Interaction of nanostructured TiO$_2$ biointerfaces with stem cells and biofilm-forming bacteria

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**Abstract**

Nanostructured TiO$_2$ nanotubes (NTs) of diameters from 15 to 100 nm were fabricated by an electrochemical anodization process. Biofilm-positive strains of Bacillus cereus and Pseudomonas aeruginosa behaved similarly on all TiO$_2$ NTs as well as on native titanium (Ti) foil. The adhesion and growth of mesenchymal stem cells (MSc), embryonic stem cells (Esc), and pure cardiomyocytes derived from ESC exhibited significant differences. MSc as well as Esc were, in contrast to cardiomyocytes, able to adhere, and grow on TiO$_2$ NTs. A correlation between NTs diameter and cell behaviour was however observed in the case of MSc and Esc. MSc were not in a physiological state in the case of 100 nm TiO$_2$ NTs, while Esc were not able to grow on 15 nm TiO$_2$ NTs. It can be stated that these differences can be assigned to different diameters of the NTs but not to the chemistry of the surface. This is the first study describing the comprehensive behaviour of both eukaryotic and prokaryotic cells on TiO$_2$ NTs. On the basis of obtained results, it can be concluded that new generation of medical devices providing selective cell behaviour can be fabricated by optimizing the nanoscale morphology of TiO$_2$.

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1. Introduction

Titanium and its alloys are commonly employed in medical implants, but progress in nanotechnologies together with new findings indicating the importance of nanoscale morphology on cell behaviour; which open the door to new applications utilizing nanostructured titanium surfaces. This emerges from the fact that the interaction between cells or tissues and any foreign material depends not only on the chemical composition of the material, but also on its nanoscale morphology. Because of this, the interaction of cells with nanostructured surfaces is the subject of intensive research. To improve the biological, chemical, and mechanical properties of biomaterials, significant research efforts have been made to fabricate suitable morphologies which could offer the desired biological response (reviewed by Kulkarni et al. [9]). In recent years, several methods have been developed to produce nanoscale morphologies on titanium (Ti) surfaces. One of these methods, the electrochemical anodization of Ti, is a powerful tool for controlling the nanoscale morphology of Ti. For example, titanium dioxide (TiO$_2$) NTs with well-controlled morphology can be synthesized by the electrochemical anodization of Ti using a two-electrode system. Previously, self-organized TiO$_2$ NTs layers fabricated in fluoride containing electrolytes which were studied intensively [1,12]. Later it was shown that water content in the electrolyte is the critical factor that determines whether self-ordered oxide NTs or nanopores are formed during electrochemical anodization; it is thought that tube formation originates from ordered porous oxide by a “pore-wall-splitting” mechanism [20]. By tailoring the anodization parameters (applied voltage, anodization time, and concentrations of chemicals), TiO$_2$ NTs of different diameters ranging from 15 nm to 150 nm and different lengths can be obtained.

Knowledge about the interaction of various nanostructured TiO$_2$ surfaces with cells is one of the crucial factors influencing the use of TiO$_2$ in medical applications (e.g. orthopaedic, vascular, dental implants). Recently, studies focusing on the interaction between TiO$_2$ NTs and various cell types have emerged. Among the most commonly studied cell types in this context are mesenchymal stem cells (MSc). For example, in the work of Bauer et al. [3], MSc exhibited the best adhesion on NTs of 15 nm in diameter, the adhesion decreasing as the NTs diameter gets increased further. This inverse proportionality was also confirmed in other works on MSc adhesion and proliferation [5].
2.1. Fabrication of TiO2 nanotubes to stimulate the desired biological response. (biofilm formation) was observed for 20 nm NTs. This indicates that by appropriately structuring the surface of titanium, it may be possible to stimulate the desired biological response.

2.2. Scanning electron microscopy (SEM)

High contrast images of TiO2 NTs were obtained by scanning electron microscopy (Hitachi FE-SEM S4800). All samples were used without gold sputtering as the NTs samples already display images with reasonably good contrast.

2.3. Atomic force microscopy (AFM)

Topographic features of TiO2 NTs were examined by means of Atomic Force Microscopy (Solver PRO, TiO2 NTs – MDT, Russia) in tapping mode in air. The TiO2 NTs were analysed using the standard Si cantilever with a force constant of 22 N/m, and at a resonance frequency of 325 kHz. The average surface roughness (Ra) across a 3 x 3 μm² area was measured from representative images. The results are shown as the average Ra from five different areas.

2.4. Water contact angle measurements

Surface wettability was measured 25 h after TiO2 NTs were produced by anodization and stored in sealed plastic containers at room temperature in order to prevent surface ageing effects (changes in hydrophilic character). All NT surfaces were analysed after one week of fabrication. Droplet of 3 μL DI water was placed on the surface of NTs and the contact angle was determined from high resolution images. For each sample, six measurements were performed in order to obtain statistically robust data.

2.5. X-ray photoelectron spectroscopy (XPS)

The samples were analysed by means of X-ray photoelectron spectroscopy (XPS or ESCA) using a PHI-TFA XPS spectrometer (Physical Electronics Inc.). Samples were placed on a sample holder and introduced into an ultra-high vacuum spectrometer. The analysed area was 0.4 mm in diameter and the analysed depth was about 3–5 nm; this high surface sensitivity is a general characteristic of the XPS method. X-ray radiation from a monochromatic Al source with a photon energy of 1486.6 eV was used to excite the surface, and high-energy resolution spectra were acquired by an energy analyser operating at a resolution of approximately 0.6 eV and a pass energy of 29 eV. During data processing, spectra were aligned by setting the C 1s peak at 285.0 eV, characteristic for C—C bonds. The accuracy of binding energies was approximately ±0.3 eV. The quantification of surface composition was determined from XPS peak intensities taking into account relative sensitivity factors provided by the instrument manufacturer. Three different XPS measurements were performed for each sample and the average composition was calculated.

The survey and high resolution spectra of C 1s, O 1s and Ti 2p were recorded for Ti foil and TiO2 NTs of different diameters one week after fabrication.

2.6. Biointerface properties

The biointerface properties of Ti foil and TiO2 NT surfaces were therefore determined by both biofilm positive strains of bacteria (prokaryotic and mesenchymal stem cells (Msc), embryonic stem cells (Esc), and pure cardiomyocytes derived from embryonic stem cells (eukaryotic cells). A sample of 1 cm² size was used for all tests. All tests were performed in quadruplicates and repeated for three times. Prior to testing, the samples were sterilized using UV light for 30 min.

2.6.1. Bacterial biofilm formation

To detect biofilm formation on pristine Ti foil and TiO2 NTs, two biofilm-forming bacterial strains were used, Bacillus cereus (Czech Collection of Microorganisms 2010) and Pseudomonas aeruginosa (Czech Collection of Microorganisms 3955). To inoculate the bacteria, 2 mL of Trypton soya Broth (Himedia, India) was added to each sample in the Petri dish and subsequently seeded by 20 μL of the bacterial suspensions (2. degree of McFarland standard in physiological solution). After
and visualized through nuclei staining by DAPI (10 ng mL−1 Invitrogen USA) and actin–fluorochrome (ActinRed™ 555, Thermo Fisher Scientific USA) was used to determine cell morphology. ESc and cardiomyocytes were cultivated either on native surfaces or on surfaces coated with gelatine.

ESc R1 cells were seeded at a density of 10 × E4 cells per cm². After 48 h, cells were either uploaded by Calcein AM (10 μM) (Invitrogen; US), which coloured only viable cells, or fixed by 2% formaldehyde and stained by antibody against cardiomyocyte-specific myosin heavy chain alpha promoter-dependent selection vector [7], kindly donated by Dr. Field, LJ. (For more information on the cardiomyocytes, see [6, 19]).

MSc were cultivated in ATCC–formulated Dulbecco’s Modified Eagle’s Medium (BioSera, France) containing 20% of calf serum (BioSera, France), 100 U mL−1 Penicillin/Streptomycin (GE Healthcare HyClone, United Kingdom) and 0.05 mM mercaptoethanol (Serva, Germany). ESc were propagated in an undifferentiated state by culturing on gelatinized tissue culture dishes using complete Dulbecco’s Modified Eagle’s Medium containing 15% fetal calf serum, 100 U mL−1 Penicillin, 0.1 mg mL−1 Streptomycin, 100 mM non-essential amino acids (all from Gibco–Invitrogen; US), 0.05 mM β-mercaptoethanol (Sigma–Aldrich; US), and 1000 U mL−1 of leukemia inhibitory factor (Chemicon; US). Cardiomyocytes were cultured in Dulbecco’s Modified Eagle’s Medium – F12 media supplemented with 7.5% fetal calf serum, 100 U mL−1 Penicillin, 0.1 mg mL−1 Streptomycin, 100 mM, 0.025 mM β-mercaptoethanol (Sigma–Aldrich; US), and Insulin, Transferin and Selenium (ITS supplement, Gibco–Invitrogen; US).

The cyto-compatibility test was designed as follows: MSc were seeded onto native surfaces at a concentration of 5 × E4 cells per cm² and cultivated for 48 h. Subsequently DNA staining with Hoechst 33258 (5 μg mL−1 Invitrogen USA) and actin filament staining (ActinRed™ 555, Thermo Fisher Scientific USA) was used to determine cell morphology. ESc and cardiomyocytes were cultivated either on native surfaces or on surfaces coated with gelatine.

ESc R1 cells were seeded at a density of 10 × E4 cells per cm². After 48 h, cells were either uploaded by Calcein AM (10 μM) (Invitrogen; US), which coloured only viable cells, or fixed by 2% formaldehyde and visualized through nuclei staining by DAPI (10 ng mL−1, Sigma). The mass of viable cells was also determined as the level of ATP using Cellular ATP Kit HTS (Biothema, Sweden). Cardiomyocytes were seeded on native surfaces at a density of 10 × E4 cells per cm² and, after 48 h, fixed with 2% formaldehyde and stained by antibody against cardiomyocyte specific myosin heavy chain (MF20 antibody, developed by MF20, antibody developed by Drs. Donald and Fischman, was obtained from the Developmental Studies Hybridoma Bank under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences). As a secondary antibody, donkey anti-mouse IgG Alexa Fluor 568 antibody was used. Nuclei were counterstained by DAPI (10 ng mL−1, Sigma). Micrographs were taken by means of an inverted Olympus phase contrast microscope (IX 81, Japan).

3. Results and discussion

3.1. Nanoscale morphology and surface chemistry of TiO2 NTs

The surface properties of both Ti foil and TiO2 NTs were characterized in terms of surface morphology (using SEM and AFM techniques), wettability, and surface chemistry (using XPS).

The analysis of surface morphology by SEM and AFM techniques revealed that pristine Ti foil had no special morphological features, while a uniform nanotubular structure was observed for electrochemical anodized TiO2 surfaces (Fig. 1). From SEM images it can easily be observed that TiO2 NTs are evenly distributed on the surface and that anodization potentials of 10 V, 20 V and 58 V lead to the formation of TiO2 NTs of 15, 50 and 100 nm in diameter, respectively (Fig. 1). According to the results of AFM, Ti foil is not completely flat, as the average surface roughness measured on a 3 × 3 μm² area was about 19.3 nm. In the case of NTs, the calculated average surface roughness increased with NTs diameter, from about 11.7 nm for 15 nm NTs, to about 18.6 and 27.9 nm for 50 and 100 nm NTs, respectively. Although penetration of the AFM tip inside the hollow NTs interior is limited, due to the tip radius and the size of the NTs, the calculated roughness values and images obtained provide additional information about the 3D structures of the NTs, these having the specific ability to interact with different cell types. Based on the previously published work we can conclude that the nanotubes have about 1 to 4 μm in height and are stable on the surface [8].

Another important aspect of a biomaterial surface is its wettability [13], which could, together with other surface properties, influence the biological response. Water contact angle measurements conducted on Ti foil and freshly fabricated TiO2 NTs indicate that Ti foil is poorly wettable (a water contact angle of about 78°), while TiO2 NTs are all hydrophilic (a water contact angle of ~50°). However, it should be noted that fabricated TiO2 NT surfaces tend to age if exposed to the atmosphere and become hydrophobic, as shown in our previous work [10]. Thus, to ensure the hydrophilic character of the surfaces, all biological experiments were conducted one week after fabrication.

The chemical compositions of Ti foil and TiO2 NTs of different diameters were determined from XPS survey spectra; the spectra for NT 100 is presented in Fig. 2A. No significant differences in chemical composition were observed between different diameter NTs (Table 2). Moreover, similar surface chemistry was observed for pristine Ti foil. The composition of the top surface layer of Ti foil was approximately 38% carbon, 41% oxygen, 18% titanium, and a few percent nitrogen. In the case of all nanotubular surfaces the chemical composition was very similar; the only difference was in the presence of fluorine on the surface. The fluorine content was about 3–5% and was ascribed to the remains of the electrolyte used for electrochemical anodization. In Fig. 2A, the survey spectra for 100 nm NTs are presented. It can clearly be seen that only C, O, Ti, N and F atoms are present on the surface. Similar survey spectra were also recorded for the other NT diameters as well as for the Ti foil. From the high resolution spectra of C 1s, O 1s, and Ti 2p, which are presented in Figs. 2B–D, no significant differences in peaks among different NT diameters and between NTs and Ti foil were observed. The C 1s peak has a main peak at 284.8 eV, corresponding to C—C and C—H groups, and a small shoulder peak appearing at 288.9 eV, which corresponds to C=O bonds (Fig. 2B). In the case of the O 1s high–resolution scan, a primary peak at a binding energy of about 530.2 eV can be seen, which corresponds to the TiO2 component (Fig. 2C). In the case of the Ti 2p peak, a doublet peak with maxima at 458.7 eV and 464.5 eV is observed, which is typical for the TiO2 component (Fig. 2D). Differences in high–resolution peaks among different NTs diameters were not observed, indicating that the binding of C, O and Ti is similar for all NTs.

3.2. Biointerface properties

The interaction of any biomaterial with biological fluids, cells, tissues or the immune system begins on the surface of the material. Therefore, the biointerface properties of any biomaterial or medical device are crucial for its biocompatibility. Titanium and its alloys are commonly employed for medical implants, but progress in nanotechnologies together with new findings indicating the importance of nanoscale morphology on cell behaviour opens the door for the application of titanium nanostructured surfaces.

From a practical point of view, interactions with biofilm-forming species of bacteria are highly relevant, especially in the case of nosocomial infections and implants. Therefore, interactions with B. cereus and P. aeruginosa, representing both G+ and G− biofilm forming bacteria,
were studied. The results demonstrate that both bacteria can form biofilms with slightly higher amounts on TiO₂ NTs compared to Ti foil (Table 3); however, the differences in our study were not significant. In addition, slightly higher amounts of formed biofilm were observed for Bacillus cereus compared to Pseudomonas aeruginosa; the differences, however, were also not significant. In this study, biofilm-positive bacterial strains were employed, which differ in many of their properties compared to the planktonic form of bacteria, which were preferentially used in previous works. The findings from this study are therefore comparable to only a few previous studies, mainly the work of Puckett et al., who reported that lower amounts of live Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa were found on nanorough Ti compared to conventional (non-nanorough) Ti [18]. In another paper, Anitha et al. studied the adhesion of Shewanella oneidensis MR-1 cells on TiO₂ NTs. They found increased biofilm formation with decreasing NT diameter; thus, the highest level of bacterial cell adhesion (biofilm formation) was observed for 20 nm NTs [2]. The results of the present study are therefore not in accordance with these studies, as we found no significant differences in bacterial behaviour with respect to biofilms formed on Ti.

In the present study, MSc were able to adhere and subsequently grow on all surfaces, although cells behaved differently on TiO₂ NTs of different diameters. On 15 nm TiO₂ NTs (Fig. 3b) and 50 nm TiO₂ NTs (Fig. 3c), the cells exhibited similar morphology and also their quantity was comparable to that found on Ti foil (Fig. 3a). On 100 nm TiO₂ NTs, (Fig. 3d) the cells drastically changed their morphology. The cells did not spread out and their viability was significantly lower (this is evident from the number of dead cells, as only DNA and not the cytoskeleton is seen on the micrograph). The 100 nm TiO₂ NTs can therefore be assigned as having a critical diameter, which initiates different behaviour of mesenchymal stem cells on TiO₂ NTs.

According to Fig. 4, it can be concluded that ESc were able to adhere, grow and survive in spherical compact colonies on both gelatine non-coated and coated surfaces, but with NTs diameter having an important influence. It is important to note that only viable cells were coloured with the dye, so we can also conclude that surface morphology influences the number of viable cells which adhere. ESc seem to be able to behave similarly on Ti foil, TiO₂ NTs 50 nm and TiO₂ NTs 100 nm, but on TiO₂ NTs 15 nm (Fig. 4a) the cells form smaller non-spherical colonies.

To confirm observations from fluorescence microscopy, the quantification of ESc was performed using ATP staining. The first graph (Fig. 5A) shows how the cells behave in contact with native non-gelatine-coated
surfaces. The results confirm that significantly lower amounts of viable cells was found on the TiO2 NTs 15 nm sample, compared to both tissue culture plastic and pure Ti foil. 50 nm TiO2 NTs and 100 nm TiO2 NTs exhibit comparable abilities with respect to cell adhesion and survival; however, compared to tissue culture plastic their ability is significantly lower. As the gelatinization of surfaces is a frequently used technique for improving surface cyto-compatibility, this technique was also applied on nanostructured titanium surfaces. When the surfaces were coated with gelatine (Fig. 5b), the differences between 15 nm TiO2 NTs and the other diameter NTs were insignificant. The significant difference found with tissue culture plastic, however, remained. It can be concluded that gelatinization eliminates significant differences between different nanostructures, i.e., differences that were observed previously on freshly fabricated surfaces. Although improvements in ESc cell response on gelatine-covered nanostructured surfaces were observed in the case of 15 nm TiO2 NTs, the overall influence of gelatinization was not as prominent. Thus, nanotopographic features seem to prevail over the chemical alteration of surfaces covered with gelatine, as the response of ESc to these surfaces was still lower compared to control. The small differences between the results from Calcein staining and ATP are based on the fact that Calcein loading leads to the release of cell colonies from surfaces in a time dependent manner, probably mediated by its toxicity to cells. Based on the results presented in Figs. 4 and 5, it can be concluded that ESc can adhere to and grow on TiO2 nanostructured surfaces, except on NTs of 15 nm in diameter.

In contrast to MSc and ESc, the ability of cardiomyocytes to adhere to and mainly to survive and grow on all the tested Ti based samples was very limited. From Fig. 6 it is clear that cell artefacts counterstained by DAPI are observed on the surfaces; yet, when the specific myosin heavy chain is stained, it is clear that the cells are not in their physiological state. It can be concluded that neither Ti-foil nor TiO2 NTs provide the required cues for cardiomyocytes. In addition, the quantification of cells (determined by ATP level), showed practically no viable cells on Ti-foil or TiO2 NTs (data not shown).

The ability of both prokaryotic and eukaryotic cells to adhere, survive and grow on any surface substantially depends on the surface’s chemical composition, wettability, morphology, and nanoscale morphology, and even eventually on the leaching of toxic impurities. In our study, chemical composition and wettability, as described above, did not significantly differ between the different TiO2 NTs. Interactions with the proteins of culture media, which can influence subsequent cell adhesion and growth, should therefore be similar. Thus, it can be concluded that any differences in cell reaction can be assigned to the different surface morphologies. This is also supported by the fact that gelatinization improves the cyto-compatibility of nanostructured titanium surfaces, especially in the case of 15 nm NTs. However, the overall improvement in ESc interaction with gelatinized surfaces was not as prominent as expected, as the control surfaces and gelatinized Ti-foils seemed to provide a better environment for cell growth compared to the even gelatinized nanostructured surfaces of TiO2.

It is also well known that different cell lines can react differently on the same surface topography, as every cell line has its specific cues, these provided by the extracellular matrix. For example, rat mesenchymal stem cells adhere, proliferate and migrate significantly better on TiO2 NTs of 15 nm in diameter compared to flat TiO2 [17]. With increasing diameter, cell adhesion as well as the subsequent proliferation and migration start to decline. Similarly, in the work of Bauer et al. [3], the best adhesion of MSc was detected on NTs of 15 nm in diameter, the adhesion decreasing as the NTs diameter increased further. A different situation, however, arose in a study by Lewandowska et al., in which nine different diameters of NTs (from 23 to 145 nm) were examined for

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bacillus cereus</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti foil</td>
<td>$1.24 \times 10^6$</td>
<td>$2.52 \times 10^6$</td>
</tr>
<tr>
<td>15 nm</td>
<td>$66.5 \times 10^5$</td>
<td>$7.10 \times 10^6$</td>
</tr>
<tr>
<td>50 nm</td>
<td>$16.7 \times 10^5$</td>
<td>$12.5 \times 10^6$</td>
</tr>
<tr>
<td>100 nm</td>
<td>$52.5 \times 10^5$</td>
<td>$2.42 \times 10^6$</td>
</tr>
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Fig. 2. Surface chemistry determined by XPS analysis: (A) XPS survey spectra for TiO2 NTs of 100 nm in diameter, and the high resolution scan of TiO2 NTs of 100 nm in diameter for (B) C 1s, (C) O 1s, and (D) Ti 2p peaks.
murine fibroblast adhesion and proliferation. The cell adhesion and proliferation were significantly higher for all diameters relative to pure Ti foil [11]. Remarkably, in a study by Park et al., the cell response was directly proportional to the size of NTs [17], while in a study by Lewandowska et al. this dependence was indirect. The highest level of cell proliferation was observed on the surface with a NTs diameter of 96 nm [11]. Another cell type (osteoblast) on similar diameter was used in another study [16]. It was shown that cell growth was significantly decreased after 48 h of incubation on nanostructured TiO₂ NTs with outer diameters ranging from 70 to 120 nm compared to a flat titanium surface. Thus it is not surprising that our results are in agreement with some previous studies, while some other studies present contradictory results. For example, Xu et al. studied the adhesion and proliferation of bone marrow MS cells on titanium NTs with diameters of 30, 100 and 200 nm. While proliferation on 30 and 100 nm NTs was comparable with Ti, cell proliferation on the largest diameter was considerably reduced [21]. Likewise, Zhao et al. studied bone MS cell proliferation on NTs with diameters of 25 and 80 nm, but did not observe any statistically significant difference in the cell quantities on these two diameters after 4 days of cultivation [23]. A different situation arose in a
study by Park et al., in which the MS cell count decreased with the increasing diameter of NTs. Generally, the highest cell quantity was observed on 15 nm NTs and the lowest on 100 nm NTs [17].

The above-described different behaviours of MSc, ESc and cardiomyocytes show that nanostructured titanium surfaces can be used either as selective substrates for cell cultivation, or as selective surfaces for medical devices, on which only some cell lineages will be able to grow. In the case of cardiomyocytes, nanostructured surfaces could be applied for cardiac support devices, concretely in the part of devices where interactions with cardiomyocytes are not desired.

4. Conclusions

Native tissues are highly hierarchical systems with defined structures on the nanoscale level. The nanostructures of tissues, together with the biomolecules of the extracellular matrix, growth factors, and other biologically active compounds determine cell fate. The impact of various nanostructures created on biomaterials is therefore one of the crucial parameters influencing cell behaviour and should be studied before any application is considered. In addition, the variety of cell lineages with divergent properties that specifically interact with nanotopographic features must be taken into account. In the present work, three different cell lineages – mesenchymal stem cells, embryonic stem cells, and cardiomyocytes – were seeded on nanostructured surfaces of titanium. Differences in their behaviour were studied and it was shown that mesenchymal and embryonic stem cells were able to adhere to and grow on the tested surfaces, while the contrary was observed for cardiomyocytes. Moreover, a correlation between the nanotube diameter NTs and cell behaviour was observed. The 100 nm NTs appear to present a crucial diameter for, which NTs, critically influences on mesenchymal stem cells behaviour, while the 15 nm NTs appear to present a critical diameter for embryonic stem cells. However the

Fig. 5. Amounts of ESc cells on a) native and b) gelatine coated surfaces of individual samples after 48 h cultivation (ctrl – non-coated, TC plastic). Statistical significance was determined by ANOVA and post hoc Bonferroni's Multiple Comparison test. * marks $P < 0.05$; ** marks $P < 0.01$.

Fig. 6. HGB cells on a) Ti foils; b) TiO$_2$ NTs 15; c) TiO$_2$ NTs 50; d) TiO$_2$ NTs 100 after 48 h cultivation.
behaviour of both G+ and G− biofilm-forming prokaryotic cells was similar. The results of our study provide new essential knowledge about the interaction of well-defined nanostructured titanium surfaces with two types of cell linages, which could be of significance for the development of novel nanostructured implantable devices.

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