# BIOCOMPATIBLE AND BACTERICIDAL COATINGS ON TITANIUM BASED IMPLANT MATERIALS BY USING OF PCO AND APCVD

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**Abstract**

Biocompatible and bactericidal coatings were deposited onto different titanium based implant materials. For this the plasma chemical oxidation (PCO) process as well as the atmospheric pressure plasma chemical vapour deposition (APCVD) was used successively. With the PCO technique about 10 µm thick oxide films with high amounts of amorphous calcium phosphate and a defined morphology structure can be deposited onto the titanium implant substrates. It can be shown that the morphological structure and the film composition have a positive influence on the adhesion and the growth of human cells, tested with HaCaT keratinocytes and therefore are suited to improve the biocompatibility compared to pure metals or alloys. With the APCVD thin composite SiOx films can be deposited on the modified titanium substrates. Silver, copper or zinc were used as bactericidal agent, the APCVD film thickness is in the range of less than 150 nm. It could be shown that the bactericidal efficiency of these composite coatings depends slightly on the amount of agent in the films, the agent itself and the used bacteria strains with their specific cell structure. For both of the shown agents Ag and Cu a therapeutic range could be determined, without cytotoxic but bactericidal properties. Beside the description of the coating processes and test methods diverse film properties like structural images as well as the bactericidal and cytotoxic behaviour of the coatings will be presented.

**Keywords:** Plasma chemical oxidation (PCO), Atmospheric pressure plasma chemical vapour deposition (APCVD), implant materials, titanium alloys, bactericidal behaviour

## 1. INTRODUCTION

One of the biggest challenges in orthopedics, among other things is the treatment or prevention of infections during and after an implant operation. The incidence of postoperative infections is generally low and is in the range of 0.3 - 3% [1-3]. The consequences for the affected patients are very uncomfortable, ranging from longer hospital stays, antibiotics, pain up to immobilization. Cytocompatibility is necessary for biomaterials for integration to human tissue, in particular for orthopedic implants, when the osseointegration is a major goal. So the combination of improved osseointegration and prophylaxis of implant-related infection would solve two major concerns in one step. Therefore, it was our goal, producing an additional antibacterial effect on a macroporous Ca and P containing PCO - layer. Both layers were deposited onto titanium alloy samples, first the PCO – layer to improve biocompatibility and then an extra thin bacterial active but non-toxic layer by means of APCVD.

## 2. EXPERIMENTAL PART

### 2.1. The Plasma chemical Oxidation

Plasma chemical oxidation (PCO) was used to modify the surface of titanium alloy implants (TiAl6V4), as published before [4]. In short, this technique converts the nm-thin natural occurring titanium-oxide layer on an implant to a ten µm thick ceramic coating. The surfaces have a macroporous structure and were loaded with calcium and phosphorus from the electrolyte.

The oxide film thickness increases linearly with approximately 1.5 $nm \cdot V^{-1}$ to 3.0 $nm \cdot V^{-1}$ during the anodic oxidation process. With a suitable electrolyte further layer growth beyond the coloration regime of the anodic oxidation is realizable. Above a certain voltage, mainly determined by the electrolyte, work piece and power supply settings, the oxide is insufficiently resistive to prevent increasing current. This results in an augmented oxygen formation with local field strengths above 106 $V \cdot m^{-1}$ to 109 $V \cdot m^{-1}$ and an ignition of a thermal spark discharge in pure oxygen surrounded by aqueous electrolyte. This additional layer formation pathway increases the deposition rate and leads to the incorporation of electrolyte compounds into the layer when the previously melted plasma channels and pores solidify again. With this plasma assisted electrolyte incorporation into the $TiO_2$ matrix the main technological aspect is described to dope these layers with functional elements or compounds such as Ca and P. Furthermore, the longer the discharge regime of the deposition process occurs the more dopant material is incorporated into the coating. The process of plasma chemical oxidation is also suitable for producing protective layers on biodegradable magnesium alloys [5].

The power supply for the electrolysis cell consists of a special pulsed rectifier from *Munk GmbH* in Hamm, Germany. This power supply delivers a pulsed direct current of 10 A with voltage from 0 V to 350 V and an adjustable pulse frequency from 10 Hz to 1000 Hz.

The coatings were generated by the plasma chemical oxidation process in alkaline electrolytes. The major components of the alkaline electrolyte used for the PCO process were calcium dihydrogen phosphates, sodium dihydrogen and ammonium hydroxide (CaP-electrolyte). The samples were polarised anodically with a constant current of 0.1 $A/cm^2$ until reaching a voltage of 350 V by applying pulsing DC. More details of the process are given elsewhere [4].

In figure 1 one can see by SEM the change in morphology of TiAl6V4 after treatment and deposition with the PCO process, resulting in an increase of the specific surface and the same time named changes to film and surface composition, respectively.

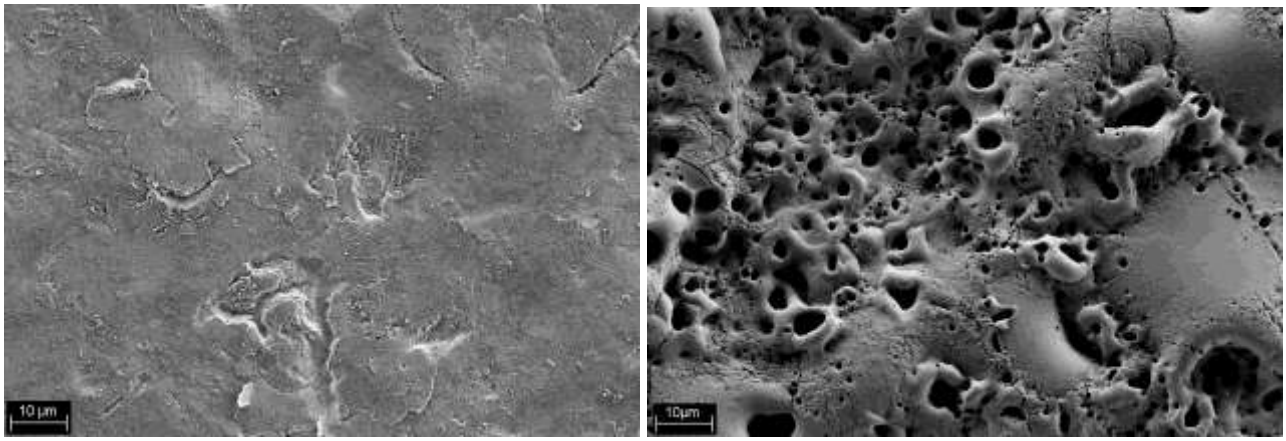


**Fig. 1** SEM surface topography of untreated TiAl6V4 alloy (left) and biocompatible modified one by plasma chemical oxidation (PCO) (right)

### 2.2. The atmospheric pressure plasma chemical vapor depostion (APCVD)

APCVD combining a typical CVD process with a plasma discharge at atmospheric pressure was used to deposit the bactericidal thin films. As plasma source for the APCVD process, a plasma jet of type BLASTER MEF (TIGRES, Marschacht, Germany) was used in combination with a modified plasma blast pipe, which enabled to feed additives as second precursors into the plasma. A pulsed dc plasma was ignited between two cylindrical electrodes, i.e. an inner stick electrode and the actual plasma nozzle as grounded outer electrode. The plasma jet was accelerated using compressed air at a pressure of 6 bar. The length of the plasma jet was controlled by the applied power set to 400 W. Further details on the APCVD process are reported in previous papers [6]. The substrates were located at a distance of 10 mm below the plasma jet. All

samples were moved in meandering patterns with respect to the stationary plasma jet, with a set grid spacing distance of 3 mm. To achieve a film thickness of ~100-150 nm, 8 runs during the deposition process were realized.

As primary precursor, evaporated hexamethyldisiloxane (HMDSO) was used to create the $SiO_x$ thin film matrix. As secondary precursors, solutions with 5% silver nitrate ($AgNO_3$) and copper nitrate ($Cu(NO_3)_2$), respectively, both in a 1:1 volume mixture of isopropanol and water, were used. The variable parameters used to adjust the Ag and Cu content in the films were the flow rates of the $AgNO_3$ and $Cu(NO_3)_2$ solutions, respectively. These were varied between 20 µl/min and 100 µl/min in steps of 20 µl/min.

### 2.3. Bactericidal and cytotoxic test methods

All these standardized measurements were done in cooperation with the University Medical Center in Jena, (Department of Dermatology), which is equipped with the crucial microorganisms responsible for infections and also test methods. Three kinds of pathogenic microorganisms were used for the antibacterial investigations: *Staphylococcus aureus* ATCC 6538 (Gram positive), *Klebsiella pneumoniae* ATCC 4352 (Gram negative) and *Pseudomonas aeruginosa* DSM 1117 (Gram negative). Tests in direct contact between the surface and the organisms, according to ISO 22196, were carried out to quantify the activity of the coatings in comparison to a specified PE-foil, which acts as reference.

Cytotoxic effects were determined on Human adult low calcium high temperature keratinocytes (HaCaT, cell line 432) with two assay kits, which were applied consecutively. After colonisation on the substrate surface for 72h (37°C), in a first step the CellTiter-Blue® Cell Viability Assay (Promega, Germany) was used and then afterwards measurements of the Adenosine triphosphate (ATP) content (ATP lite, Perkin Elmer, Germany). In both cases glass samples in the same dimensions like the substrates were references for ideal, unaffected cell growth. Results will be only shown here for ATP assays because the outcome was very similar between both test methods. The ATP concentration is a direct indicator regarding the cell viability and is measured by luminescence radiation within this assay kit. Low intensity of light means high toxicity and vice versa.

## 3. RESULTS AND DISCUSSION

Normally the APCVD deposition process of pure silicon oxide layers without other incorporated materials leads to homogeneous, dense films. With increasing dosing rates of the $AgNO_3$ and $Cu(NO_3)_2$ Precursor solutions additionally, more and more nm-sized particles and agglomerates are created in-situ inside the plasma jet, which are also recognizable on the substrate surface after the deposition. In figure 2 this can be seen for both substances in SEM surface images for the highest precursor dose, each time.

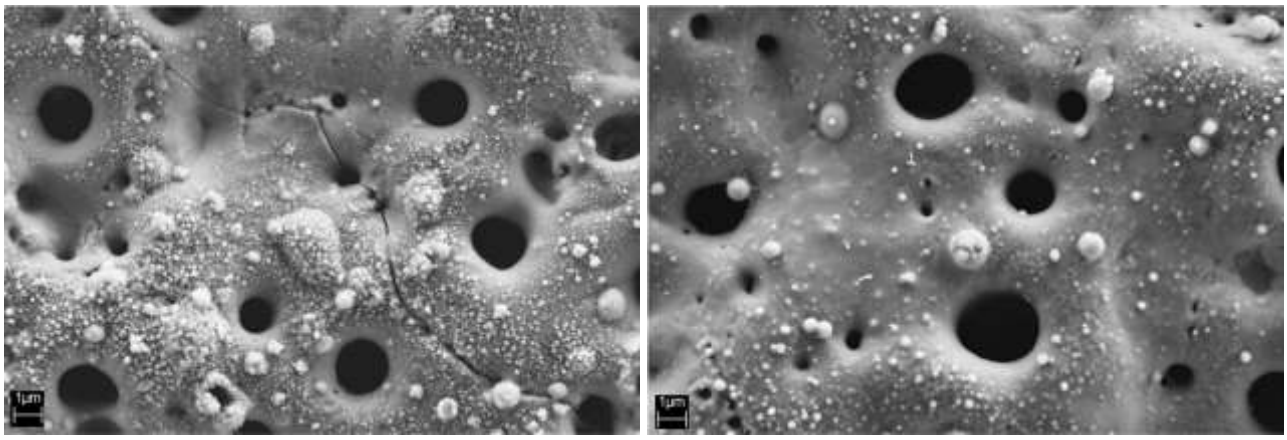

**Fig. 2** SEM surface topography after APCVD coating – $SiO_x$ / Ag with highest flow rate of $AgNO_3$ precursor (left) as well as for $SiO_x$ / Cu and the highest flow rate of $Cu(NO_3)_2$ precursor (right)

The range of these particle and cluster sizes vary from small nanometers up to the some micrometers in the case of agglomerates. In previous publications it could be clarified that the small sizes up to about 100 nm are mostly embedded into the silicon oxide matrix and the bigger ones are preferably located on top of the layers. So in case of abrasive wear to the surface first these on top will be removed, the remaining composite layer is quite stable and releases the active agents continuously when interactions with microorganisms in direct contact or bacteria suspensions occur [7]. XPS measurements reveal full oxidation of copper in the layers by using the $Cu(NO_3)_2$ precursor, but also residuals of the initial nitrate precursor can be found [8]. For silver it is expected that mostly metallic silver nanoparticles are created besides the nitrate residuals [7], but up till now it is not completely confirmed. The antibacterial assays (Fig. 3 and 4) show that the antibacterial effect on the gram negative *Klebsiella pneumoniae* was significant stronger, especially for the silver precursor, than for the gram positive *Staphylococcus aureus*. In tendency higher precursor doses are leading to slightly higher antibacterial activity, as expected.

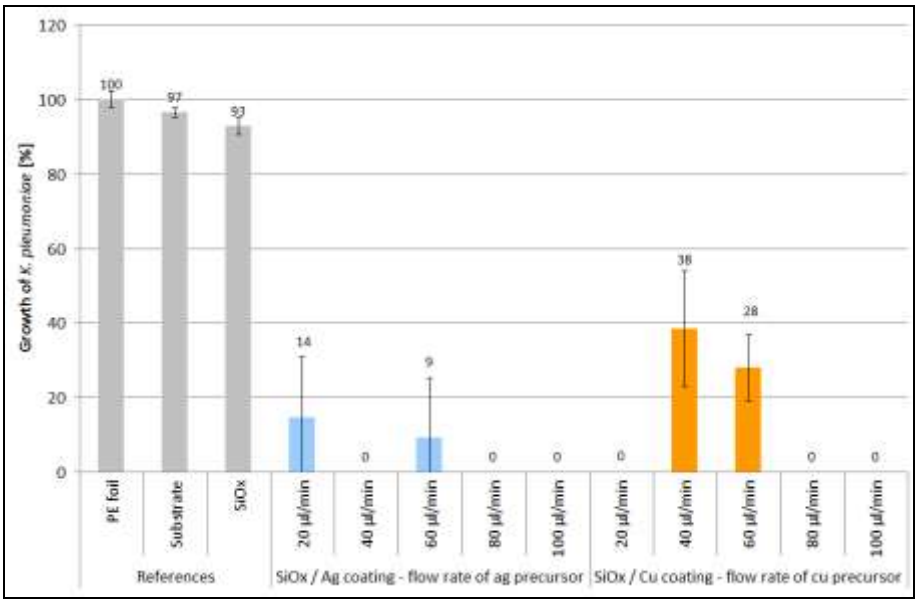


**Fig. 3** Growth of *Klebsiella pneumoniae* ATCC 4352 in respect to PE foil as reference, tested according to ISO 22196, incubation time of 24h

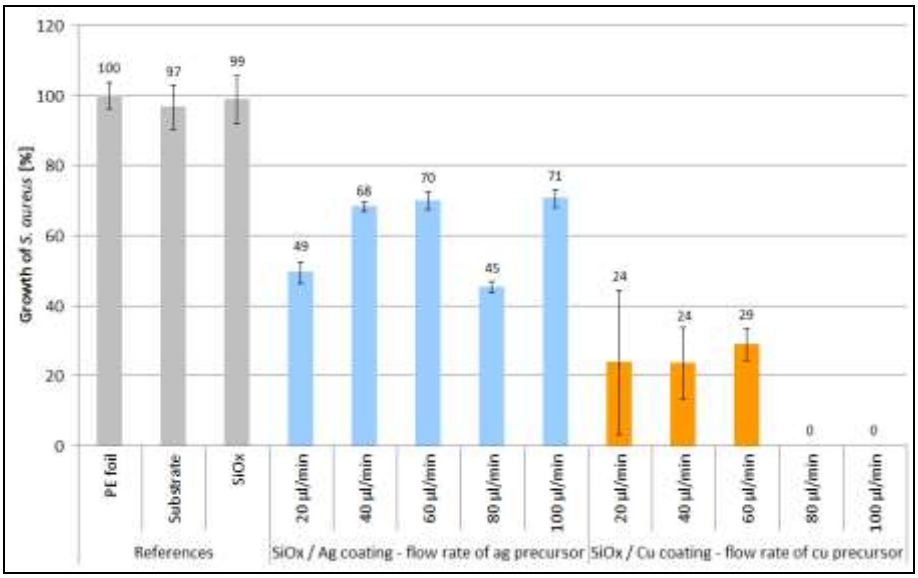


**Fig. 4** Growth of *Staphylococcus aureus* ATCC 6538 in respect to PE foil as reference, tested according to ISO 22196, incubation time of 24h

The growth reduction of *K. pneumoniae* was up to 100%, dependent of the antibacterial agent and concentration. On the other side the antibacterial effect on *S. aureus* was slightly lower except for copper as an antibacterial agent. These differences in activity for silver between gram-positve and gram-negative bacteria are in good accordance to observations from Kawahara et al. [9]. They suppose that gram-positive bacteria are more resistant against silver ions due to cell walls which contain three to twenty times more peptidoglycan than gram-negative bacteria. Peptidoglycan is negatively charged and can bind a higher percentage of the positive charged silver ions, which are released of the nanoparticle surfaces [9].

The cytotoxic ATP assay performed with HaCaT showed that the PCO coating had a positive effect on the growth rate of the cells. The number of viable cells was almost doubled with the pure PCO coating. Higher concentrations of silver precursor starting at 80 µl/min showed a strong cytotoxic impact on the HaCaT cells. Copper showed in comparison to the uncoated reference a good compatibility despite high concentrations of precursor. In comparison to the reference material the PCO layer compensates the cytotoxicity of antibacterial layers and enhanced the cell growth rate slightly.

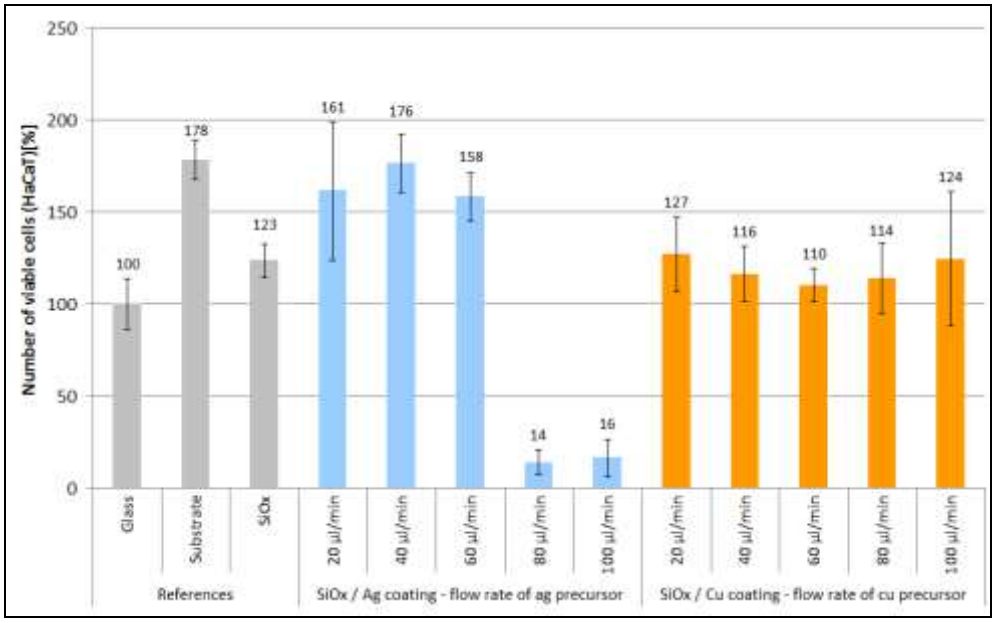


**Fig. 5** Cytotoxic behaviour against HaCaT keratinocytes compared to glass references, ATP assay

## 4. CONCLUSION

The investigations have shown that it is possible to combine the excellent biocompatibility of PCO calcium phosphorus layers on titanium based surfaces with the antibacterial effect of APCVD coatings. Different antibacterial agents such as silver and copper have achieved a good antibacterial effect (very strong to significant reduction) against pathogenic gram positive and gram negative bacteria but negligible cytotoxicity, at the same time. Therefore, a therapeutic range could be found for both active agents.

So these APCVD layers show good promise for medical applications like implants as shown here, but also temperature sensitive surfaces like textile wound dressings can be modified by this technique [10].

## ACKNOWLEDGEMENTS

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