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TiO₂ and Ag-TiO₂ nanohybrid films on titanium dental implant material: an *in vitro* cell culture study

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Abstract

Failure of dental implants is caused mainly by peri-implant infections resulting in loss of supporting bone. Our aim was to examine the attachment and proliferation of primary epithelial and MG63 osteosarcoma cells on Ti dental implants coated with two photocatalytic nanohybrid films, which could eliminate bacterial contamination. Commercially pure (CP4) sand blasted, acid etched and machined Ti discs were used as control surfaces. Two copolymer based layers were investigated: 60 % TiO₂/ 40 % copolymer and 60% AgTiO₂/ 40 % copolymer ([Ag] = 0,001 m/m %). Surface properties were examined by scanning electron microscopy (SEM) and profilometry. *In vitro* attachment and proliferation of epithelial and MG63 cells were investigated via MTT and visualized with fluorescence microscopy. SEM and profilometry revealed significant changes in surface roughness and morphology of the AgTiO₂ layer compared to the control surface. MTT results demonstrated that the attachment (24h) of the epithelial cells was significantly higher on the Ag-TiO₂ coated samples than on the control surfaces, whereas MG63 cells did not show any difference in attachment between the groups. No significant difference was found in the proliferation of the cells on the covered samples (72h, 168h). After one-week, epithelial cells showed slightly increased survival as compared to MG63 cells. The potential antibacterial effect of the silver containing nanohybrid layer makes it a promising surface coating.

Keywords: nanoparticles, silver, MG63 osteoblasts, epithelial cells, titanium (alloys)

1. Introduction

In spite of their widespread application, dental implants are still in the focus of intense research. Dental implants belong to the most frequently used biomaterials, since they have become the first choice for tooth replacement¹⁻³. The last few decades have seen the development of a host of surface modifications aimed at shortening the healing period and accelerating osseointegration. These modifications have ranged from the macro to the micro scale, but research is increasingly concentrating on the nano scale⁴.

Titanium is still the number one material for dental implants, thanks to its excellent physical, mechanical and chemical properties. A high weight to strength ratio, excellent corrosion resistance and biocompatibility are well known properties of this metal^{5,6}. Commercially pure (CP 1 - 4) titanium is still the most widely used material for implants, but titanium alloys, such as Ti6Al4V (CP 5) also have great potential⁷.

Although the success rate of titanium implants is generally high, peri-implant inflammation, the most common factor leading to implant failure, is still a problem. In the case of peri-implant mucositis, only the soft tissue is affected and it is reversible with efficient conservative therapy. The more progressive form is peri-implantitis, which is an irreversible disease of the soft and hard tissues that surround the implant⁸. Of all the implantation cases, 10% ends up in peri-implantitis, provided that the patient has not suffered from periodontal disease previously. Periodontal disease in the patient's history increases this to approximately 20%⁹.

Peri-implantitis is treated either in a conservative (manual debridement, antibiotics, laser supported and photodynamic therapy) or a surgical (resective or regenerative) way. The ultimate purpose is to clean the contaminated implant surface and remove the bacterial biofilm. Both approaches have their drawbacks, though. Mechanical root scaling and air polishing can modify the surface characteristics of the implant, the use of antibiotics can lead to antibiotics resistance, while a resective surgical therapy can result in extensive postoperative recession⁸. Since there is no ideal treatment for peri-implantitis, prevention should be assigned priority. Antibacterial surfaces represent a promising approach to reach that end. Such surfaces can prevent the attachment of first colonizing bacteria, inhibit the formation of biofilm and kill bacteria by releasing free radicals or even an antibacterial agent^{10,11}.

Photocatalytic surfaces are widely studied as antibacterial surfaces^{12,13}. The principle of photocatalysis is electron excitation in the catalyzator molecule, resulting in redox reactions with the surrounding adsorbed water which leads finally reactive oxidizing species (hydroxide, superoxide radicals) releasing. These free radicals can induce the destruction of the adsorbed organic pollutants and bacterial contamination^{14,15}. Because of its high photoactivity, titanium dioxide (TiO₂) is one of the most intensively studied photocatalysts¹⁶. It is also a promising semiconductor, because of its low toxicity, chemical inertness and low price. However, due to its large electron band gap energy, TiO₂ can only be excited by UV light ($\lambda < 380$ nm)^{17,18}. It is possible, though, to shift its light absorption into the visible range by the application of metallic and non-metallic substances and elements.

Veres et al. used nanosilver to modify and improve the photocatalytic activity of TiO₂. They used polyacrilate resin [poly(ethyl acrylate-co-methylacrylate; p(EA-co-MMA))] to provide a polymer bed for TiO₂ and Ag-modified TiO₂ nanoparticles, and they prepared thin hydrophilic photocatalytic film coatings with photodeposition¹⁹. This polymer matrix started to decompose after one hour of UV irradiation, which led to higher free catalyst concentration on the surface.

As a result of nanoparticles' small (10^{-9} m) size, they have specific properties. After oral, cutaneous exposition or inhalation uptake, they can penetrate across different barriers into the whole human body. Intracellular uptake can occur by endocytosis, and they can interact with proteins or even with the nucleic acids²⁰. The opinions about Ag nanoparticles (AgNP-s) are not unequivocal. Some authors reported high bactericidal activity and biocompatibility as compared to other nanoparticles²¹⁻²³. On the other hand, depending on the size and concentration, AgNP-s show considerably higher toxicity than silver ions. For instance, they exert their toxic effect immediately upon contact in cancer cell lines²⁴.

We studied two newly developed photocatalysts/polymer nanohybrid films: TiO₂-copolymer and Ag-TiO₂-copolymer containing 0.001% silver nanoparticles. Both were applied with the spray coating technique, and activated with LED light ($\lambda = 405$ nm). The photocatalytic antibacterial activity of TiO₂ and Ag-TiO₂ coatings was established previously, and it was found that the role of photocatalytic activity was dominant over the antibacterial effect of silver ions themselves²⁵. Györgyey et al. investigated the antibacterial effect of 0.5% Ag- containing TiO₂ and TiO₂ copolymer films on titanium implant material. Their results suggested that this type of surface modification might be of good use in the therapy of peri-implantitis²⁶.

Our main goal was to examine the attachment and proliferation of primary human epithelial cells and MG63 osteosarcoma cells on two nanohybrid surface coatings: TiO₂-copolymer and Ag-TiO₂-copolymer containing 0.001% silver nanoparticles. *In vitro* cell culture experiments have several advantages compared to *in vivo* animal studies. Cell-biomaterial interactions can be examined in detail, they are maintained under controlled conditions, have better reproducibility and are even cost-efficient. Primary cells are more convenient models for *in vivo* simulation compared to immortalized cells. They have a finite life span and their characteristics in culture can change over time. Therefore earlier passages are recommended for experiments with primary cells. Their diversity is a big advantage compared to cancer cell lines, which origins from only one person. Reactions from different donors in different ages and genetics could be observed. According to De Saint Jean et al. primary epithelial cells showed higher sensitivity than immortalized cell lines to inflammatory cytokines²⁷. Endosseous dental implants are placed into the alveolar bone, but the transgingival parts are surrounded by soft tissues. For long term success, tight and healthy peri-implant soft tissue seal is required. Primary epithelial cells were used for the evaluation of soft tissue response. Based on the literature polished Ti discs were used as control samples for promoting better epithelial cell adhesion and spreading^{28,29}. On the other hand, one of the most popular cell culture models for *in vitro* osseointegration studies is MG63 human osteoblast-like cell³⁰. This tumor cell line shows osteoblast-like characteristics, hence it is an appropriate model for studying integrin expression and osteocalcin production, but not so suitable for examining alkaline phosphatase activities. The MG63 cells represent a suitable model for the *in vitro* study of osseointegration³¹. The response of epithelial cells as primary cells imitates the response of the peri-implant tissues better than that of the MG63 cells. If these coatings can preserve their antibacterial properties in spite of saliva and/or blood contamination during the surgical placement procedure and osseointegration occurs, the photocatalytic properties of the films could serve as promising therapy against peri-implant infections.

2. Materials and methods

2.1 Preparation of Ti samples

Ti sample discs (1.5 mm thick and 9 mm in diameter) were cut from commercially pure (CP 4) titanium rods (Denti[®] System Ltd. Hungary). Epithelial cell culture studies were carried out with these discs with machined surface ($R_a < 0.5 \mu\text{m}$). For the MG63 cell culture studies sandblasted and acid-etched discs were used to model the surface of the dental

implant's fixture. Before the treatment, all samples were cleaned in acetone and then in 70% ethanol ultrasonically, and finally rinsed in ultrapure water. After this preparation process, the sandblasted and acid-etched discs were coated with the copolymer-based nanohybrid layers.

2.2 Preparation of Ag-TiO₂ copolymer films

Polished Ti discs for epithelial cells and sandblasted, acid-etched Ti discs for MG63 cells served as control samples. Two photocatalytic layers: 60 % TiO₂/ 40 % copolymer and 60% AgTiO₂/ 40 % copolymer ([Ag] = 0.001 m/m %) were investigated. We reduced the amount of nanosilver from 0.5 % to 0.001% compared to our previous experiments²⁶. A hydrophilic polyacrylate resin [poly (ethyl-acrylate comethyl-methacrylate) (p(EA-co-MMA)) binder material (obtained from Evonik Industries, Germany) was used providing the polymer bed for TiO₂ and Ag photocatalyst nanoparticles. The process of producing these TiO₂ and Ag-TiO₂ nanohybrid layers was described in the study of Veres et al¹⁹.

Before Ti discs were coated with these photocatalyst layers, the suspensions were sonicated for 15 minutes, then sprayed onto the Ti discs with an AD-318 spray gun (Alder, USA) and dried at high temperature (120 °C). 2 ± 0.05 mg polymer suspension was sprayed onto each Ti discs. After this process, the discs were sterilized in hot air sterilizer at 180 °C for 45 minutes. The last step was to illuminate the coated discs with UV-light for 60 minutes in order to decompose the upper layer of the polymer film to uncover the silver and the TiO₂ nanoparticles¹⁹.

2.3 Scanning electron microscopy

The morphological characteristics of the coatings were investigated before UV treatment, without cells. A field emission scanning electron microscope (SEM) (Hitachi S4700, Japan) was applied to record images with ×500, ×1000 and ×5000 magnifications in secondary electron imaging mode. The samples were tilted at 45° for better surface visualization.

2.4 Profilometry measurements

Average surface roughness (R_a (μm)) of sandblasted and acid-etched Ti, TiO₂ and Ag-TiO₂ copolymer coated Ti discs was measured with a Veeco, Dektak 8 Advanced Development Profiler® (Veeco Instruments, USA). 500×500 μm² areas were recorded at three different places, on two samples of each group. The resolution along the scanning direction

was 0.17 μm and the spacing was 6.33 μm between the scanned lines. The vertical resolution was 4 nm. Macro-roughness line profiles were recorded also. Profiles were analyzed on computer, using Vision 3D Image and Analysis Software (Veeco Instruments, USA).

2.5 Cell culture studies

Two different cell culture models were used: primary human epithelial cells and MG63 osteosarcoma cells. Gingival tissues were obtained from healthy adult patients undergoing routine dento-alveolar surgery. Epithelial cells were isolated from inflammation-free mucosa after obtaining the written consent from the patients. The study protocol conformed to the tenets of the Declaration of Helsinki in all respects, and it was approved by the Regional Research Ethics Committee for Medical Research at the University of Szeged. The process of primary epithelial cell isolation was described in detail in Ungvári et al.³².

One frozen ampoule of MG63 osteoblast-like cells was placed into a water bath (37 °C) for a few minutes. When the contents of the ampoule thawed, we centrifuged the cells in phosphate-buffered saline (PBS) (PAA Laboratories GmbH, Germany) and fetal bovine serum (FBS) (PAA Laboratories GmbH, Germany). Cells were pipetted into a 25 cm² flask and were passaged at least three times before the investigations.

The complete culture medium for oral epithelial cells consisted of keratinocyte serum-free medium (KSFM) with L-glutamine (Gibco BRL, Eggenstein, Germany), supplemented with recombinant epidermal growth factor 2.5 $\mu\text{g}/500\text{ ml}$ (Gibco BRL, Eggenstein, Germany), bovine pituitary extract 25mg/500 ml (Gibco) and 1 % Antibiotic Antimycotic Solution (penicillin, streptomycin, amphotericin B, Sigma-Aldrich GmbH, Germany). The medium for MG63 cells consisted of Eagle's Minimal Essential Medium (EMEM) (Sigma-Aldrich GmbH, Germany), supplemented with 10 % FBS, 1 % L-glutamine, 1 % nonessential amino acids (Sigma-Aldrich GmbH, Germany), and 1 % Antibiotic Antimycotic Solution (penicillin, streptomycin, amphotericin B, Sigma-Aldrich GmbH, Germany). The culture medium was refreshed three times per week. Cultures were grown under standard conditions in humidified atmosphere (5 % CO₂) at 37 °C.

Cells were inoculated onto the Ti discs in a density of 10⁴ cells / well, and cultured in 48-well plates. Plate wells (henceforth plate) were used as control surface with the same amount of cells. The attachment of the cells was determined after 24 h, and the proliferation rate was measured after 72 and 168 hours via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich GmbH, Germany) assay. The

experiments were repeated four times, and four samples were used for each assay. Beside the MTT measurements, we also captured fluorescent images, one sample per each group, for the visualization of the cells. Experiments with two different types of cells were carried out in 4 replicates.

2.5.1 MTT assay

The viability of cultured cells was assessed by a common colorimetric assay, which determines the living cell number by the reduction of MTT³³. Cells were inoculated into 48-well plates at a density of 10⁴ cells /well, and grown on the Ti sample discs in culture medium for 24, 72 and 168 h. The culture medium was removed and replaced with 1mg/ml MTT solution in EMEM/KSFM. After four hours of incubation under standard culturing conditions, this medium was removed from each well. The formazan crystals, which are the crystallized form of the dye, produced by the living cells, were solubilized with 0.04 mM HCl in absolute isopropanol and 10 % sodium dodecylsulfate (SDS). The optical density was determined at 540 nm (OD₅₄₀) by an Organon Teknika Reader 530 spectrophotometer (Anthos Labtec Instruments GmbH, Austria). Tissue culture plates without discs served as positive control as well.

2.5.2 Visualization with fluorescent microscopy

We used fluorescent dyes for the visualization of the cells. Representative images of the discs were taken after 24, 72 and 168 h incubation with MG63 and epithelial cells, in parallel with the MTT assay. The cells were fixed on the surface with 4% formaldehyde. Cell nuclei were labeled with Bisbenzimidazole Hoechst 33342 dye (blue, Merck Millipore, Germany) and the cytoskeleton with Phalloidin–Tetramethylrhodamine B isothiocyanate (TRITC-phalloidin, red, Sigma-Aldrich GmbH, Germany). The images were taken with a Nikon Eclipse 80i fluorescent microscope (Nikon Corporation, Japan) at a magnification of ×100. In each case, we took two images with two different filters (DAPI, ex. 320,520 nm; TRITC, ex. 510-560 nm) with the position of the samples unaltered. To produce composite pictures, we used ImageJ 1.47v software (National Institutes of Health, USA).

2.6 Statistical analyses

After normality testing, the data yielded by optical densitometry were compared via one-way analysis of variance (ANOVA), followed by Tukey's HSD, LSD and Scheffé post hoc tests (SPSS 21, Chicago, Illinois, USA). The level of significance was set at $\alpha=0.05$.

3. Results

3.1 SEM images of the surfaces

SEM images (Fig.1) revealed significant differences in the surface morphology and roughness of the nanohybrid layers as compared to the uncovered Ti surface. The acid-etched, sandblasted Ti discs exhibited typical surface characteristics. The TiO₂ copolymer film showed an amorphous surface pattern, similar to the control. The silver-containing polymer film was different. On these surfaces characteristic grains appeared.

3.2 Surface profilometry

Profilometry images (Figs. 2 and 3) confirmed the above mentioned differences in surface morphology and roughness. Uncovered Ti (sandblasted-, acid-etched) and TiO₂ copolymer coated discs had similar roughness values (for Ti: $R_a = 1.26 \mu\text{m}$; for TiO₂: $R_a = 1.79 \mu\text{m}$), but Ag-TiO₂ coated samples had considerably rougher surfaces (for Ag-TiO₂: $R_a = 5.76 \mu\text{m}$), with considerable height differences along the scan direction.

3.3 MTT assay

The results of the MTT measurements related to cell attachment (24h observation) and cell proliferation (72 and 168h) are shown on the bar graphs in Fig. 4

Attachment of epithelial cells on the Ag-TiO₂ modified surface was significantly higher ($p = 0.013$) compared to the polished control Ti surface. The level of attachment almost reached the control plate values. No significant differences were found in the attachment of cells between the two copolymer-coated discs. The early proliferation of epithelial cells was similar on all of the investigated discs. No significant differences were found between the polished and either of the the polymer- coated groups after 168h.

From a different perspective, we observed a significant increase in the cell count on the polished Ti surface after one week, while, at the same time point, a significant decrease

was found in the cell count of TiO₂ -modified samples. Epithelial cells did not show a growing tendency on the Ag-TiO₂ -coated discs, but mitosis and apoptosis was well balanced.

First, the OD₅₄₀ values of MG63 cells on different surfaces were compared at the same time points (24 h, 72 h and 168 h). No significant difference was found in the attachment (24 h) of the MG63 cells between the treated and the control surfaces. However, significantly more cells ($p = 0.03$) attached to the surface of the nanosilver- modified discs compared to the TiO₂ -modified discs. Early proliferation of the cells (72 hours) was significantly higher on the control discs than on the TiO₂ -copolymer and nanosilver coated samples ($p < 0.001$). After 168 hours, there was a considerable increase in the proliferation rate of MG63 cells on the uncoated Ti surfaces, in contrast to the TiO₂ and Ag-TiO₂ -modified surfaces, where the cells did not show any growing tendency. No statistical differences were found between the copolymer- treated groups after 72 and 168 hours. We found the smallest number of cells on the TiO₂ polymer- coated surfaces.

Secondly the OD₅₄₀ values of each group were evaluated over time. The definite increase in the cell number of the plate and the untreated Ti discs proves the viability of MG63 cells. In case of the two control groups there was a significant increase in the cell number between the three investigation points ($p < 0.001$). According to our results these cells did not show any sign of proliferation on the polymer coated discs. There was no significant difference in the cell number of the modified samples under the investigated period (24, 72 and 168 h).

3.4 Fluorescent images

Composite images of epithelial and MG63 cells are presented in figure 5. The actin cytoskeleton is red (TRITC-phalloidin) and the DNA content of cell nucleus is blue (Hoechst 33342).

We found no considerable difference in the attachment of epithelial cells on the three investigated groups, but the cells covered the surface of the silver- modified discs more profusely. Typical polygonal cell morphology with a few filopodia was characteristic in all three groups. After 72 h we observed an increased proliferation on the polished and on the Ag-TiO₂ samples with well-spread morphology, whereas a decrease was observable on the TiO₂ polymer modified samples. On the Ag-TiO₂ samples, not every cytoskeleton was noticeable between the grains, in spite of the good visibility of cell nuclei. One week later,

polygonal cells covered the whole surface of polished Ti discs, with several filopodia. The cell counts remained unchanged on the Ag-modified surfaces, while just a few poorly spread cells were detected on the TiO₂-copolymer-coated discs. A remarkable number of rounded cells were observed on the polymer- covered discs, signifying cell detachment.

The attachment of MG63 cells was similar in the three investigated groups. Cells showed flat morphology with short and little processes. They covered the surfaces dispersedly, without any orientation. We found the most cells with rounded morphology on the silver- modified surface. After 72 h, we observed a higher cell density on the untreated Ti discs. Cells formed bigger groups, and they had more and stronger processes. Their characteristic triangle shape was noticeable. MG63 cells did not show any sign of proliferation on the TiO₂ and Ag-TiO₂ samples. They had some processes, but we did not observe any mitotic cells. After 168 h the cells covered almost the whole surface of the uncovered Ti sample discs, and we found multiple layers of cells near the edge of the discs. On the TiO₂ and Ag-TiO₂ copolymer discs, only a few cells were observed.

4. Discussion

In our study the viability (attachment and proliferation) of epithelial cells and MG63 osteosarcoma cells was compared. Our results showed that the attachment of epithelial cells on the Ag-TiO₂ modified samples was significantly higher compared to the polished Ti control samples. However, epithelial cells did not show a growing tendency on the Ag-TiO₂ - coated samples, while the rate of mitosis and apoptosis was well balanced. MTT test showed that MG63 cells were not capable of proliferation on the TiO₂ and Ag-TiO₂-coated Ti discs. We have found decreasing cell numbers. Nevertheless, we found living cells on both of them after one week incubation.

Using polymer-embedded nanoparticles on the surface of titanium dental implants is a relatively new approach, which means that literature on this topic is scarce. Still, it is possible to interpret our findings with the help of similar studies.

Regarding the surface roughness of the discs, our results were not in accordance with the results of previous studies^{30,34,35}. They found that MG63 cells preferred rougher surfaces ($R_a = 4\text{--}5\text{ }\mu\text{m}$). Increased surface roughness led to increased differentiation and decreased cell proliferation. Although the average roughness of the silver modified discs was almost ideal,

MG63 cells could not proliferate. This inhibitory effect could be explained by the presence of the nanoparticles.

The attachment of epithelial cells on the nanoparticle- modified surfaces was relatively high, but the proliferation was partially inhibited. *In vitro* epithelial cell responses of nanoscale modified dental implants were investigated by several authors ³⁶. Rossi et al. reported better epithelial attachment on the nanoporous sol–gel-derived TiO₂ surface-treated implants as compared to untreated titanium implants *in vivo* ³⁷. In an *in vitro* biocompatibility study of Ag-embedded TiO₂-nanotubes on Ti dental implants Mei et al. found that epithelial cell viability and proliferation was better on a silver modified nanotube surface (plasma implanted at 1kV) than on pure titanium ³⁸.

The Ag-TiO₂ and TiO₂ modified titanium implant surfaces in this study showed exerted considerable inhibitory effect on the proliferation of MG63 cells. A number of studies have investigated the effect of Ag nanoparticles on osteoblast-like cells. De Giglio et al. used silver nanoparticles immobilized on Ti implant material in hydrogel coating. They found that there was a progressive release of silver from the coating, which led to long-term antibacterial activity. In accordance with our results, the MTT tests showed reduced MG63 osteoblast-like cell viability after 24 and 48 h of incubation. Interestingly, after 7 days the cell proliferation increased ³⁹. Moaddab and colleagues reported that nano-Ag influenced negatively the growth of G292 cancer cell line. This phenomenon was concentration dependent (IC₅₀ value of 3.42 µg/ mL) and had great selectivity to cancer cells. These findings might indicate the potential of nano-silver in the field of anti-cancer therapies ⁴⁰.

It must be taken into consideration, however, that some other studies came to the opposite conclusion. In a recent study, Ferraris et al. investigated the viability of MG63 osteoblast-like cells on different titanium surfaces. CP Ti and Ti6Al4V samples were treated with acid etching and controlled oxidation process in hydrogen peroxide, which was completed with silver ions. These nanostructured surfaces showed good antibacterial properties and good viability of MG63 cells on CP Ti surfaces with low concentration of silver (0.001M), but Ti6Al4V surfaces were more cytotoxic with the same amount of silver ⁴¹. Tilmaciu et al. investigated MC3T3-E1 preosteoblast cell viability and bacterial adhesion on nanocoatings with minimal silver loading. They found that these monolayers significantly inhibited the adhesion of *E. coli* and *S. epidermidis*, while not significantly interfering with the attachment and proliferation of MC3T3-E1 cells ⁴².

We found decreasing cell counts (both cell types) on the TiO₂-polymer modified discs. This result is in contrast with the literature. Ivask et al. concluded that TiO₂ nanoparticles,

among other metal nanoparticles (Al_2O_3 , Fe_3O_4 , MgO , SiO_2 , WO_3), had no toxic side effect on epithelial and fibroblast cells⁴³. Similarly, Choi et al. found that TiO_2 films on Ti alloys were not toxic to L-929 mouse fibroblast cells⁴⁴. A cytotoxicity study was carried out with different metal oxide nanoparticles by Jeng et al., who found that TiO_2 nanoparticles had no measurable effect on Neuro-2A cells under 200 $\mu\text{g/mL}$ ⁴⁵. In all of these studies, cancer cell lines were used as cell culture models. One possible explanation to this discrepancy in the proliferation of epithelial cells that in this study primary cells were investigated. High sensitivity to the polymer matrix might also be an explanation.

The viability of the epithelial cells was more promising on the Ag- TiO_2 modified discs according to the fluorescent pictures. The difference between the OD_{540} values of the MTT assay and the fluorescent images is one that is difficult to explain without going into speculations. MG63 cells proved to be still too sensitive to nanosilver, in spite of reduced AgNP concentration.

5. Conclusions

Taking all aspects of our findings into consideration, new nanoscale surface modifications of titanium dental implants have great antibacterial potential and they also appear to be biocompatible. However, the observed effect on cell proliferation indicates that the process of cell-nanoparticle interaction needs to be better understood, biocompatibility investigations with other cell types and increasing the investigation time are also needed.

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Figure legends:

Figure 1. SEM images of the surfaces without cells before UV irradiation ($\times 500$ and $\times 5000$ magnification)

Figure 2. Characteristic line profiles of the uncovered Ti, TiO₂ copolymer coated and Ag-TiO₂ coated surfaces (R_a (μm))

Figure 3. Average surface roughness values of the samples (R_a (μm)). Data are given as mean \pm SEM. Asterisk denotes significant difference at $p < 0.05$.

Figure 4. 24, 72 and 168 h OD₅₄₀ values of epithelial cells (a) and MG63 cells (b) incubated with MTT on the control discs (plate and uncovered Ti) and on the TiO₂ and Ag-modified TiO₂ polymer covered discs. Data are presented as mean \pm SEM.

Figure 5. Fluorescent images of primary epithelial (a) and MG63 cells (b) on Ti, TiO₂ and Ag-TiO₂ surfaces at 24 h, 72 h and 168 h at a magnification of $\times 100$.

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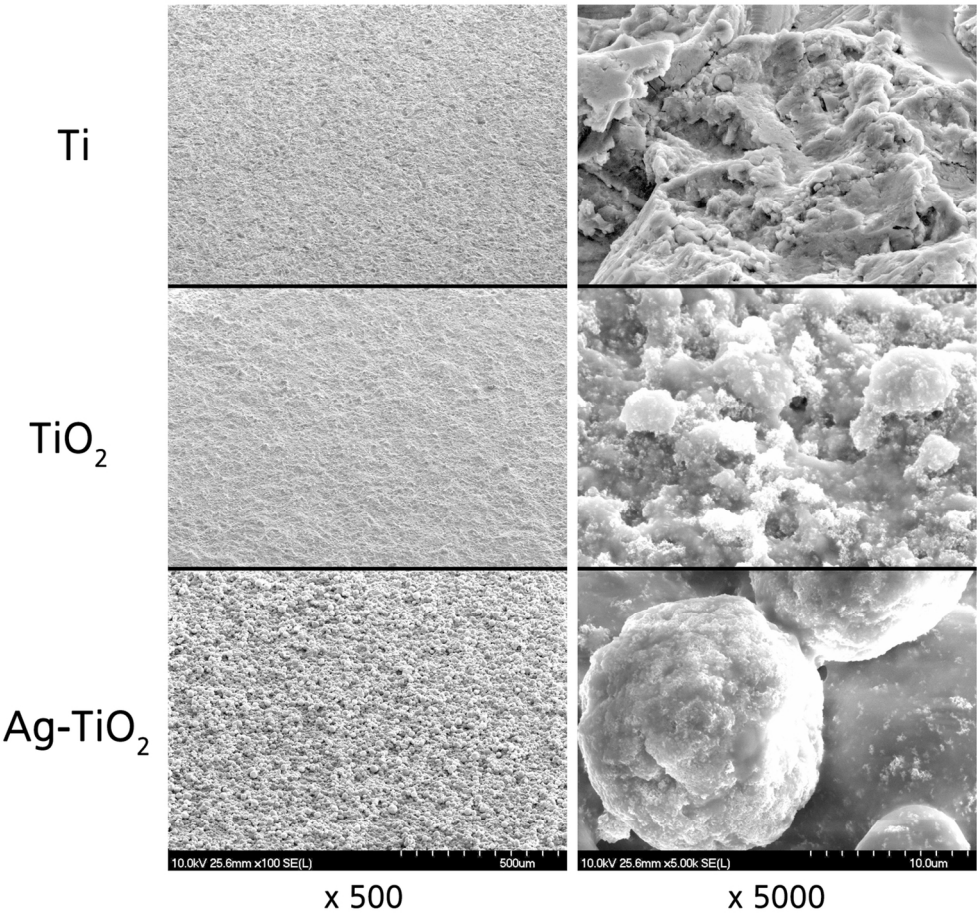


Figure 1. SEM images of the surfaces without cells before UV irradiation (×500 and ×5000 magnification)

SEM images (Fig.1) revealed si
124x115mm (300 x 300 DPI)



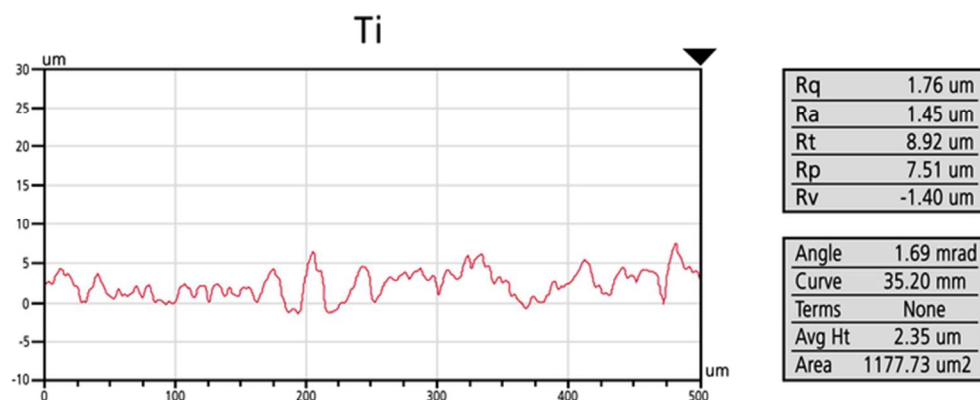


Figure 2. Characteristic line profiles of the uncovered Ti, TiO₂ copolymer coated and Ag-TiO₂ coated surfaces (R_a (μm))

Profilometry images (Figs. 2 a
59x25mm (300 x 300 DPI)

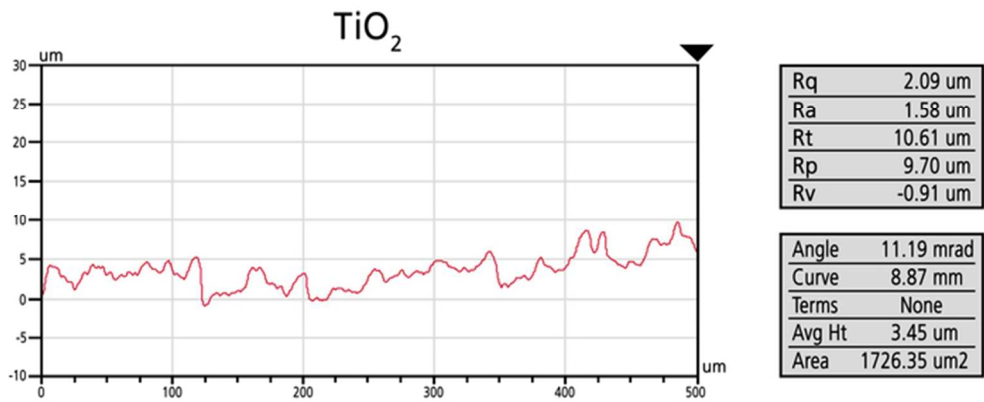


Figure 2. Characteristic line profiles of the uncovered Ti, TiO_2 copolymer coated and Ag- TiO_2 coated surfaces (R_a (μm))
Profilometry images (Figs. 2 a
59x25mm (300 x 300 DPI)

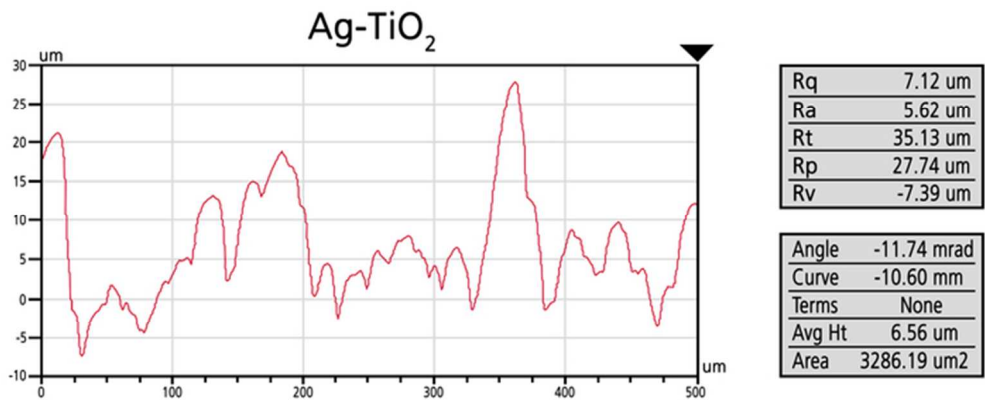


Figure 2. Characteristic line profiles of the uncovered Ti, TiO₂ copolymer coated and Ag-TiO₂ coated surfaces (R_a (μm))
Profilometry images (Figs. 2 a
59x25mm (300 x 300 DPI)

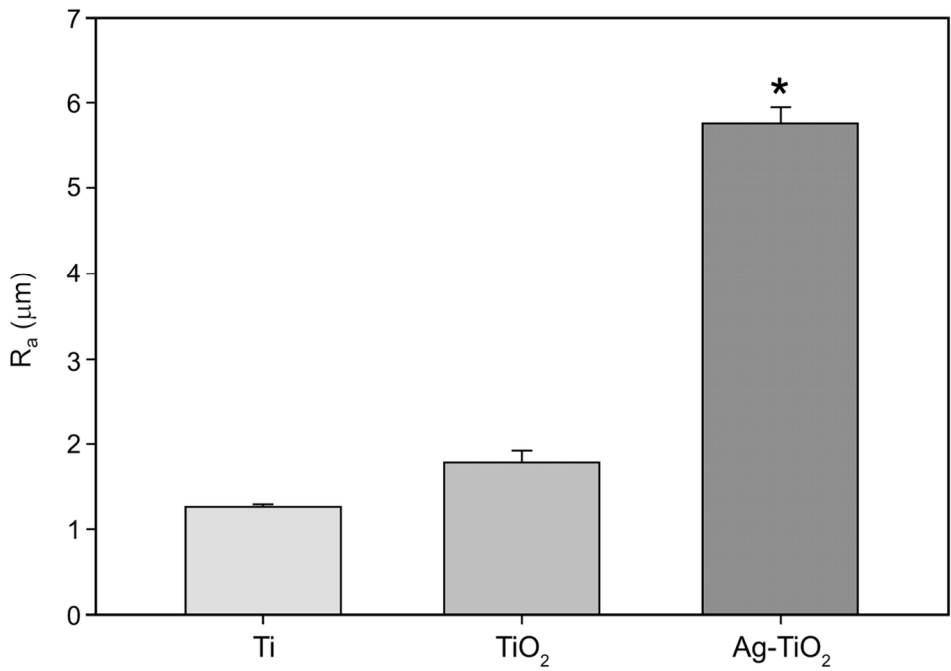


Figure 3. Average surface roughness values of the samples (R_a (μm)). Data are given as mean \pm SEM. Asterisk denotes significant difference at $p < 0.05$.
Profilometry images (Figs. 2 a
109x80mm (300 x 300 DPI)

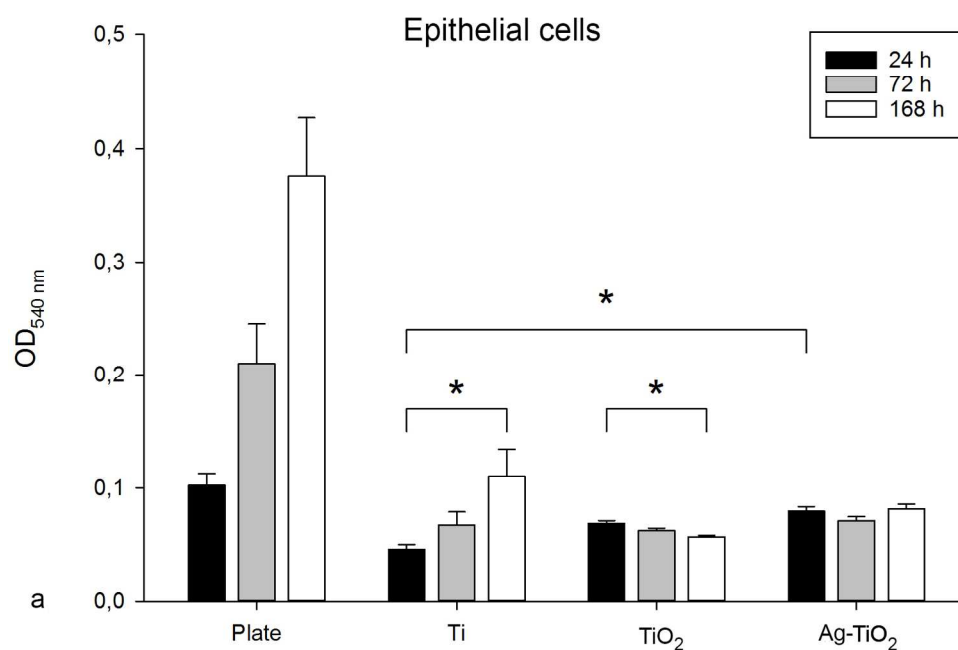


Figure 4. 24, 72 and 168 h OD₅₄₀ values of epithelial cells (a) and MG63 cells (b) incubated with MTT on the control discs (plate and uncovered Ti) and on the TiO₂ and Ag-modified TiO₂ polymer covered discs. Data are presented as mean \pm SEM.

The results of the MTT measure
157x110mm (300 x 300 DPI)

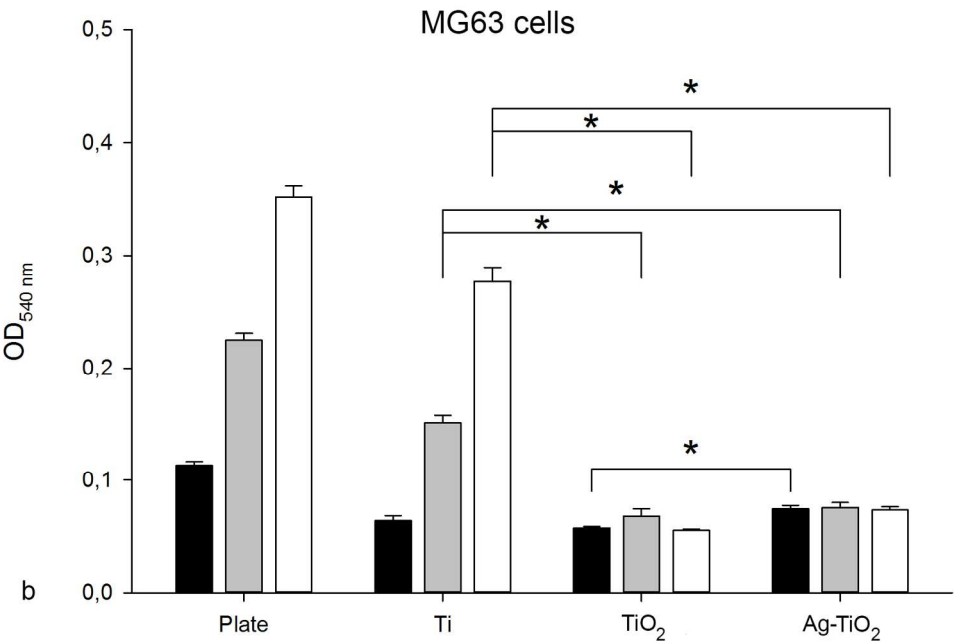


Figure 4. 24, 72 and 168 h OD₅₄₀ values of epithelial cells (a) and MG63 cells (b) incubated with MTT on the control discs (plate and uncovered Ti) and on the TiO₂ and Ag-modified TiO₂ polymer covered discs. Data are presented as mean ± SEM.
The results of the MTT measure
158x110mm (300 x 300 DPI)

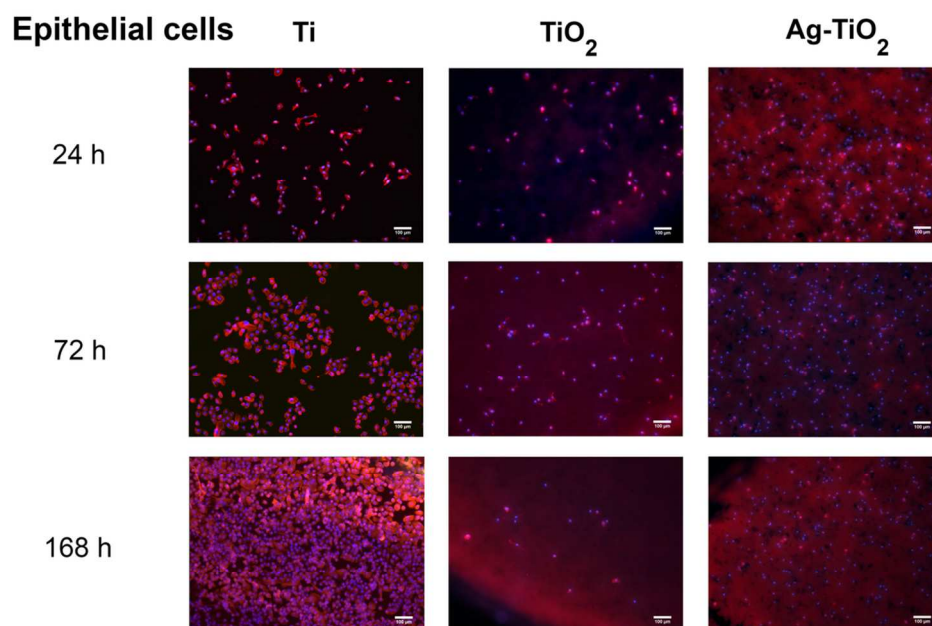


Figure 5. Fluorescent images of primary epithelial (a) and MG63 cells (b) on Ti, TiO₂ and Ag-TiO₂ surfaces at 24 h, 72 h and 168 h at a magnification of $\times 100$.
Composite images of epithelial
105x72mm (300 x 300 DPI)

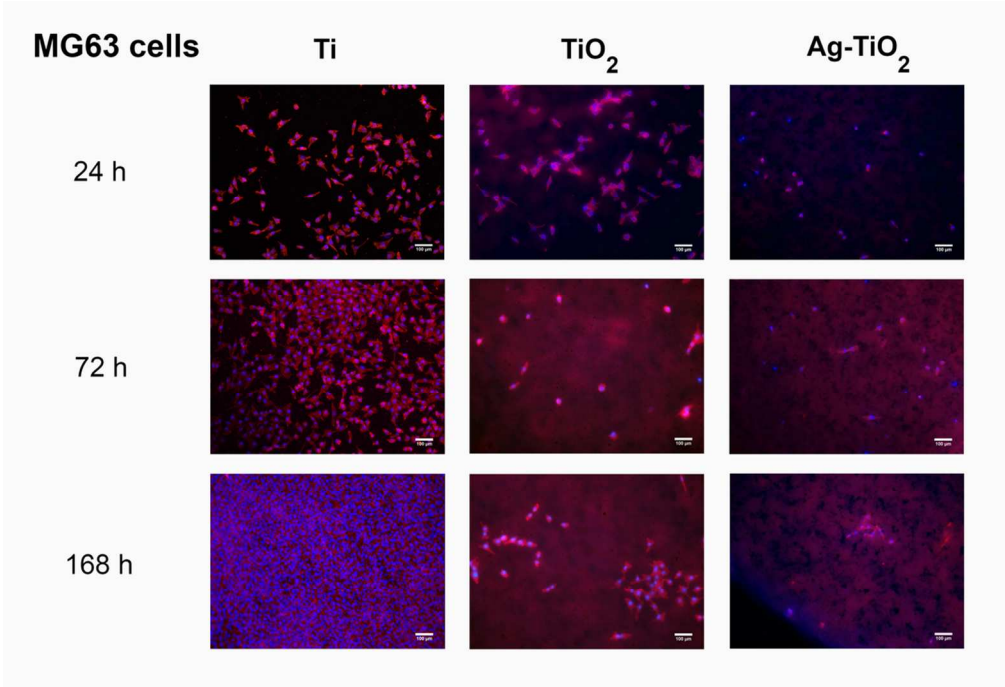


Figure 5. Fluorescent images of primary epithelial (a) and MG63 cells (b) on Ti, TiO₂ and Ag-TiO₂ surfaces at 24 h, 72 h and 168 h at a magnification of ×100.
Composite images of epithelial
105x72mm (300 x 300 DPI)