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# Tunable Functionality and Toxicity Studies of Titanium Dioxide Nanotube Layers

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## Abstract

In this **study**, we have developed a **simple** process to **fabricate** scalable titanium dioxide nanotube layers which show a tunable functionality. The titanium dioxide nanotube layers were prepared by electrochemical anodization of Ti foil in 0.4 wt% hydrofluoric acid solution. The nanotube layers structure and morphology were characterized using x-ray diffraction and scanning electron microscopy. **The surface topography and wettability were studied according to the anodization time. The sample synthesized displayed a higher contact angle while the current density reached a local minimum.** Beyond this point, the contact angles decreased with anodization time. Photo-degradation of acid orange 7 in aqueous solution was used as a probe to assess the photocatalytic activity of titanium dioxide nanotube layers under UV irradiation. We obtained better photocatalytic activity for the sample **fabricated** at higher current density. Finally we used the Ciliated Protozoan *T. pyriformis*, an alternative cell model used for in vitro toxicity studies, to predict the toxicity of titanium dioxide nanotube layers in a biological system. We did not observe any characteristic effect in the presence of the titanium dioxide nanotube layers on two physiological parameters related to this organism, non-specific esterases

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activity and population growth rate.

*Keywords:*

Anodization, Titanium Dioxide Nanotubes, Contact Angle, Photodegradation, Toxicity.

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## 1. Introduction

2        Research into developing nanotubes with **interesting** properties by con-  
3        trolling the nanostructure topography has attracted great interest because of  
4        their variety of applications. In 2001, Gong and co-workers [1] reported the  
5        fabrication of vertically oriented highly ordered TiO<sub>2</sub> nanotube arrays up to  
6        approximately 500 nm length by anodization of titanium foil in an aqueous  
7        HF electrolyte. Since then, substantial efforts have been devoted to the self  
8        organisation and growth of TiO<sub>2</sub> [2, 3, 4]. Titanium dioxide nanotube layers  
9        are used as photo-catalysts in water and environmental purification, as well  
10       as **biological** and biomedical applications [5, 2, 6]. In particular, Titanium  
11       dioxide nanotubes are used as a **biomaterial** for implants, drug delivery plat-  
12       forms, tissue engineering and bacteria killing [7, 8, 9, 10, 11, 12, 13]. Another  
13       interesting propriety of TiO<sub>2</sub> is its tunable wettability effect [14, 15]. The  
14       ability to modify surface topography and to control wetting behavior is useful  
15       for biomedical applications. Surface roughness, contact angle, surface energy  
16       are the main factors in understanding the biology media and material inter-  
17       action. In this work, we present recent results on TiO<sub>2</sub> nanotubes fabricated  
18       by anodization of Ti foil in 0.4 wt% hydrofluoric acid solution to produce a  
19       self-organized porous film structure versus the anodization time. Such TiO<sub>2</sub>  
20       nanotube surfaces are of interest in order to change the wettability proper-  
21       ties of titanium oxide films. The nanotube layers were characterized using  
22       x-ray diffraction and scanning electron microscopy. We have investigated the  
23       surface wettability of as-anodized samples obtained at different anodization  
24       times. The as-grown sample synthesized while the current density **reached**  
25       a local minimum displayed higher contact angle. The surface of the oxide  
26       was covered at this point with a high density of fine pits. **We report on**  
27       **the photo-degradation of acid orange 7 (AO7) in aqueous solutions**  
28       **which was used as a probe to assess the photocatalytic activity of**  
29       **titanium dioxide nanotube layers under UV irradiation.** We obtained  
30       better photocatalytic activity for the sample **fabricated** at higher current  
31       density. Finally we **used** the Ciliated Protozoan *T. pyriformis* to predict the

32 toxicity of titanium dioxide nanotube layers towards biological systems.

## 33 **2. Experimental Details**

### 34 *2.1. Sample preparation*

35 To fabricate anodic TiO<sub>2</sub> nanotube layers, we used Ti foil (Goodfellow  
36 99.6% purity) with a thickness of 0.1 mm. The Ti foils were degreased by  
37 successive sonication in trichloroethylene, acetone and methanol, followed by  
38 rinsing with deionized water, dried in the oven at 100°C and finally cooled  
39 in the desiccator. Anodization was carried out at room temperature (20°C)  
40 in 0.4 wt% HF aqueous solution with the anodizing voltage maintained at  
41 20 V.

### 42 *2.2. Surface characterization*

43 The surface topography characterization was performed using a Zeiss  
44 Supra 55 VP scanning electron microscope (SEM) **with secondary emis-**  
45 **sion and in lens detector. The accelerating voltage and the work-**  
46 **ing distance are respectively 3kV and 5mm. The crystalline struc-**  
47 **ture and phase of the TiO<sub>2</sub> nanotube layers were determined by X-**  
48 **ray diffraction (XRD) using a Scintag XRD X'TRA diffractometer**  
49 **with CuK<sub>α</sub> ( $\lambda = 1.54 \text{ \AA}$ ) radiation. The CuK<sub>β</sub> radiation is filtered**  
50 **through a nickel filter. The diffraction pattern was achieved be-**  
51 **tween 20 and 80° with a step angle of 0.05° and a scanning speed**  
52 **of 0.01° per second.**

### 53 *2.3. Contact angles*

54 Surface wettability **was** investigated using a drop shape analysis system  
55 (EasyDrop, Kruss, Hambourg, Germany). The contact angle of 3  $\mu\text{L}$  ses-  
56 sile droplet of deionized water was measured on the surface under ambient  
57 conditions.

### 58 *2.4. Photo-degradation*

59 Photocatalytic experiments were conducted in 3 mL of AO7 solution  
60 (from Acros Organics) with a concentration of  $5.0 \cdot 10^{-5}$  mol/L, placed in a  
61 cylindrical Pyrex glass reactor. The surface area of the anodized samples was  
62 approximately 3.5 cm<sup>2</sup>. The glass reactor was irradiated with polychromatic  
63 fluorescent UV lamps (Philips TDL 8 Watt (total optical power 1.3 Watt),  
64 300 mm long, wavelength range 315-400 nm) in a configuration providing

65 about 0.9 mW/cm<sup>2</sup> at the sample surface. The photocatalytic decomposi-  
66 tion of AO7 was monitored by the decrease in the solution's absorbance at a  
67 wavelength of 485 nm using a UV-Vis spectrometer (Perkin Elmer Lambda  
68 35).

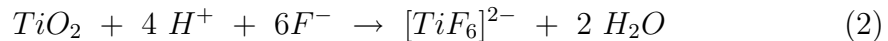
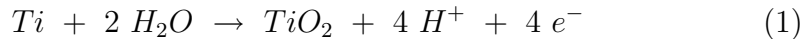
### 69 2.5. Toxicity assessment

70 The potential toxicity of TiO<sub>2</sub> nanotube surfaces was evaluated with  
71 *Tetrahymena pyriformis* using two tests previously validated : inhibition of  
72 an enzymatic activity and effect on population growth rate. For non-specific  
73 esterases activities quantification, a *T. pyriformis* culture in an exponential  
74 growth phase (in PPYS medium) was centrifuged at 300 rpm, and the su-  
75 pernatant was discarded. The *T. pyriformis* pellet was suspended in Volvic  
76 mineral water. After counting cells under a microscope, dilution was done to  
77 obtain about 4000 cells/mL. 1 mL of this dilution was incubated for 1h with  
78 the different Ti layers at 28 °C under UV or without UV irradiation. After  
79 incubation, Ti layers samples were removed and 1 mL of **Fluorescein diac-**  
80 **etate (FDA)** at 4.8 μM was added (2000 cells/mL in final). Each toxicity  
81 test included two controls : FDA in Volvic water to measure self degradation  
82 of this substrate and FDA with *Tetrahymena pyriformis* (untreated cells).  
83 After 30 min, the fluorescence was measured by a spectrofluorimeter (Kon-  
84 tron SFM 25, Kontron, Milan, Italy) with a 485 nm excitation filter and  
85 a 510 nm emission filter. Experiments were repeated three times for each  
86 sample. To test the inhibition of development of populations in an exponen-  
87 tial growth phase, we prepared 8 erlenmeyer flasks (40 mL): 2 for control  
88 cultures and 6 for the samples to be tested (Ti foil, unannealed TiO<sub>2</sub> and  
89 TiO<sub>2</sub> annealed at 500 °C). The samples were deposited at the bottom of the  
90 erlenmeyer flasks and 3 mL were **removed** at 0h, 3h, 6h and 9h to measure  
91 the optical density (OD) at 535 nm.

## 92 3. Results and discussion

### 93 3.1. TiO<sub>2</sub> Nanotube growth process and Layer Characteristics

94 The anodization growth was governed by a competition between anodic  
95 oxide formation and chemical dissolution [16] of the oxide as soluble fluoride  
96 complexes according respectively to reactions (1) and (2) :



97 Figure 1 shows a characteristic density current time curve for Ti anodiza-  
 98 tion in our operating conditions and figure 2 shows SEM images of the TiO<sub>2</sub>  
 99 grown at different stages of growth corresponding to the points a, b, c and d.  
 100 We can notice that after an initial exponential decay of the current density  
 101 to a local minimum around 10 mA.cm<sup>-2</sup> about 70 s. The structure of the  
 102 film at this point led to the formation of randomly generated pits on the  
 103 oxide which were shown in figure 2a. The pits were approximately 30 nm in  
 104 diameter.

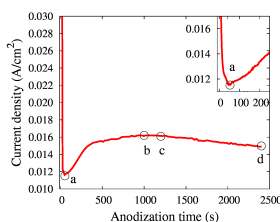


Figure 1: Typical density current time curve for Ti foil anodization. Anodization was carried out at room temperature (20°C) in a 0.4 wt% HF aqueous solution with the anodizing voltage maintained at 20V. Inset in upper right hand corner shows a blow up of the time behavior from 0 to 250 s.

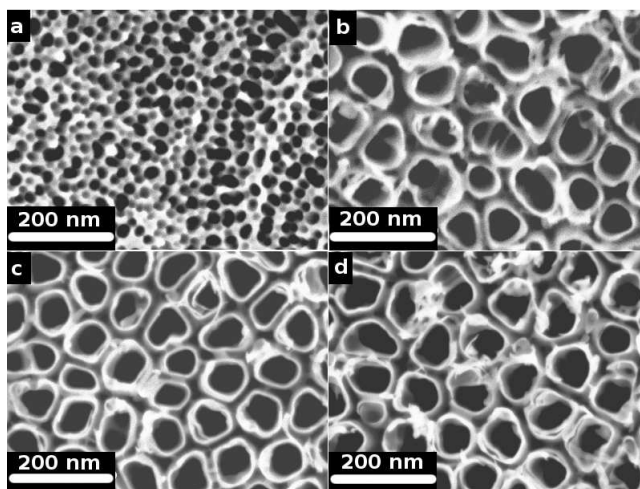


Figure 2: SEM top-view images of samples taken while density current was at local minimum (a), local maximum (b) and at the anodization time of 20 min and 40 min respectively (c, d).

105 After the current density increased to a local maximum of 12,7 mA.cm<sup>-2</sup>

106 in 1000 s, we **observed** ordered nanotube arrays with approximately 85 nm  
 107 in diameter as evidenced by figure 2b. After 20 min and 40 min of growth,  
 108 we observed in figure 2c and 2d ordered nanotube arrays with approximately  
 109 100 nm and 105 nm in diameter respectively. We summarize in figure 3, the  
 110 evolution of the diameter of the pores. Between 70 and 1000 s, we **observed**  
 111 a linear evolution of the diameter versus time with a high slope. Similarly,  
 112 the trend is weak between 1000 and 2400 s. At 2400 s, we reach the maximum  
 113 diameter. This shows that the dissolution rate of oxide is predominant over  
 114 the oxide growth velocity. So in a steady state, the pore diameters do not  
 115 depend on the anodization time despite enlargement of the pore diameters  
 116 due to the dissolution of the mouth of the nanotubes as reported elsewhere  
 117 [17].

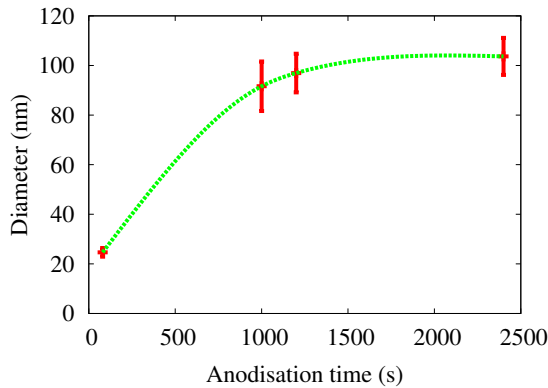


Figure 3: The evolution of the diameter of the pores of **samples** (a), (b), (c) and (d).

118 We summarize in figure 4 the X-ray diffraction patterns of Ti foil and  
 119  $\text{TiO}_2$  nanotube layers anodized for 40 min before and after annealing at  
 120  $500^\circ\text{C}$  in oxygen for 2 h according to the paper published elsewhere [2]. The  
 121 unannealed  $\text{TiO}_2$  nanotube layer exhibits only the peaks from titanium metal  
 122 foil under the nanotube layer, while the annealed sample exhibits the main  
 123 lattice phases of anatase and rutile (figure 4).

### 124 3.2. Contact angles

125 The influence of  $\text{TiO}_2$  nanotubes on Ti on bone cell-materials interac-  
 126 tion has been reported [18]. In this study, we have evaluated the contact  
 127 angle behavior on  $\text{TiO}_2$  nanotube layers which have pronounced topologi-  
 128 cal features and increased surface areas. Contact angles were measured for

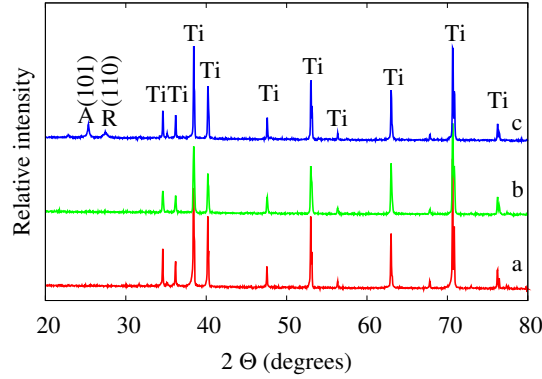


Figure 4: X-ray diffraction patterns of Ti foil (a), as grown during 40 min (b) and annealed TiO<sub>2</sub> nanotube layers at 500°C in oxygen for 2 h (c). Lattice planes indicate anatase (A), rutile (R) and titanium (Ti).

129 each as-anodized sample. Each sample was dried in the oven for 30 min and  
 130 cooled 15 min in the desiccator before measurements. We observe in figure 5,  
 131 optical images of water droplets on as-grown TiO<sub>2</sub> nanotube layers. Results  
 132 indicated a higher contact angle for the sample covered with pits obtained  
 133 at local minimum density current (70 s). Beyond this particular point, the  
 134 contact angles value decreases with anodization time. So we have performed  
 135 a scalable surface functionality without any chemical treatment.

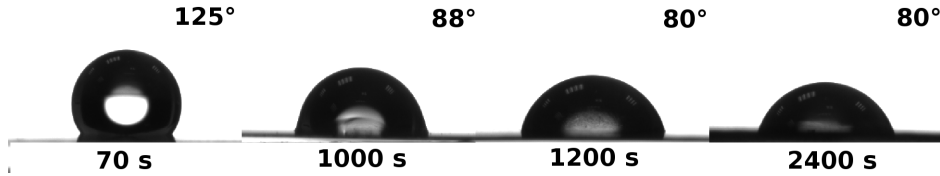


Figure 5: Optical images of water droplets on as-grown TiO<sub>2</sub> nanotube layers. Surface at local minimum density current (70 s); surface at local maximum density current (1000 s); surface after 20 min of growth (1200 s) and surface after 40 min of growth (2400 s).

### 136 3.3. Photo-catalytic activity measurement

137 The photo-degradation of AO7 in the presence of TiO<sub>2</sub> nanotubes under  
 138 different conditions is summarized in figure 6. This shows the AO7 concen-  
 139 tration versus time as determined by the solution's absorbance at 485 nm.  
 140 The initial concentration of the AO7 was  $5.0 \cdot 10^{-5}$  mol/L.  $C(0)$  is the initial



141 concentration of AO7 while  $C(t)$  is the concentration after time,  $t$ , of con-  
 142 stant UV irradiation in 315-450 nm wavelength range. The variation of the  
 143 concentration of AO7 in the presence of the  $\text{TiO}_2$  layer without irradiation  
 144 after 5 h is less than 1%. Thus the effect of adsorption of the dye on the  $\text{TiO}_2$   
 145 surface is negligible. Curve 6(e) shows the variation in the concentration of  
 146 AO7 in the presence of unannealed  $\text{TiO}_2$  nanotubes under UV irradiation.  
 147 This result indicates that AO7 is not substantially degraded in the presence  
 148 of amorphous  $\text{TiO}_2$  nanotube layers. Curves 6(a), 6(b), 6(c) and 6(d) corre-  
 149 sponding to the  $\text{TiO}_2$  nanotube layers grown during 70 s, 1000 s, 1200 s, 2400  
 150 s respectively and annealed at  $500^\circ\text{C}$  illustrate photo-degradation of AO7.  
 151 These results show the decay of organic molecules with UV irradiation in  
 152 the presence of the annealed nanotubes. We observed the strongest photo-  
 153 catalytic activity for the sample grown during 1200 s. We **cannot** draw any  
 154 correlation between surface wettability and photocatalytic activity.

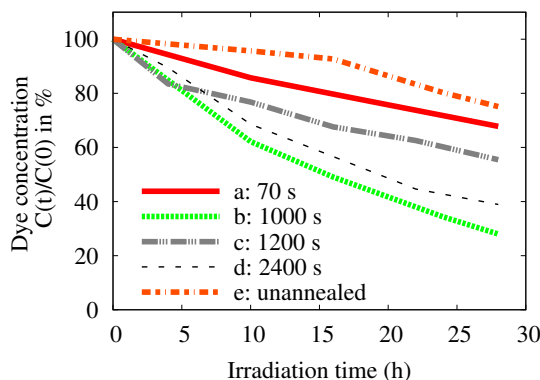


Figure 6: Photo-degradation of acid orange 7 (AO7) dye under UV-lamp irradiation at wavelengths of 315-400 nm in the presence of a  $\text{TiO}_2$  nanotube layer, as measured by the absorbance of the irradiated dye at 485 nm.  $C(0)$  is the initial concentration of AO7 ( $5 \times 10^{-5}$  M) and  $C(t)$  is the concentration after time,  $t$ , of irradiation. (e) unannealed  $\text{TiO}_2$  nanotube layer; (a), (b), (c), (d)  $\text{TiO}_2$  nanotube layers grown during 70 s, 1000 s, 1200 s, 2400 s respectively and annealed at  $500^\circ\text{C}$ .

155 The effect of irradiation of the sample grown for 1000 s with polychro-  
 156 matic light (315-400 nm) on the UV-Vis spectrum is **shown in figure 7**.  
 157 Absorbance for increasing irradiation time decreases from the upper curve  
 158 toward the lower curve.

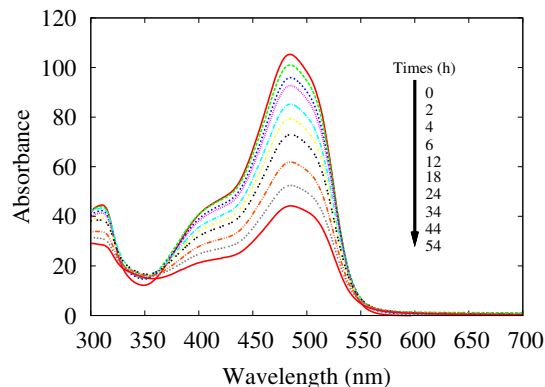


Figure 7: Effect of irradiation with polychromatic light (315-400 nm) of AO7 in the presence of TiO<sub>2</sub> nanotube layers grown during 1000 s and annealed at 500°C on the UV-Vis spectrum in the 300-700 nm range.

### 159 3.4. Toxicity tests

160 The cellular toxicity of TiO<sub>2</sub> based nanofilaments [19], nanoparticles [20]  
 161 was studied. The authors concluded that these nanofilaments and nanopar-  
 162 ticles are cytotoxic. However, in other studies, no toxic effects were observed  
 163 with TiO<sub>2</sub> nanoparticles [21, 22]. Similarly the effects of radical reactivity  
 164 generated by TiO<sub>2</sub> on cells under UV irradiation **is** still controversial [23, 24].  
 165 **As far as we know**, no study has been conducted on the toxicity of TiO<sub>2</sub>  
 166 nanotube layers. In this work two different tests of toxicity have been made  
 167 with the Ciliated protozoan *T. pyriformis* as described in detail elsewhere  
 168 [25]. This organism is an alternative eukaryotic cell model including the es-  
 169 tablished fibroblastic cell lines used **in vitro** toxicity studies. All the tests  
 170 were carried out with titanium foil, amorphous and crystalline TiO<sub>2</sub> nan-  
 171 otube layers. The cristalline samples showed photocatalytic activities. We  
 172 studied their influence under UV radiation on the toxicity tests. The test  
 173 of inhibition of non-specific intracellular esterase activity was based on the  
 174 hydrolysis of fluorescein diacetate (FDA) by *T. pyriformis* and quantifica-  
 175 tion of fluorescein released **for** 30 min. Esterases are ubiquitous enzymes  
 176 present in all living organisms and are considered as good biomarkers of cel-  
 177 lular activities. The aim of this test was to determine the evolution of the  
 178 percentage of the *T. pyriformis* activity over the control in the presence of  
 179 Ti foils, amorphous and crystalline TiO<sub>2</sub> nanotube layers without UV irra-  
 180 diation **without UV**) and with constant UV irradiation (**With UV**) at a  
 181 wavelength of 315-400 nm. We can observe an effect of titanium foil but

182 no significant effect of the amorphous and crystalline TiO<sub>2</sub> nanotube layers.  
 183 The UV light radiation did not disturb the results (Figure 8) **despite** the  
 184 photocatalytic activities of cristalline samples. Furthermore, *T. pyriformis*  
 185 population growth rate test allows us to include different physiological distur-  
 186 bances which could have been caused by the three types of layers. Growth  
 187 was followed photometrically with a measure of optical density ( $\lambda = 535$   
 188 nm) every 3 hours. Reduction in growth compared to a control culture is  
 189 indicative of toxicity.

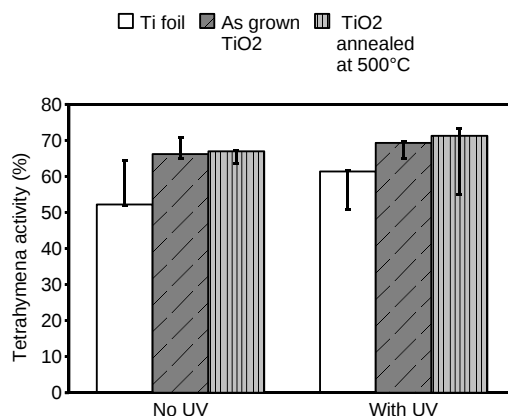


Figure 8: *T. pyriformis* activity over the control in the presence of Ti foil, amorphous and crystalline TiO<sub>2</sub> nanotube layers without UV irradiation (No UV) and with constant UV light (With UV) at wavelength range 315-400 nm.

190 The purpose of this test was to determine a 50% inhibition of growth rate  
 191 in treated cultures (increase in 50% of the generation time compared to a  
 192 control culture). We did not observe any characteristic effect related to the  
 193 inhibition of protozoa growth for Ti foils, amorphous and crystalline TiO<sub>2</sub>  
 194 nanotube layers (Figure 9). We can conclude through these two tests that  
 195 the TiO<sub>2</sub> nanotube layers are not toxic.

#### 196 4. Conclusions

197 We have demonstrated the fabrication of controllable as-grown surfaces  
 198 of titanium dioxide nanotube layers. The contact angles measurements show  
 199 clearly the correlation between surface topography and surface wettability.  
 200 We have shown the ability of the titanium dioxide nanotube layers to degrade

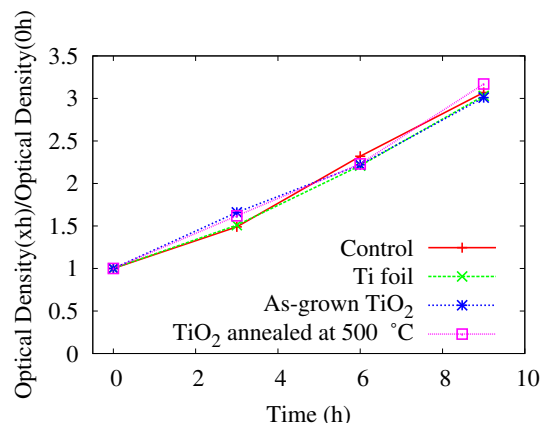


Figure 9: Growing populations of *T. pyriformis* in the presence of titanium foil, amorphous and crystalline titanium dioxide nanotube layers.

201 AO7. Such surfaces **do not** show any characteristic *in vitro* toxicity effect in  
 202 a biological system. In conclusion, we have developed **a simple process** to  
 203 **fabricate** active surfaces of titanium dioxide with scalable nanotube layers  
 204 and tunable functionalities.

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