer is not in the traditional sense a barrier layer which prevents the diffusion of ions and molecules from one side to another. Instead, in the present sense of the word, the "barrier" coating merely slows down the diffusion of ions and molecules in order to regulate the release of an antimicrobial substance and at the same time offers an uppermost interface that controls or regulates cell adhesion. Thus, less ions and molecules, in particular Zn

ions, are released from the multilayer or bilayer coating in a given time period as compared to the release from the Zn-release layer without such a functional barrier layer deposited thereon.

The functional barrier layer may promote cell adhesion (as compared to a reference surface without this layer, in particular a surface coated only with the Zn release layer or any other reference surface such as a Ti or Teflon surface) either as such, due to its inherent surface characteristics, and/or by further immobilisation of cell adhesion motifs, e.g. fibrinogen, fibronectin, RGD.

Alternatively, the functional barrier layer may reduce the adhesion of cells (as compared to a reference surface without this layer, in particular a surface coated only with the Zn release layer or any other reference surface such as a Ti or Teflon surface) without being cytotoxic due to the chemical nature of the surface.

Thus, the term "regulating cell adhesion" comprises in particular the alternatives "reducing cell adhesion" and "promoting cell adhesion".

This functional barrier layer or coating can be designed in different ways. Preferably, the present invention involves the deposition of a plasma polymerised functional thin layer from precursors such as allylamine (already well known to promote cell adhesion, refs. from Förch CVD Journal 2007 and others), maleic anhydride (refs. from Förch, Jenkins, and others, above) and pentafluorophenyl methacrylate (refs. from Förch & Borros, 2005, 2007, above). Plasma polymer layers deposited from these monomers are highly reactive thin films and can be further derivatised to attach adhesion peptides

and proteins such as RGD or fibrinogen. It has been shown (Förch et al. unpublished) that such surface derivatised layers can further improve the adhesion of P19 progenitor cells as well as fibroblasts, endothelial cells and osteoblasts.

The inventors have demonstrated that plasma polymerised hexamethyldisiloxane exhibits reduced cell adhesion properties (see also refs. from Wei, J. Biomed. Mater. Research Part B: Applied Biomaterials, 2006 as well as Diploma theses of Lotz, MPIP Germany, 2009 and Kroth, MPIP Germany, 2009) and have further found that this material can be advantageously used for providing a functional barrier layer suitable for the multifunctional coating of the invention which functional barrier layer additionally exhibits said antiadhesive surface characteristics.

Typically, the functional barrier layer has a thickness in the range of 1 to 500 nm.

The present invention relates also to a convenient and very effective method for producing the multifunctional coating as disclosed above.

A preferred method for producing the multifunctional coating of the invention comprises the steps:

- a) providing an optional anchoring layer on a substrate surface, e.g. by plasma polymerisation or surface modification;
- b) plasma deposition and simultaneous polymerisation of a Zn-containing organic monomer to provide a Zn-release layer;
- c) plasma deposition and simultaneous polymerisation of one or more organic monomers capable to be polymerised into a

polymer which regulates cell adhesion and provides a functional barrier layer.

More specifically, in the method for producing the multifunctional coating of the invention the optional anchoring layer is provided in step a) by polymerising, preferably plasma assisted polymerising, a siloxane compound, preferably hexamethyldisiloxane (HMDSO) or a structurally similar compound, or by plasma treatment of the substrate surface with gases, e.g. argon, oxygen, nitrogen.

Preferably, the Zn-containing organic monomers deposited and polymerised in step b) are selected from the group of $\text{Zn}(\text{acac})_2$, bis(pentafluorophenyl)-zinc, zinc-hexafluoroacetylacetonate or structurally related compounds.

In order to achieve further improvements of the properties of the Zn-release layer, the Zn containing organic monomers such as $\text{Zn}(\text{acac})_2$ may be mixed with other gases such as fluorocarbons, hydrogen, oxygen, argon, carbon dioxide, carbon monoxide. This allows for the modification of the polymer network structure and can be used to control or tune the diffusion of Zn within and out of the polymer matrix.

Typical, but non-limiting working conditions of the method for producing the multifunctional coating of the present invention are as follows: plasma powers in the range of 5 to 150 Watts, duty cycles for pulsed plasma conditions in the range of 1 to 50 %, working pressures in the range of 0,001 to 0.5 mbar.

Specifically, the organic monomers polymerised in step c) of the present method are selected from the group of allylamine,

maleic anhydride, pentafluorophenyl methacrylate and hexamethyldisiloxane.

A further related aspect of the invention relates to a substrate, more specifically a medical device, e.g. an implant or surgical equipment, comprising the multifunctional coating as disclosed above.

The invention is illustrated in more detail by the following non-limiting examples and figures.

FIGURES

Fig. 1. Schematic cross-sections of 2 variants of a multifunctional coating of the present invention

Fig. 2. IRRAS spectra of pp-Zn(acac)₂ films showing changes in chemical structure with increasing input powers

Fig. 3. TEM micrographs of the same films demonstrating 2-4 nm sized ZnO/Zn nanoparticles within the films

Fig. 4. Zn content of the films; (a) XPS elemental scans showing the presence of Zn in the Zn(acac)₂ plasma polymer; (b) summary of Zn content in the film as a function of position in reactor and input power

Fig. 5. Kinetics of Zn release from two plasma polymerised Zn(acac)₂ films of different thickness

Fig. 6. IRRAS spectra of multilayers of Zn(acac)₂ and ppAA

Fig. 7. SEM image of a multilayer of Zn(acac)₂ and ppAA

Fig. 8. Metal release from an inventive multilayer as a function of adlayer thickness

Fig. 9. ICP-OES measurements of Zn released as a function of time and adlayer thickness

Fig. 10. Antimicrobial activity (a) measured by colony counting of an untreated substrate, plasma deposited acetyl acetate and plasma deposited $\text{Zn}(\text{acac})_2$ films. (b) measured by optical density measurements to compare the antimicrobial activity of $\text{ppZn}(\text{acac})_2$ and $\text{ppZn}(\text{acac})_2$ mixed with hydrogen towards *S. aureus* and *P. aeruginosa*

Fig. 11. Cell surface coverage on unmodified Teflon and Teflon modified with plasma polymerised allylamine, derivatized with RGD and Fibronectin

Fig. 12. Adhesion of human umbilical vein endothelial cells (HUVECs) on different surfaces; **12a:** micrographs (immunostainings): a) titanium; b) ppAA; c) 500 nm $\text{Zn}(\text{acac})_2$; (d) $\text{Zn}(\text{acac})_2$ + 15 nm ppAA; **12b:** Quantitative evaluation after image processing of a-d

Fig. 13. Adhesion of *E. coli* bacteria on different surfaces

Fig. 14. Growth of fibroblast cells (NIH 3T3 cells) and *E. coli* cells on different surfaces: a) ppAA; b) 500 nm $\text{Zn}(\text{acac})_2$ + 15 nm ppAA

Fig. 15. Adhesion of fibroblasts (NIH 3T3 cells) on different surfaces: A) glass; B) ppAA; C) 500 nm $\text{Zn}(\text{acac})_2$; D) $\text{Zn}(\text{acac})_2$ + 15 nm ppAA; E) fibronectin bound to multilayer (D)

Example 1*Preparation of an antimicrobial and cell adhesion promoting coating*

In a first step a polypropylene substrate surface was coated with an anchoring layer of a plasma polymerised hexamethyldisiloxane film which bonds to an implant surface via Si-O bridges on the implant side and via Si-C- and Si-O bridges on the multilayer side. It improves the stability of the multilayer on the implant material in aqueous medium.

Subsequently, plasma deposition of the different monomers in steps b) and c) of the inventive method was effected. Plasma deposition was carried out in a home-built capacitively coupled cylindrical radio frequency (rf, 13.56 MHz) plasma reactor, 30 cm in length and 10 cm in diameter. The power was generated by an rf generator, and was coupled into the reactor chamber via two concentric, external ring copper electrodes placed 12 cm apart. Input powers during this work ranged from 30 to 50W. The samples were placed within the chamber 1 cm from the leading hot electrode. Because of the low volatility of the monomers used, the reactor inlet was equipped with a home-built heating jacket. The monomer supply vessel was heated close to the boiling point of the monomer using a thermal bath. The temperature of the glass reactor was monitored using a thermo sensor (PT-100) mounted on the arm inlet, and was controlled using a home built temperature controller. The reaction chamber was evacuated to 1×10^{-3} mbar using a rotary pump (Leybold Trivac, D16BCS PTFE). A liquid nitrogen cold trap was used to collect excess vapour. A baratron (MKS, Type 627B) placed near the monomer inlet was used to monitor the pressure inside the chamber. The monomer pressure was typically 0.02 mbar, and the deposition time was adjusted to obtain the desired thickness.

Using the above process conditions a typical deposition rate of the $\text{Zn}(\text{acac})_2$ monomer was 12 nm/min. For comparison and to validate the role of the ligand, the acetylacetonate ligand (acac) was also polymerised using the same process conditions. Some improvement over properties was achieved by mixing $\text{Zn}(\text{acac})_2$ with other gases such as fluorocarbons or hydrogen. This allows for the modification of the polymer network structure and can possibly influence the diffusion of Zn within and out of the polymer matrix.

Example 2

Characterisation of the antimicrobial and cell adhesion promoting coating

Chemical composition of the pp- $\text{Zn}(\text{acac})_2$ films

The IRRAS spectrum of the plasma polymerized pp- $\text{Zn}(\text{acac})_2$ films (Fig. 2) shows bands at 1250 cm^{-1} , 1400 cm^{-1} , 1445 cm^{-1} , 1520 cm^{-1} and 1600 cm^{-1} with a significant relative intensity, suggesting that complexing has been at least partially retained during the plasma deposition (Ref.: Inagaki, N.; Tasaka, S.; Nozue, Y. *Journal of Applied Polymer Science* 1992, 45, 1041; Luis Duque, Renate Förch, *Plasma Processes and Polymers* 2011, Volume 8, Issue 5, pages 444-451). The appearance of the additional band around 1720 cm^{-1} indicates -C=O groups in the uncomplexed state and is in agreement with bands observed in the IRRAS spectrum of pp-acac.

The IRRAS spectra of pp- $\text{Zn}(\text{acac})_2$ deposited using different input powers are shown in Figure 2. No significant differences between the low power deposits (20 W and 50W) were observed and the peaks associated with complexed C=O and C-C bonds suggest a similar chemical structure. When increasing the input power to 100W, however, the changes

observed in the spectra suggest a loss of the complexed structure. The intensity of the bands at 1260 cm^{-1} , 1400 cm^{-1} , 1440 cm^{-1} , 1520 cm^{-1} was seen to decrease close to background intensities. The shoulder around $1680\text{--}1730\text{ cm}^{-1}$ indicates substantial conversion of complexed C=O to uncomplexed C=O in the higher power deposits.

TEM micrographs of the same films showed 2-4 nm sized nanoparticles within the films (Fig. 3).

X-Ray Photoelectron Spectroscopy (Fig. 4) showed that the metal content can be variable and is dependent on the deposition conditions used. In the work to date 10 % Zn was the maximum. The Zn appears to be present within the film predominantly as small (2-4 nm) sized ZnO/Zn nanoparticles as shown by TEM (Fig. 3).

Chemical composition and structure of multilayers

Fig. 6. shows IRRAS spectra of a 500 nm ppZn(acac)₂ layer and of multilayers composed of a 500 nm ppZn(acac)₂ layer plus 10 nm or 50 nm ppAA, respectively. The ppZn(acac)₂ layer was produced under conditions of 30 W CW for 45 minutes at 150°C heating and ~ 0.02 mbar pressure. The ppAA layer was generated under conditions of 40 W CW at ~ 0.15 mbar for either 5 or 10 minutes depending on the different desired thickness. The pure ppZn(acac)₂ layer showed the typical absorption bands as described above (Fig. 2). Upon deposition of the pp-allylamine layer additional bands that are characteristic for the pp-AA film are observed at wavenumbers around 3300 cm^{-1} indicating the additional presence of amine groups on the surface. The intensity of this band increased as the thickness of the layer was increased from 10 nm to 50 nm.

Fig. 7 shows SEM images of a multilayer of Zn(acac)₂ (~ 200 nm) and ppAA (~ 200 nm).

The Zn(acac)₂ layer was produced under conditions of 30 W CW for 20 minutes at 150°C heating and ~ 0.02 mbar pressure. The ppAA layer was generated under conditions of 40 W CW for 5 minutes at ~ 0.15 mbar.

Zinc release properties

Zinc release properties of the pure pp-Zn(acac)₂ films were analysed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). It was observed that the Zn is released from a PECVD Zn(acac)₂ layer slowly over approximately 2 days (Fig. 5). The rate of release is dependent on the process conditions used and thus the chemical structure of the surrounding polymer network. The amount of Zinc stored in the pure Zn(acac)₂ films was found to be dependent on the film thickness. The release dose of Zinc was found to be dependent on the film thickness, such that the thicker the film, the more is released, see Fig. 5.

When depositing a plasma polymerised layer of allylamine, maleic anhydride, pentafluorophenyl methacrylate or related monomers on top of the zinc release system, the release of the zinc into the surrounding environment can be slowed down. Fig. 8 shows pp-allylamine films of different thickness deposited on top of the Zinc release system and the effect this has on the release of zinc from the multilayer system. Similar results are to be expected using other functional surface layers.

Fig. 9 shows the amount of Zinc released from multilayers with top layers of different thickness as measured by ICP-OES

measurements. Measurements were conducted with ml of Milli-Q water in a 5 cm diameter polystyrene Petri dish. For each point in time 0.5 ml samples were taken. It can be seen that at time=2 hours the multilayer with the thickest top layer has released the lowest amount of Zinc into the solution, whereas that with no top layer has released the greatest amount of Zinc into solution. After time=24 hours the multilayer with the thickest top layer shows a similar release rate as at t=2 hours. On the other hand, the multilayer with no top layer has already lost much of its available Zinc. After time = 120 hours, again we observe that the multilayer with the thick top layer continues to release a relatively small, but constant amount of Zinc into the solution, whereas the multilayer with no top layer has by that time probably released everything into solution. The data clearly shows that with increasing thickness of top layer the release of the Zn into solution can be reduced significantly. The optimum solution is probably for a thickness of top layer of around 50-100 nm.

The $\text{Zn}(\text{acac})_2$ layer was produced under conditions of 30 W CW for 45 minutes at 150°C heating and ~ 0.02 mbar pressure. The ppAA layers were generated under conditions of 40 W CW for at ~ 0.15 mbar.

Cell adhesion and antimicrobial activity

Antimicrobial activity was tested i.a. towards *Staphylococcus aureus* and *Pseudomonas aeruginosa* using $\text{Zn}(\text{acac})_2$ and $\text{Zn}(\text{acac})_2$ mixed with H_2 during the deposition (Fig. 10). The microbiological results show a very clear trend and no growth of MRSA was observed for the $\text{Zn}(\text{acac})_2$ films, whereas some growth of *Pseudomonas aeruginosa* was still observed. This shows that the film is more effective towards *S. aureus* than towards *P. aeruginosa* and has been reproduced several times.

When comparing pure pp-Zn(acac)₂ films and plasma polymer layers from mixtures of Zn(acac)₂ and Hydrogen (Figure 10b) it can be observed that there are no significant differences and both surfaces show significant antimicrobial behaviour.

Preliminary results show that a relatively low concentration of Zn release is effective in significantly reducing the attachment of *S. aureus*.

Further experiments demonstrate both the antimicrobial activity and the cell adhesion/growth promoting activity of a multilayer/bilayer according to the present invention.

In particular, Fig. 12 shows the growth of human umbilical vein endothelial cells (HUVECs) on 4 different surfaces: a) titanium; b) ppAA; c) 500 nm Zn(acac)₂; Zn(acac)₂ + 15 nm ppAA.

As evident from both the micrographs of immunostainings (Fig. 12a) as such and the corresponding quantitative evaluation after image processing of said micrographs (Fig. 12b), the ppAA coating alone results in a strong growth of the mammalian cells, whereas the ppZn(acac)₂ surface alone appears to be toxic (virtually no growth). The combination of ppZn(acac)₂ + 15 nm ppAA, however, results in a surface which is no longer toxic and cells are able to grow well (even better than upon an untreated Ti surface).

Fig. 13 illustrates the antimicrobial effect of these surfaces when exposed to *E. coli* bacteria.

7x10⁴ CFU *E.coli* pTNT-VSV-OR5 in 200 ml 1/10 LB medium on 1 cm² samples were cultured overnight and 20 µl were plated out on agar in dilutions of 1/1, 1/2500, 1/5000 and 1/10000.

Samples were a) glass; b) 500 nm $\text{Zn}(\text{acac})_2$; c) $\text{Zn}(\text{acac})_2$ + 15 nm ppAA.

The corresponding data show the effect of the untreated surface (control) versus the effect of a $\text{Zn}(\text{acac})_2$ surface and a $\text{Zn}(\text{acac})_2$ surface with an additional pp-AA top layer on the bacterial growth. The two latter ones show no bacterial growth and this is particularly remarkable for the multilayer c) which exhibited adhesion of mammalian tissue cells in Fig 12.

In a further experiment when a sample surface was seeded with both bacteria and mammalian cells, this combined effect was demonstrated more clearly (Fig 14).

E.coli pTNT-VSV-OR5 (10^3 CFU/ml) and NIH 3T3 fibroblasts (10000 cells/cm²) were cultured overnight in Dulbeccos Modified Eagle Medium (DMEM) (Invitrogen) and stained with Toto-1 iodide (nuclei), Rohodamine phalloidin (actin) and DiD (membrane) and photographed with a confocal laser scanning microscope (Leica TCS-SP2).

In panel a) of Fig. 14, the growth of both bacteria and mammalian cells can be observed on a typical single pp-Allylamine film - the bacteria are seen as small green rods distributed randomly over the surface, the cells have been stained to show the nucleus and the cytoskeleton. On the other hand, in panel b) which shows the multilayer of pp $\text{Zn}(\text{acac})_2$ and pp-allylamine it can be seen that only cells are growing whereas the bacteria have been killed by the Zn leaching out.

Fig. 15 shows the growth and adhesion of the NIH 3T3 fibroblasts on different surfaces in more detail.

Fibroblasts (100000 cells/ml) were cultured in 1 ml medium (DMEM) on A) glass; B) ppAA; C) 500 nm $\text{Zn}(\text{acac})_2$; D) $\text{Zn}(\text{acac})_2$ + 15 nm ppAA; E) fibronectin bound to multilayer (D)

From a comparison of panels C) (very low growth) and D) (considerably enhanced growth relatively to C)) a similar effect as for the HUVEC cells in Fig. 12 is evident.

Furthermore, panel E) shows the enhanced adhesion/growth of mammalian cells on a surface with additional fibronectin adhesion motifs.

CLAIMS

1. A multifunctional coating having antimicrobial activity and the capability to regulate cell adhesion, comprising, deposited on a substrate surface,
 - i) an optional anchoring layer,
 - ii) a Zn-release layer deposited on the anchoring layer or directly on the substrate surface and
 - iii) a functional barrier layer deposited on the Zn-release layer, wherein the functional barrier layer is capable to slow down the diffusion of Zn from the Zn-release layer through the barrier layer and wherein the surface of the functional barrier layer regulates cell adhesion.
2. The multifunctional coating according to claim 1, wherein the optional anchoring layer is produced by plasma polymerisation of hexamethyldisiloxane or plasma treatment of the substrate surface with gases, e.g. argon, oxygen, nitrogen.
3. The multifunctional coating according to claim 1 or 2, wherein the Zn-release layer is a polymeric film comprising nanoparticles of ZnO and/or Zn as well as complexed zinc ions.
4. The multifunctional coating according to any one of claims 1 to 3, wherein the polymeric film of the Zn-release layer has been formed by plasma assisted polymerisation of zinc-organic precursors, e.g. $\text{Zn}(\text{acac})_2$, bis(pentafluorophenyl)-zinc, zinc-hexafluoro acetylacetonate.
5. The multifunctional coating according to any one of claims 1 to 4, wherein the functional barrier layer comprises an organic polymer which promotes cell adhesion.

6. The multifunctional coating according to any one of claims 1 to 4, wherein the functional barrier layer comprises a polymer which reduces cell adhesion.

7. The multifunctional coating according to claim 5 or 6, wherein the organic polymer of the functional barrier layer comprises a polymer film produced by plasma assisted polymerisation of monomers selected from the group consisting of allylamine, maleic anhydride, pentafluorophenyl methacrylate, hexamethyldisiloxane.

8. The multifunctional coating according to claim 5 or 7, wherein the organic polymer of the functional barrier layer comprises a polymer film produced by plasma assisted polymerisation of monomers and on which polymer film further cell adhesion molecules or motifs, e.g. fibrinogen, fibronectin, RGD, are immobilised.

9. The multifunctional coating according to any one of claims 1 to 8, wherein the Zn-release layer comprises ZnO/Zn nanoparticles having a diameter of 1 to 50 nm as well as complexed zinc ions.

10. A substrate comprising the multifunctional coating according to any one of claims 1 to 9.

11. A medical device, e.g. an implant or surgical equipment, comprising the multifunctional coating according to any one of claims 1 to 9.

12. A method for producing the multifunctional coating according to any one of claims 1 to 9, comprising the steps:
a) providing an optional anchoring layer on a substrate surface;

- b) plasma deposition and simultaneous polymerisation of a Zn-containing organic monomer to provide a Zn-release layer;
- c) plasma deposition and simultaneous polymerisation of one or more organic monomers capable to be polymerised into a polymer which regulates cell adhesion and provides a functional barrier layer.

13. The method of claim 12, wherein the anchoring layer is produced by plasma polymerisation of hexamethyldisiloxane or by plasma treatment of said substrate surface with gases.

14. The method according to claim 12 or 13, wherein the Zn-containing organic monomers in step b) are selected from the group of plasma polymerisable zinc-organic precursors, e.g. $\text{Zn}(\text{acac})_2$, bis(pentafluorophenyl)-zinc, zinc-hexafluoroacetylacetonate.

15. The method according to any one of claims 12 to 14, wherein the organic monomers in step c) are selected from the group of allylamine, maleic anhydride, pentafluorophenyl methacrylate and hexamethyldisiloxane.

16. The method according to any one of claims 12 to 15, wherein in step b) the Zn-containing organic monomer is mixed with additional gases, e.g. fluorocarbons, hydrogen, oxygen, argon, carbon dioxide, carbon monoxide.

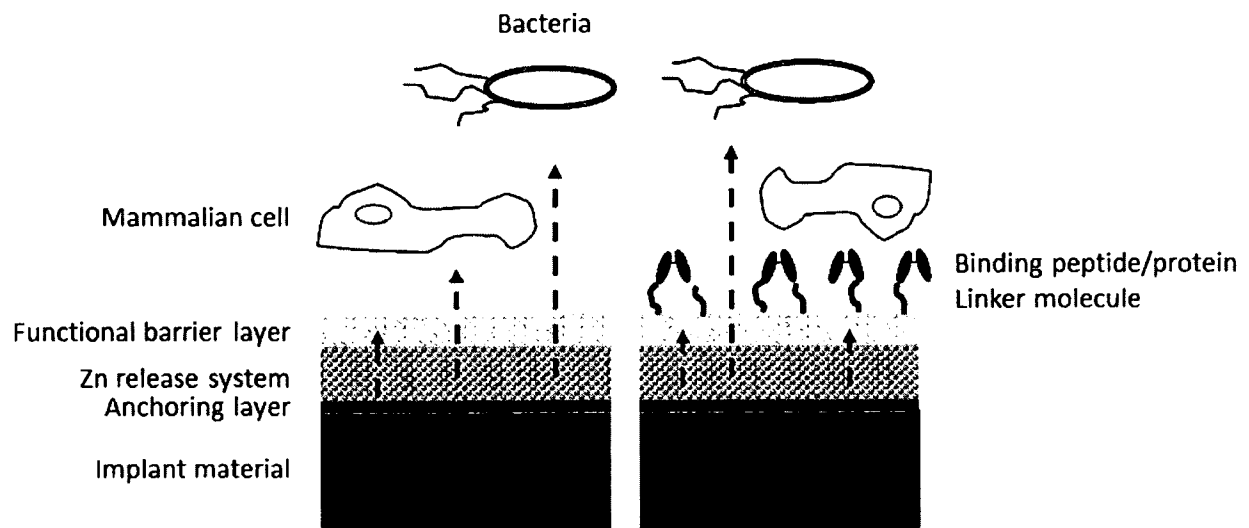


Fig. 1

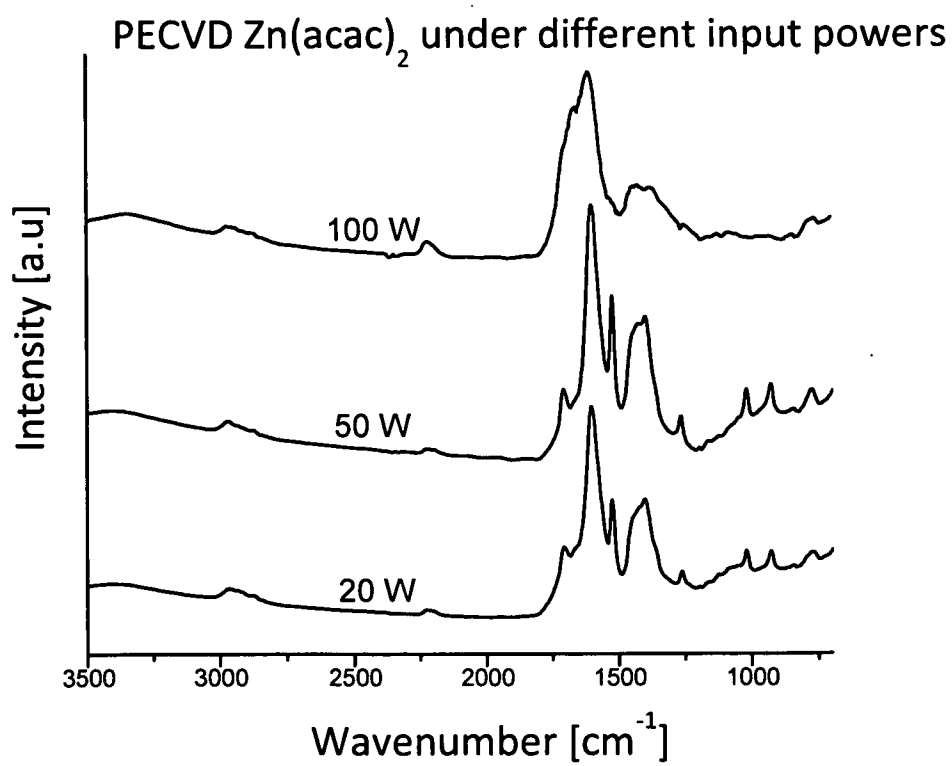


Fig. 2

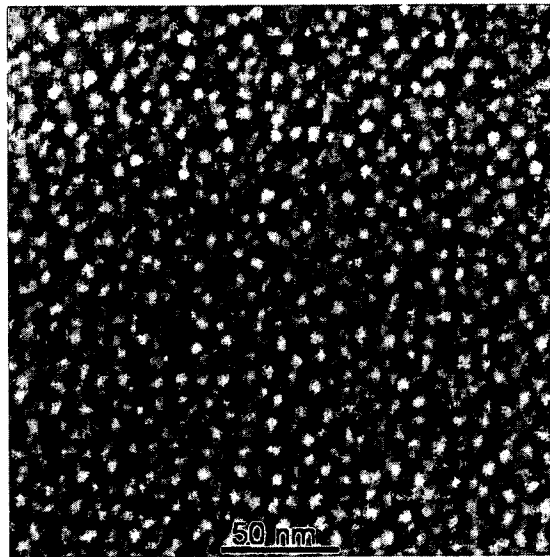


Fig. 3

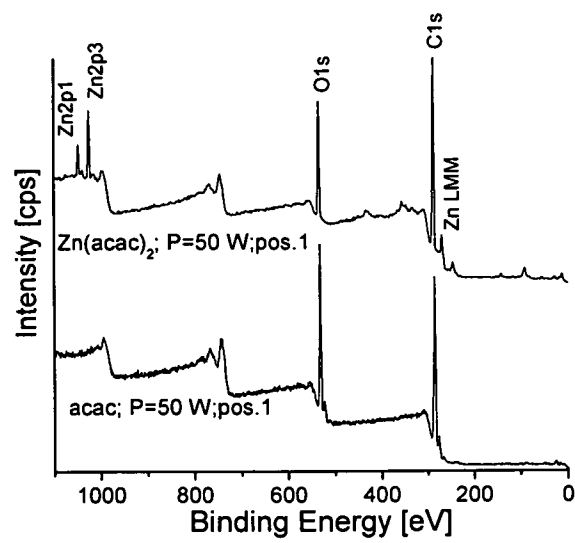


Fig. 4a

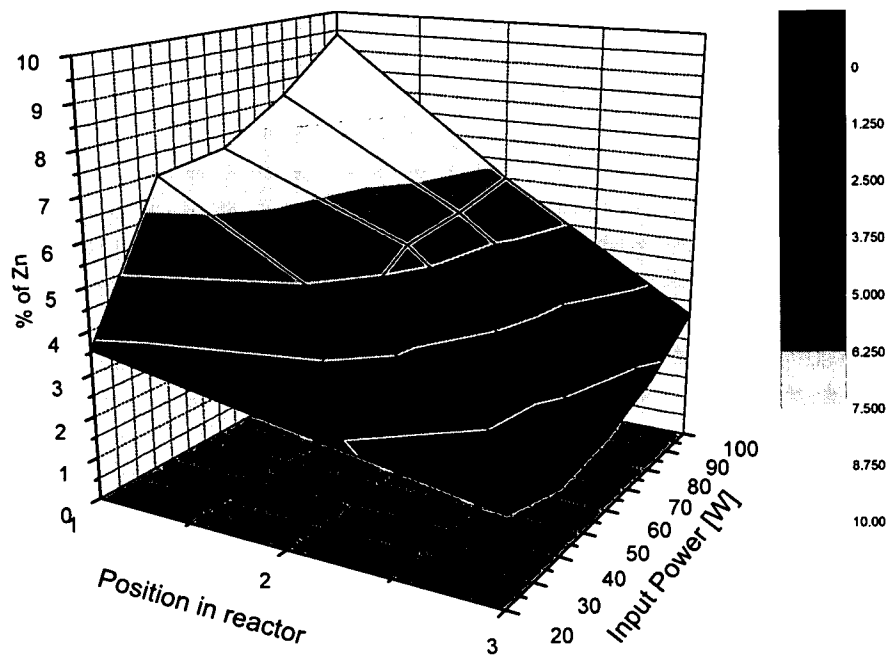


Fig. 4b

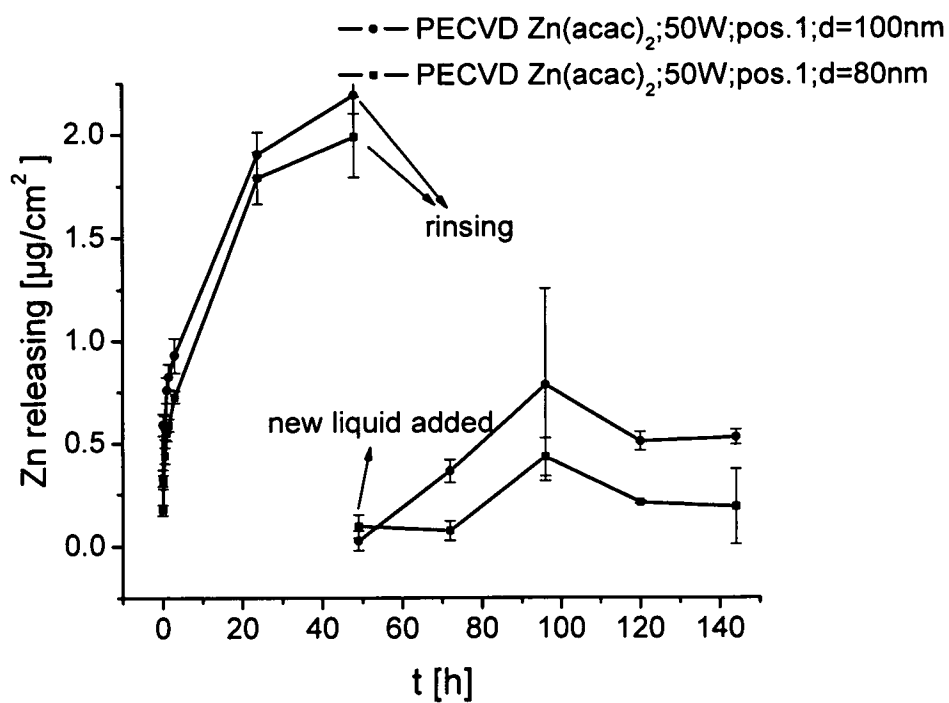


Fig. 5

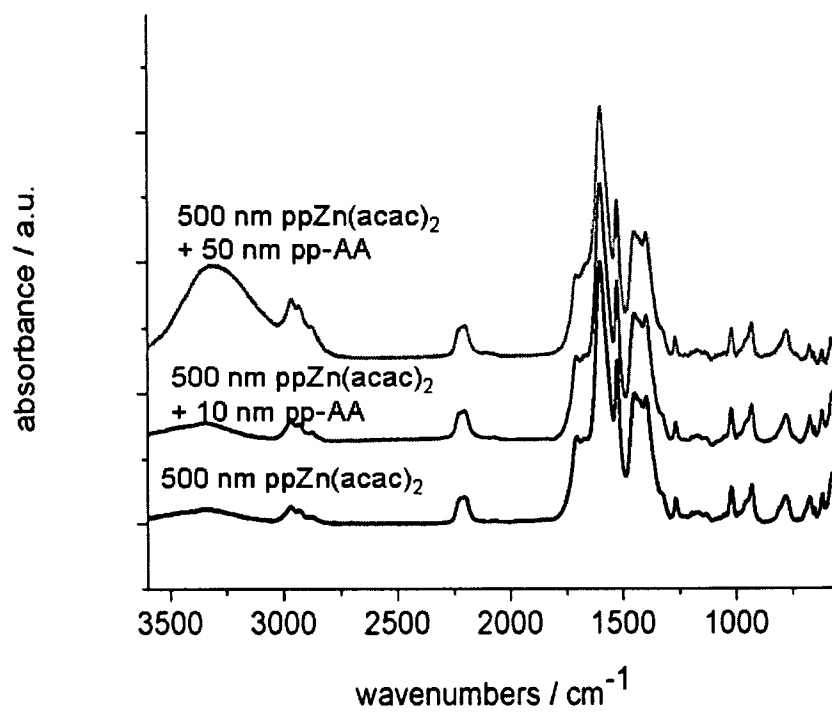


Fig. 6

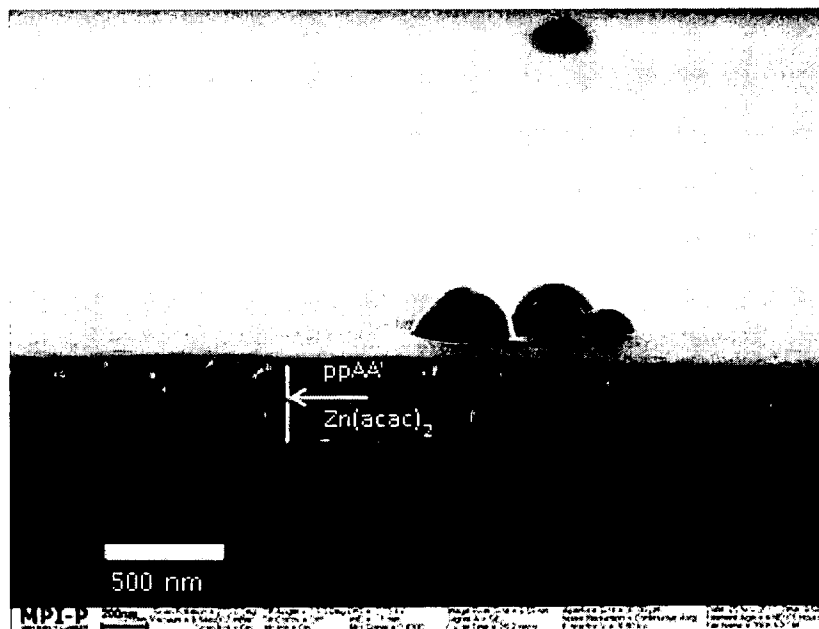


Fig. 7

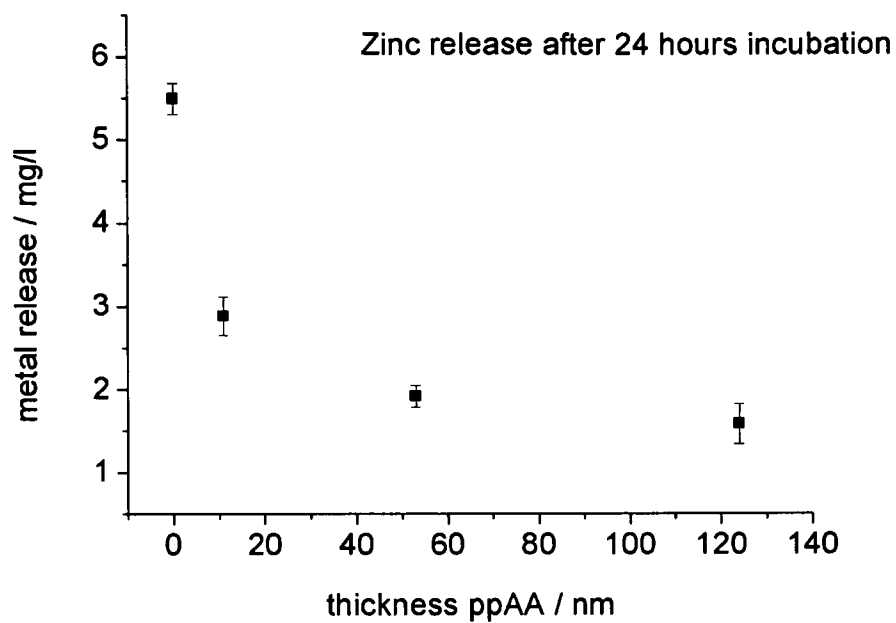


Fig. 8

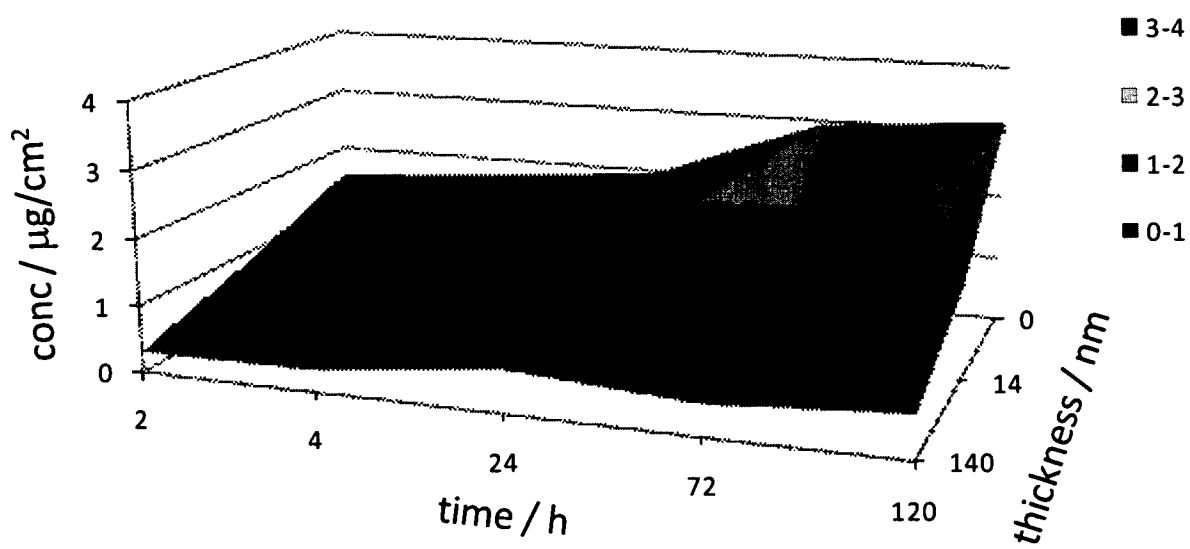


Fig. 9

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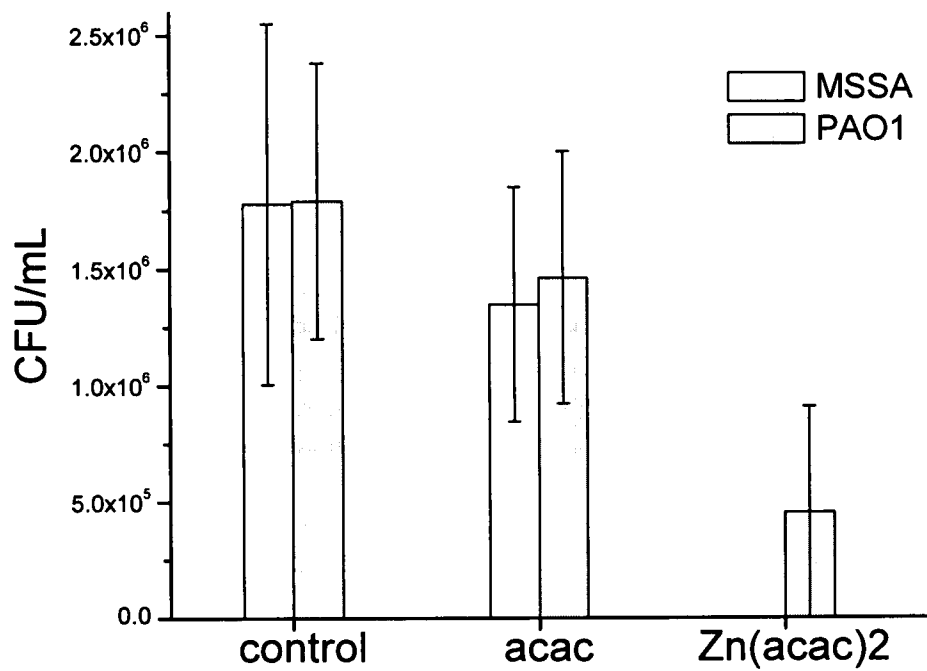


Fig. 10a

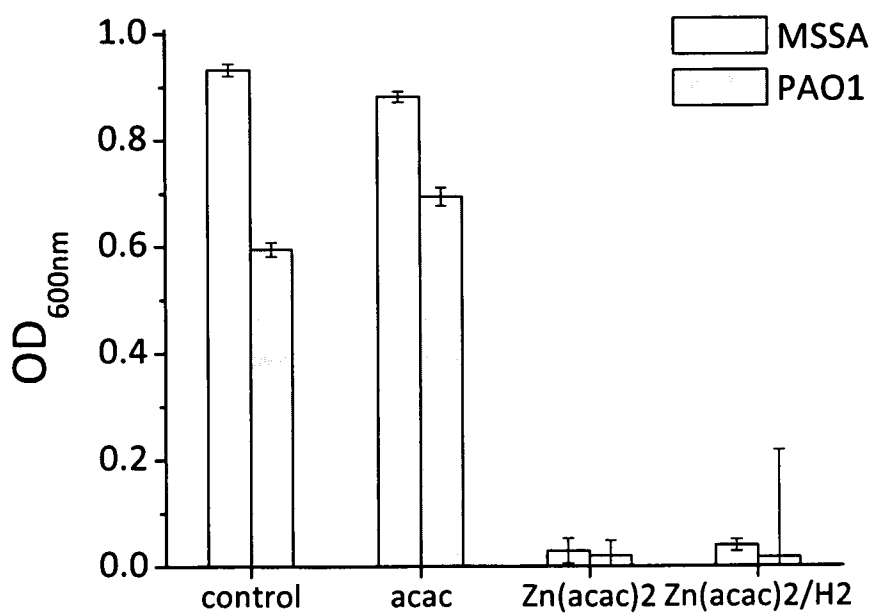


Fig. 10b

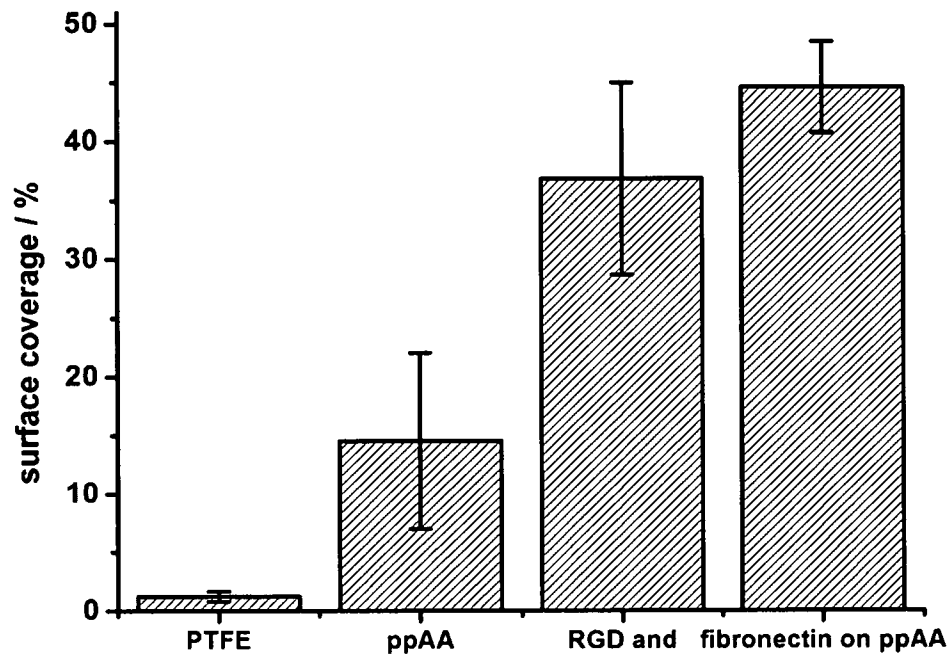


Fig. 11

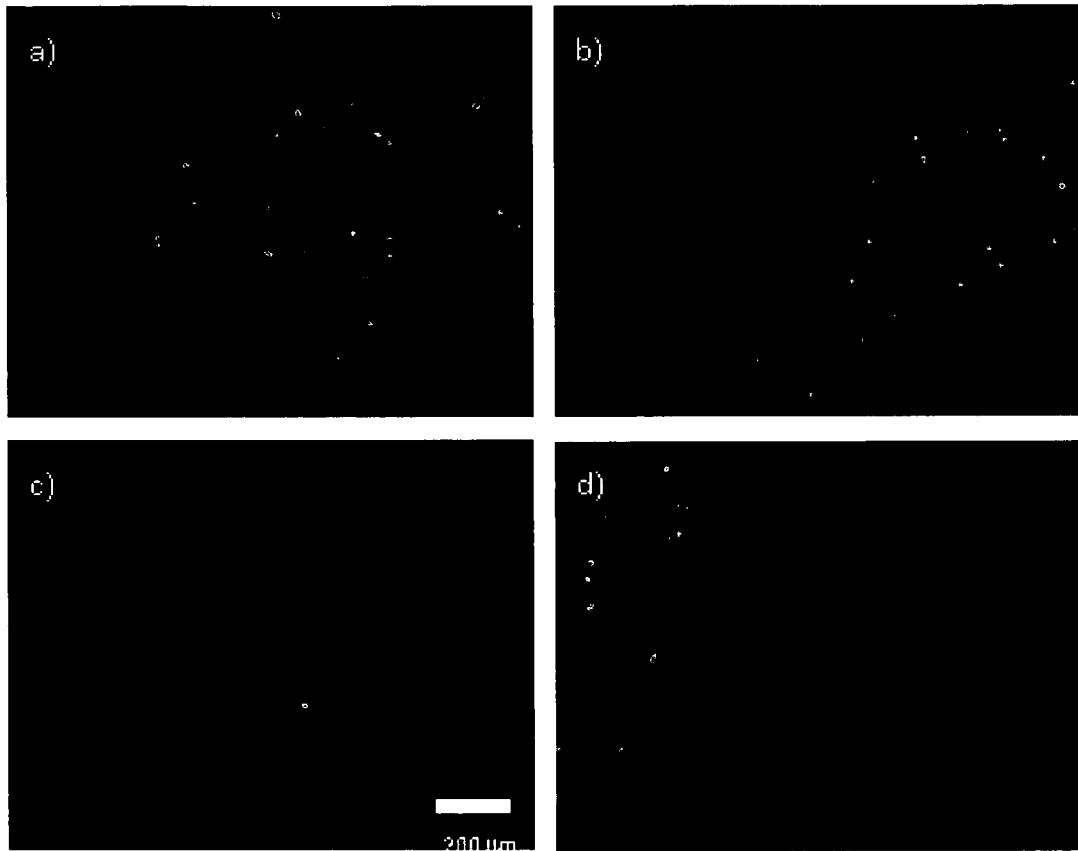


Fig. 12a

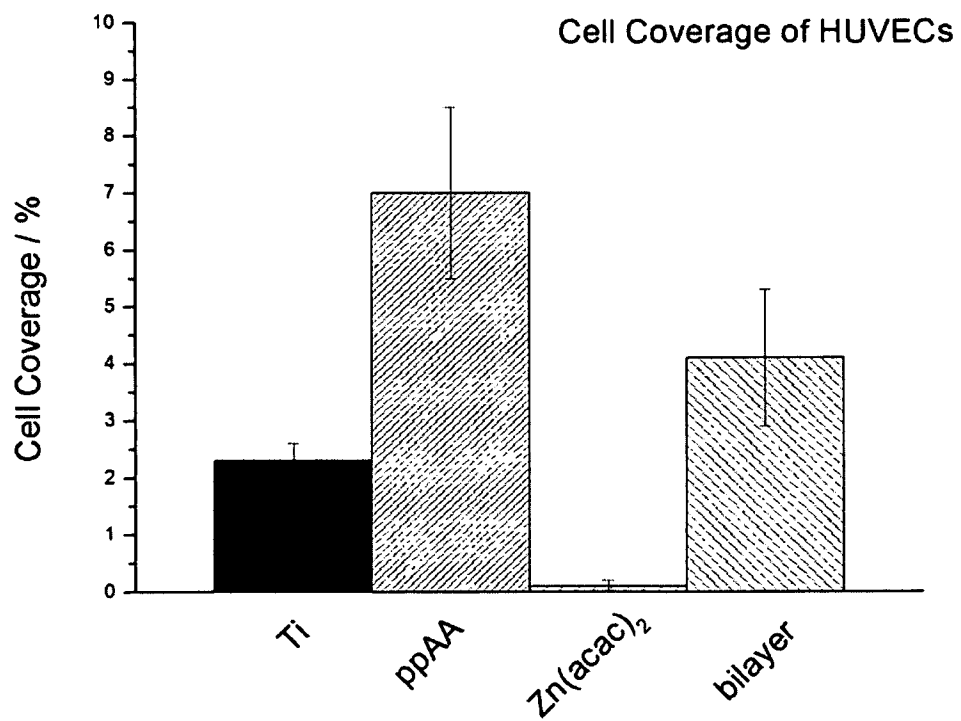


Fig. 12b

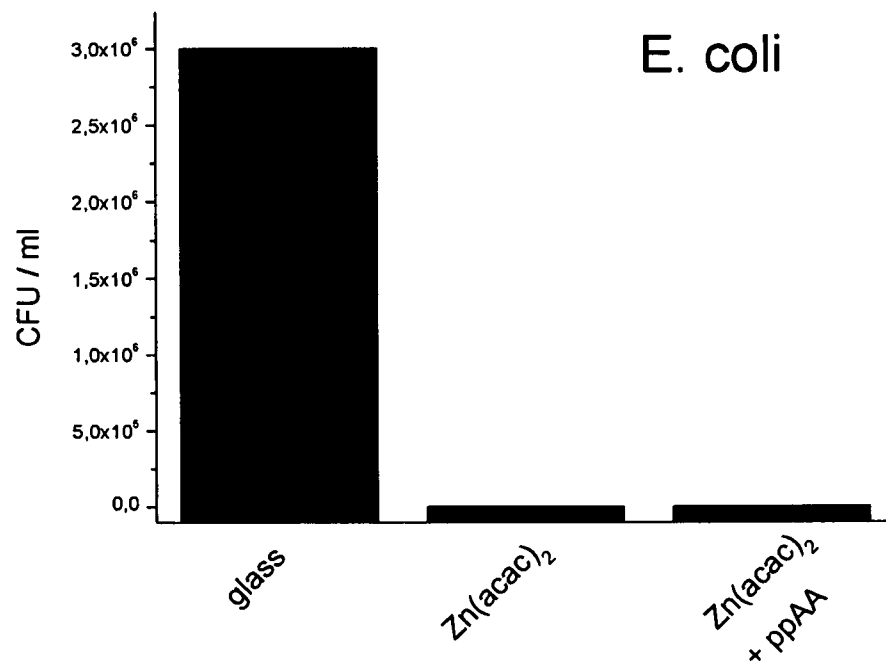


Fig. 13

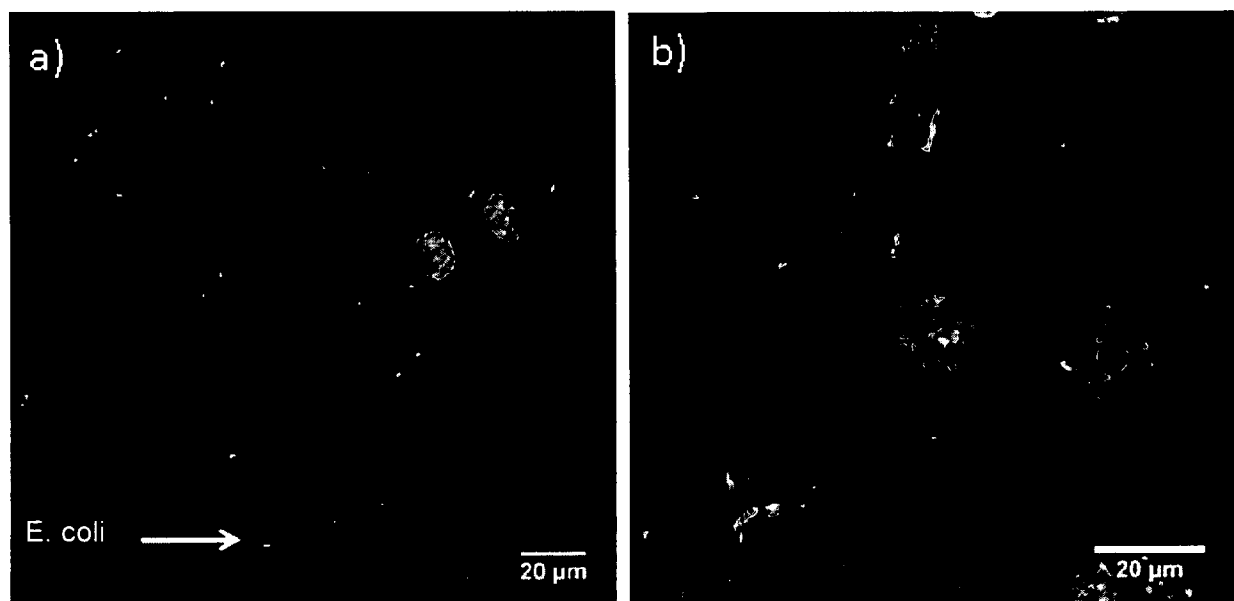


Fig. 14

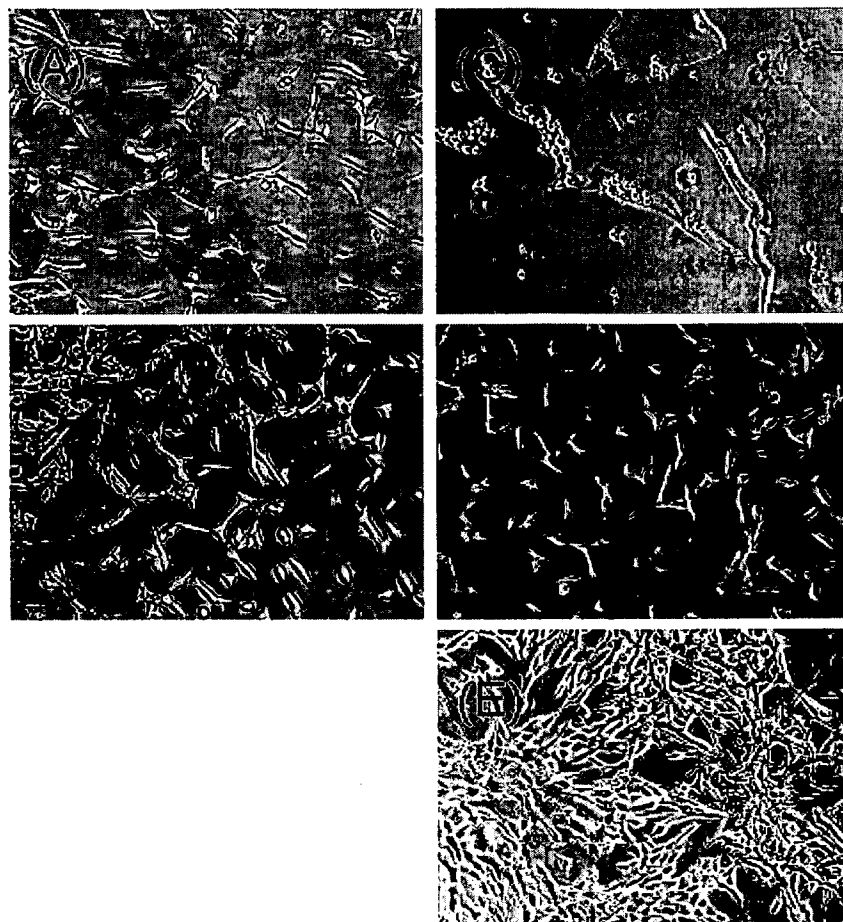


Fig. 15

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/003934

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61L27/34 A61L29/08 A61L31/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/068985 A1 (UNIV SOUTH AUSTRALIA [AU]; VASILEV KRASIMIR ATANASOV [AU]; GRIESSER HA) 24 June 2010 (2010-06-24) claims 1-20	1-16
A	US 6 495 613 B1 (GANGNUS BERND [DE]) 17 December 2002 (2002-12-17) claims 1-16	1-16
A	US 2005/064176 A1 (TERRY RICHARD N [US]) 24 March 2005 (2005-03-24) claims 1-21	1-16
A	US 2009/035341 A1 (WAGENER MICHAEL [DE] ET AL) 5 February 2009 (2009-02-05) claims 1-35	1-16



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

16 September 2011

Date of mailing of the international search report

26/09/2011

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Schneider, Aurore

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2011/003934

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