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Simona Popescu, Ioana Demetrescu, V. Mitran, Alain Gleizes. MOCVD-Fabricated TiO<sub>2</sub> Thin Films: Influence of Growth Conditions on Fibroblast Cells Culture. *Molecular Crystals and Liquid Crystals*, 2008, 483, pp.266-274. 10.1080/15421400801914301 . hal-03578423

**HAL Id: hal-03578423**

**<https://hal.science/hal-03578423>**

Submitted on 17 Feb 2022

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**To link to this article :**

URL : <http://dx.doi.org/10.1080/15421400801914301>

**To cite this version :** Popescu, Simona and Demetrescu, Ioana and Mitran, V. and Gleizes, Alain ( 2008) *[MOCVD-Fabricated TiO<sub>2</sub> Thin Films: Influence of Growth Conditions on Fibroblast Cells Culture.](#)* Molecular Crystals and Liquid Crystals, vol. 483 . pp. 266-274. ISSN 1542-1406

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## MOCVD-Fabricated TiO<sub>2</sub> Thin Films: Influence of Growth Conditions on Fibroblast Cells Culture

S. Popescu<sup>1</sup>, I. Demetrescu<sup>1</sup>, V. Mitran<sup>2</sup>,  
and A. N. Gleizes<sup>3</sup>

<sup>1</sup>Faculty of Applied Chemistry and Materials Science, UPB, Polizu, Bucharest, Romania

<sup>2</sup>Faculty of Biology, University of Bucharest, Bucharest, Romania

<sup>3</sup>Institut Carnot – Centre Interuniversitaire de Recherche et d'Ingénierie des Matériaux (CNRS-INPT-UPST) ENSIACET, Toulouse cedex, France

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*TiO<sub>2</sub> thin films with various morphologies were grown on Ti substrates by the LP-MOCVD technique (Low Pressure Chemical Vapour Deposition from Metal-Organic precursor), with titanium tetra-iso-propoxide as a precursor. All the films were prepared in the same conditions except the deposition time. They were characterized by X-ray diffraction, scanning electron microscopy, optical interferometry, water contact angle measurements. MOCVD-fabricated TiO<sub>2</sub> thin films are known to be adapted to cell culture for implant requirements. Human gingival fibroblasts were cultured on the various TiO<sub>2</sub> deposits. Differences in cell viability (MTT tests) and cell spreading (qualitative assessment) were observed and related to film roughness, wettability and allotropic composition.*

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**Keywords:** biocompatibility; fibroblasts; MOCVD; titanium; titanium oxide

## INTRODUCTION

Due to its very good mechanical properties, corrosion resistance and biocompatibility, titanium has been the most commonly used metallic material for biological implant devices. The role played by a naturally or artificially grown thin film of TiO<sub>2</sub> on its surface is crucial for both the

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We thank to European Commission for supporting this Transfer of Knowledge (No 014059) in the frame of Marie Curie actions. We thank also Yannick Thébaud for assistance in scanning electron microscopy.

Address correspondence to A. N. Gleizes, Institut Carnot – Centre Interuniversitaire de Recherche et d'Ingénierie des Matériaux (CNRS-INPT-UPST) ENSIACET, 118 route de Narbonne, F-31077 Toulouse cedex 4, France. E-mail: Alain.Gleizes@ensiacet.fr

protection against corrosion by the physiological fluids and the viability of the tissue cells [1]. A good deal of scientific and technical knowledge on biocompatible oxide films grown on metallic biomaterials with application is available [2–5]. Film thickness and film morphology are key factors. For instance a good protection against corrosion needs non porous, adherent and compact coatings. On the other hand, cells are sensitive to surface topography and chemistry [6–8]. Fibroblasts have been claimed to better develop on smooth surfaces whilst osteoblasts would prefer rough surfaces [1,9–11]. Size and arrangement of surface features such as holes and dots seem to play a role in cell adhesion mechanisms [12–14]. It is therefore important to be able to prepare TiO<sub>2</sub> thin films with varied surface morphologies and chemistries in order to study their influence on cells behaviour.

The technique of chemical vapour deposition is one of the various industrial processes to deposit a thin film on a substrate. The variant consisting in using a metal-organic compound as a precursor (MOCVD) results in relatively low deposition temperatures and generally nature-friendly processes [15]. The surface of objects with complicated shapes (as are implants and prostheses) can be conformally coated by operating at low pressure (LP-MOCVD) [16,17]. Varied film morphologies and microstructures can be obtained by playing with the several experimental parameters: nature of the precursor, nature of the carrier gas, optional use of an additional reactive gas, deposition temperature ( $T_{\text{deposition}}$ ), total pressure ( $P_{\text{total}}$ ), precursor molar fraction ( $X_{\text{precursor}}$ ), gas flow rates ( $Q_{\text{total}}$ ,  $Q_{\text{precursor}}$ ) [18,19].

We have been using the LP-MOCVD technique to grow TiO<sub>2</sub> thin films on titanium with varied morphologies and micro- (nano-) structures with the view of studying the influence of film characteristics on cells behaviour in culture systems and on the protection of Ti substrates against corrosion by biological fluids [20,21]. The aim of the present study was to grow various thin films of TiO<sub>2</sub> on titanium substrates using the MOCVD technique and to assess *in vitro* compatibility of the films by using a human gingival fibroblast cell line (HGF-1; ATCC). The films were prepared in the same conditions except the deposition time in order to possibly get films differentiating from each other by the thickness only, therefore by the roughness.

## MATERIALS AND METHODS

### Films Preparation

The substrates were 1 mm thick discs sliced from 10 mm diameter titanium rods (99.6% purity, Good Fellow Ltd.). The deposition surface

was mechanically polished with SiC paper grade 180 (grain size 76  $\mu\text{m}$ ). Prior to deposition, the discs were ultrasonically cleaned in acetone, then in boiling alcohol, and rinsed with distilled water.  $\text{TiO}_2$  thin films were grown using low pressure, metal-organic chemical vapor deposition (LP-MPOCVD) with titanium tetra-isopropoxide (TTIP) as a precursor, and nitrogen as both carrier and dilution gas. Deposition parameters were: TTIP temperature 25°C,  $\text{N}_2$  flow in the precursor container 20 sccm, dilution  $\text{N}_2$  flow 575 sccm, total pressure 20 torr, TTIP molar fraction in the gas phase  $76 \times 10^{-6}$ , deposition temperature 400°C, deposition time ranging from 90 to 500 minutes (Table 1).

## Films Characterization

Film thickness was deduced from the weight of deposit (this technique does not take into account the porosity of the film and occasional deposit on disc edge). Film topography was examined with a LEO-435 scanning electron microscope (SEM) operating at 15 kV. Roughness parameters  $R_a$  and  $R_q$  were measured before and after coating on five surface samples of 0.5  $\text{mm}^2$  with a profilometer Tencor. Contact angle measurements were measured using a DIGIDROP Contact Angle Meter (GBX Scientific Instruments). For each specimen, the measurement was effected at three places of the surface by depositing 10  $\mu\text{l}$  of distilled water. The contact angle was measured immediately after drop deposition with a digit camera using the program Windrop. Phase identification was made from grazing incidence ( $2^\circ$ ) and theta-theta X-ray diffraction patterns recorded on a vertical diffractometer (Seifert 3000t) equipped with a graphite monochromator and a Cu anticathode ( $\lambda \text{CuK}_\alpha = 1.5418 \text{ \AA}$ ).

## Fibroblast Cell Cultures

Human gingival fibroblasts (HGF-1, CRL-2014, American Type Culture Collection) were seeded at an initial density of  $10^4$  cells/ $\text{cm}^2$  in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100  $\mu\text{g}/\text{ml}$  streptomycin, in a humidified atmosphere containing 5%  $\text{CO}_2$ . Prior to cell culture, the titanium supports were sterilized by exposing both sides to ultraviolet light for 12 h and then being maintained in phosphate buffer saline (PBS) for another 12 h.

## Cell Morphology and Viability

The evolution of the cell culture was monitored after 24 h and 48 h of culture with an inverted phase-contrast microscope Nikon Eclipse

**TABLE 1 A** Summary of Films Properties

Sample codes	Non coated Ti	T-9	T-12	T-14	T-15	T-13
Deposition time (min)	-	90	250	360	430	500
Thickness (nm)	native oxide	200	600	1000	1100	1600
Roughness $R_a$ (nm)	$764 \pm 10$	$712 \pm 16$	$715 \pm 66$	$768 \pm 299$	$1166 \pm 318$	$1345 \pm 323$
Roughness $R_q$ (nm)	$966 \pm 30$	$892 \pm 36$	$929 \pm 72$	$1200 \pm 342$	$1627 \pm 271$	$1837 \pm 302$
Contact angle (deg)	60	55	9.3	7.5	7.2	7.9
before sterilization						
Composition*	native A	a-R	a-R	A-R	A-r	A-R
Cell viability	95	82	77	95	100	88
Cell spreading**	+	++	++	+++	+++	+

\*A(a) = majority (minority) of anatase; R(r) = majority (minority) of rutile.

\*\*Qualitative assessment.

TS 100. Cell morphology was assessed by fluorescent staining with hypericin (1 microM) for 15 minutes. Cell viability was tested through the MTT dye reduction assay [22]. This method is based on the reduction of yellow, water-soluble 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into dark-blue, water insoluble formazan. The reduction is produced by a mitochondrial enzyme (succinat dehydrogenase) and is a parameter for cellular mitochondrial integrity. The amount of formazan generated is directly proportional to the number of viable cells. 105 110

## RESULTS

Table 1 summarizes the properties of five MOCVD TiO<sub>2</sub>-coated titanium samples prepared for this study, in comparison with a non coated sample. 115

### Film Structure and Morphology

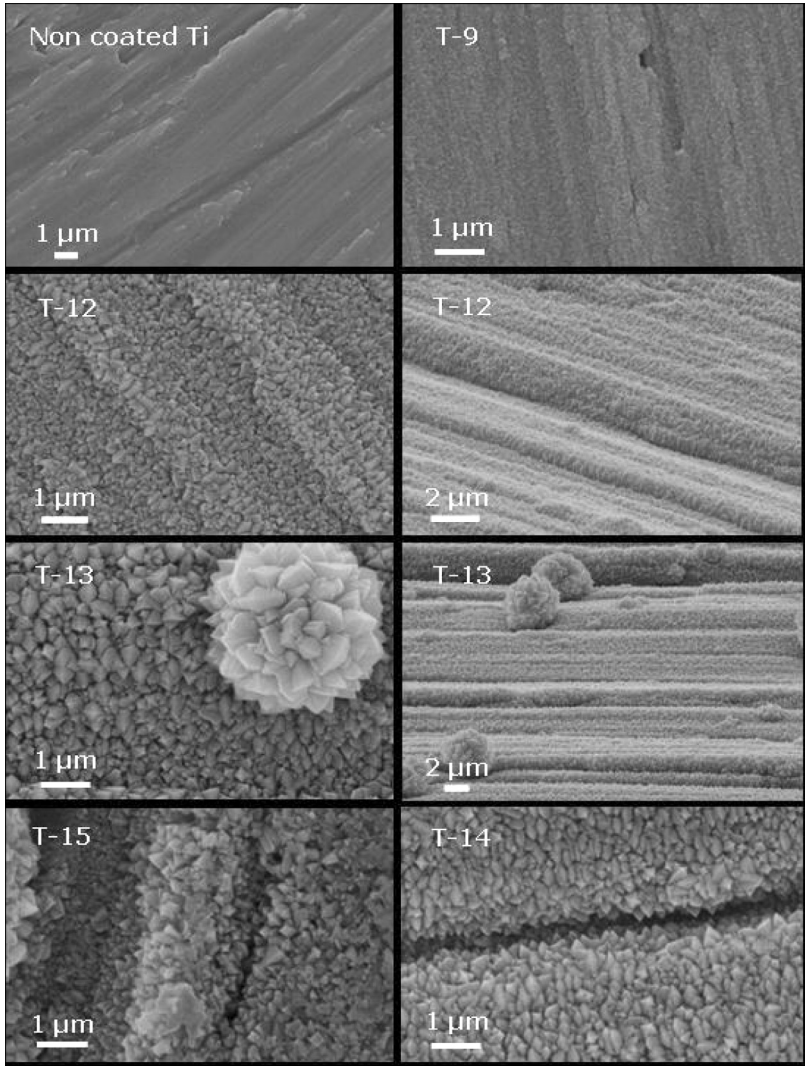
The films are made of crystallized TiO<sub>2</sub>. From X-ray diffraction patterns, they were mixtures of anatase and rutile. Qualitative estimations the allotropic composition reported in Table 1 were derived from  $\theta$ - $\theta$  patterns. 120

SEM micrographs (Fig. 1) evidence different film morphologies from fine grained to flower-like crystal clusters depending on the deposition time. Both the film thickness and the crystal size increase with increasing deposition time. As a consequence of the deposition from a vapour phase at low-pressure, the films are conformal even if the surface is rough: they match the shape of the underneath surface induced by polishing. The roughness of the substrates before being coated was  $R_q = 0.966 \pm 0.030 \mu\text{m}$  resulting from polishing with 76  $\mu\text{m}$  grain size SiC paper. The roughness measured in several parts of the TiO<sub>2</sub> films proved homogeneous. Roughness increases with deposition time therefore thickness (Table 1). Long deposition times favour the formation of big crystal clusters that will increase the roughness (Figs. 1d and 1f). 125 130

The films proved very hydrophilic as shown in Figure 2. The hydrophilic character is due to the presence of -OH groups resulting from the photo reduction of adsorbed water molecules [23]. 135

The samples were not kept in the dark, so that the presence of such groups is most likely. The hydrophilic character is enhanced by the state of division of the surface: the higher the surface area, the larger the number of attached -OH groups. Indeed there is an inverse proportionality between water contact angle and surface roughness (Fig. 2). 140

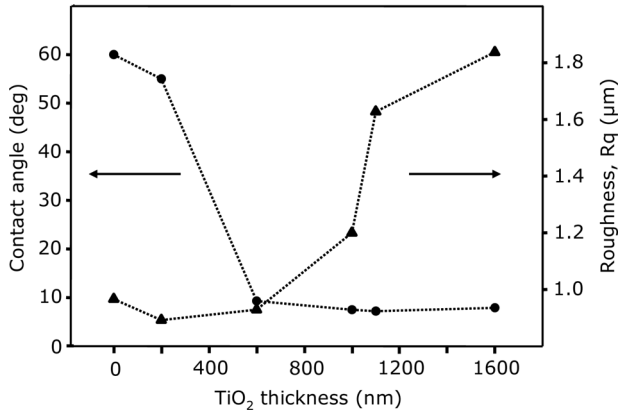




**FIGURE 1.** Surface SEM micrographs of  $\text{TiO}_2$  films grown using different deposition times. Perspective views are also shown for samples T-12 and T-13 (right).

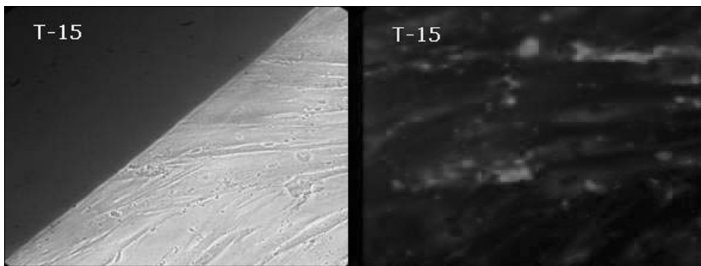
### Cell Culture and Viability

A non coated titanium disc and the five coated samples prepared in this study (Table 1) were seeded with fibroblasts (gingival fibroblast 145



**FIGURE 2.** Variation of the water contact angle and variation of the roughness with increasing TiO<sub>2</sub> film thickness.

morphology). Few cells were found on the non coated titanium disc. The cells were generally well spread and almost confluent on the plastic supports and on samples T-14 and T-15 (Fig. 3). On each sample, the cells exhibit a typical elongated morphology with no preferential orientation. Thus, there were no marked morphological differences between cells grown on the plastic support and on the coated titanium discs. Table 1 shows results of the MTT tests in terms of viability, and a qualitative assessment (optical examination) of the cell spreading. The viability varies from 77 to 100%, depending on the sample.



**FIGURE 3.** Morphology of adhered human gingival fibroblasts at the interface titanium disc/plastic support for sample T-15: phase contrast microscopy (left), fluorescence microscopy (right) [magnification  $\times 100$ ]. Cells were cultured for two days.

## DISCUSSION

The present work shows that the MOCVD method may produce TiO<sub>2</sub> coatings with various surface chemistries and surface topographies. The aim was to investigate the behaviour of human gingival fibroblasts on such surfaces and to possibly establish a correlation between one of the surface features, namely the roughness, and cells response. An approach currently used in the domain of biomaterials is to vary the surface topography because some cell types implied in implant integration process prefer smooth surfaces, while others prefer rough surfaces or surfaces with a gradient of roughness [11,24]. The search for the optimal surface roughness has been the aim of many studies but the question is still not answered. As a matter of fact, it is often difficult to obtain surfaces differing only in roughness. This is particularly true with the MOCVD process. The Ti substrates used in this study had the same roughness resulting from the polishing pre-treatment. They were coated in the same conditions of temperature, pressure, gas flow, and precursor molar fraction. The deposition time was varied to obtain various film thicknesses, therefore various film roughnesses. However, the results show that the film thickness has an influence not only upon the roughness but also on the wettability and on the allotropic composition that both influence the behaviour of cells [25]. It is therefore difficult to relate cell viability to surface roughness only.

Table 1 gathers measured roughnesses, contact angles, cell viabilities and quantitatively estimated allotropic compositions and cell spreading assessments for five coated samples and a non coated one. Cell viability and cell spreading appear to be favoured by (i) the presence of anatase, (ii) an hydrophilic surface, and (iii) not too high a surface roughness. It is noteworthy that the best viability was not observed for the smoother surfaces. So, despite the fact that some papers [1,9–11] claim that, in contrast with osteoblasts, fibroblasts prefer smooth surfaces, the present results suggest that the relation between surface roughness and biocompatibility is not so straightforward. On the other hand, a viability of 88% and a poor cell spreading were observed for the roughest surface sample, namely sample T-13. This sample showed large crystals clusters loosely spread on the surface, generating an ‘inhomogeneous’ roughness. It is therefore difficult to decide whether the poor biocompatibility is due to the roughness or to the presence of the crystals clusters.

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