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Effects of LP-MOCVD prepared TiO$_2$ thin films on the in vitro behavior of gingival fibroblasts

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**Abstract**

We report on the in vitro response of human gingival fibroblasts (HGF-1 cell line) to various thin films of titanium dioxide (TiO$_2$) deposited on titanium (Ti) substrates by low pressure metal-organic chemical vapor deposition (LP-MOCVD). The aim was to study the influence of film structural parameters on the cell behavior comparatively with a native-oxide covered titanium specimen, this objective being topical and interesting for materials applications in implantology. HGF-1 cells were cultured on three LP-MOCVD prepared thin films of TiO$_2$ differentiated by their thickness, roughness, transversal morphology, allotropic composition and wettability, and on a native-oxide covered Ti substrate. Besides traditional tests of cell viability and morphology, the biocompatibility of these materials was evaluated by fibronectin immunostaining, assessment of cell proliferation status and the zymographic evaluation of gelatinolytic activities specific to matrix metalloproteinases secreted by cells grown in contact with studied specimens. The analyzed surfaces proved to influence fibronectin fibril assembly, cell proliferation and capacity to degrade extracellular matrix without considerably affecting cell viability and morphology. The MOCVD of TiO$_2$ proved effective in positively modifying titanium surface for medical applications. Surface properties playing a crucial role for cell behavior were the wettability and, secondarily, the roughness, HGF-1 cells preferring a moderately rough and wettable TiO$_2$ coating.

1. Introduction

Nowadays, biomaterials are used in a different way compared to just a decade ago. Despite this fact, implantable medical devices are still important, and medical technologies and tissue engineering still encompass bio inert metals, activated or/and bioactivated, taking into account that expectations of their performance also have been changed [1]. In this context, Ti and Ti alloys are the most commonly used metal materials to manufacture orthopedic and dental implants owing to its excellent physical, chemical, mechanical properties, and biocompatibility with receiving living tissues after implantation [2]. An essential surface feature of Ti is the capacity to spontaneously form a stable amorphous TiO$_2$ film under exposure to the atmosphere and/or physiological fluids. This passive, 4–6 nm thick film [3] protects Ti from corrosion, delays the release of Ti ions [4,5], and promotes a favorable osseointegration. In dentistry, the passive TiO$_2$ film formed on titanium seems to be more stable and protective than that formed on the Ti alloys, usually used in other medical applications.

The evolution of biocompatibility concept [6] is based on the idea that the response to specific individual materials could vary from one application to another, biocompatibility being dependent on the material characteristics but only in a specific application. In such a way, dental implants are unique in the body when compared to other medical implant types, in that they create two interfaces: the implant bone interface (at the radicular portion of the implant) and the gingival tissue implant interface. The maintenance of this last interface seems to be as important as bone maintenance adjacent to the implant. The area of gingival tissue at the point where the natural tooth emerges from the bone has to regenerate around the neck of an implant in order to protect the underlying connective tissue and to prevent the implant from moving and loosening eventually. Thus, the response of gingival epithelial and connective tissue cells to implant surface is very important.

The long-term success of implants most importantly requires stable interfacial interactions between biomaterial and the surrounding tissue. Therefore, different surface modification approaches have been attempted to improve the bioactive bone-binding capacity of titanium metal [6–13]. They have been applied
especially to form a bioactive TiO₂ layer on the metal surface.

A technique that has scarcely been applied to the deposition of titanium dioxide for clinical purposes is metal-organic chemical vapor deposition (MOCVD) [14–16]. This technique is widely used to prepare TiO₂ thin films at relatively low temperature. It is well adapted to the coating of complex-shaped surfaces, especially at low pressure. The microstructural parameters (crystallinity, growth mode, grain size, texture, roughness, allotropic composition, surface energy . . . ) of the deposit prepared by MOCVD sensibly depends on the numerous experimental parameters of the technique, mainly: surface state of the substrate, growth temperature, total pressure, partial pressure and molar fraction of the precursor, time of deposition. Therefore varied surface microstructures can be obtained just by playing with the parameters. The MOCVD technique thus offers the possibility to study the influence of various surface structure parameters on the biocompatibility of cells.

To our knowledge, the few studies devoted to the elucidation of in vitro interactions of the cells with TiO₂ in different physical states deal with TiO₂ particles blasted on Ti substrates [17], oxide layers prepared by MOCVD [11,14–16] or anodic oxidation [18,19], cluster-assembled nanostructured TiO₂ films [20], and sol–gel–derived titanium oxide [21,22].

In this context, the present study was undertaken to evaluate the biocompatibility of various TiO₂ thin films deposited on titanium substrates using MOCVD technique. Biocompatibility was evaluated in culture of human gingival fibroblasts, by addressing cell viability, cytomorphology, adhesion cell–biomaterial interactions mediated through fibronectin (FN) matrix, proliferation capacity and potential for degradation of the extracellular matrix (ECM), as indicators of the biological performance of studied materials.

2. Experimental

2.1. Preparation of materials

The substrates were pure Ti discs of 1 mm thickness and 10 mm diameter (99.6% purity, Good Fellow Ltd.). Deposition surfaces were mechanically polished with 180-grit SiC-paper. Before TiO₂ deposition, the discs were ultrasonically cleaned in acetone, then in boiling alcohol, and rinsed with distilled water. Control discs were cleaned in the same way and used as covered with their native oxide. TiO₂ thin films were grown using LP-MOCVD with titanium tetra-isopropoxide (TTIP) as a precursor, and nitrogen as both carrier and dilution gas. The procedure and the experimental set-up were described in a previous paper [10].

Deposition parameters were: TTIP temperature, 25 °C; N₂ flow in the precursor container, 20 sccm; dilution N₂ flow, 575 sccm; total pressure, 20 Torr; TTIP molar fraction in the gas phase, 76 × 10⁻⁴; deposition temperature, 400 °C; deposition time ranging from 50 to 360 min to vary deposit thickness.

2.2. Characterization of TiO₂ films

The three LP-MOCVD TiO₂-coated Ti samples prepared for this study are characterized by the following parameters: (1) film thickness, deduced from the weight gain of deposited TiO₂; (2) film transversal morphology, examined using a LEO-435 scanning electron microscope (SEM) operating at 15 kV; (3) roughness (Rₚ), measured on 0.5 mm² of samples surface area with an optical interferometer MetroProAtt, Zygo New View 100 equipment; (4) water contact angles measured with a Digidrop Contact Angle Meter (GBX Scientific Instruments).

Deposited phases were identified from X-ray diffraction patterns recorded with a vertical, theta-theta geometry diffractometer (Seifert XRD 3000 TT) equipped with a graphite monochromator and a Cu anticathode (λ, CuKα = 1.5406 Å). Both grazing incidence X-ray diffraction (GIXRD) patterns (grazing angle of 2°) and θ–2θ patterns were recorded.

2.3. Cells and cell culture conditions

HGF-1 cells (American Type Culture Collection) were seeded on TiO₂-coated and native-oxide covered Ti discs, placed into Permanox Lab-Tek Chamber Slides (Nunc), at an initial density of 1.2 × 10³ cells cm⁻². They were grown in a Dulbecco’s Modified Eagle Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) at 37 °C in a standard incubator with 5% CO₂ in air. Prior to cell culture, all these materials were sterilized at 180 °C for 30 min. All experiments were repeated 3 times.

2.4. Assay of cell viability

The eventual toxic effects of the analyzed coatings on cultured gingival fibroblasts were evaluated by detecting lactate dehydrogenase (LDH) activity released into the media as a marker of cell death and plasma membrane lysis. This study was performed in triplicate samples from the cell culture media maintained in contact with TiO₂-coated and native-oxide covered Ti discs for 72 h, by using a cytotoxicity detection kit (TOX-7, Sigma–Aldrich Co.) according to the manufacturer’s protocol. High OD₄₉₀ values are indicative of a reduction in the cell viability.

2.5. Immunocytochemical staining of actin and fibronectin

At the end of the incubation period (72 h and, respectively, 120 h for studies of actin and fibronectin expression), cells were fixed with 4% paraformaldehyde in PBS for 20 min and observed by phase contrast microscopy. Staining of actin was performed with fluorescein isothiocyanate (FITC)–phalloidin (20 μg·mL⁻¹; Sigma–Aldrich Co.) at 37 °C for 1 h. Before this, they were permeabilized for 15 min with 0.1% Triton X-100/2% BSA and washed in PBS. For FN visualization, fixed and permeabilized specimens were incubated with anti-FN (mouse) primary antibody (EP5 clone from Santa Cruz Biotechnology, dilution 1:50) for 2 h, and then incubated with anti-mouse secondary antibody labeled with FITC (Santa Cruz Biotechnology, dilution 1:200) for 1 h. In this case, the nuclei were counterstained with DAPI to reveal cell density.

All the Ti specimens were viewed with an inverted fluorescent microscope Olympus IX71. Excitation wavelengths were 495 nm for FITC and 358 nm for DAPI. The images were captured by means of Cell F Image acquiring system.

2.6. BrdU immunocytochemistry

After 6 h treatment of HGF-1 cells (at 24 h post-seeding) with 5-bromo-2'-deoxyuridine (BrdU) at a final concentration of 10 μM, TiO₂-coated and native-oxide covered Ti discs were maintained in 4% paraformaldehyde for 20 min. To enable antibody binding to the incorporated BrdU into the DNA of dividing cells, cells were permeabilized with 1% Triton X-100 and the DNA was denatured by sequential treatment with 1 N HCl/20 min on ice and 2 N HCl/10 min at room temperature and then 40 min at 37 °C. After washing in borate buffer (0.1 M, pH 8.5) and blocking with 1% Triton X-100/5% BSA, samples were incubated for 2 h with a monoclonal mouse anti-BrdU antibody (Sigma–Aldrich Co.) diluted 1:100. Antibody binding was detected by incubating discs for 1 h at room temperature with FITC–conjugated goat anti-mouse IgG (Santa Cruz Biotechnology) used at 1:100 dilution. After washing with PBS, samples were examined under an inverted fluorescent microscope Olympus IX71.

In this study and in the immunocytochemical assays, previously presented, negative controls were realized by substitution of the primary antibodies with PBS. At least 10 fields from three independent experiments were examined.

2.7. Western blot assay

Total cell lysates containing 30 μg protein, as determined by Bradford method [23], were separated by SDS-PAGE on 10% polyacrylamide gels. After electrophoresis, proteins from the gel were transferred to nitrocellulose membranes (Invitrogen 0.45 μm pore nitrocellulose/filter paper sandwich; XCell II™ Blot Module). Membranes were blocked with 5% nonfat milk, and probed with proliferating cell nuclear antigen (PCNA) mouse monoclonal antibody (1:200) for at least 2 h. In order to detect the antibodies which have bound, the membranes were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (1:7500) for 1 h, and developed with NBT/BCIP solution. The blots were reprobed with anti-α-actin antibody to confirm equal protein loading. Both primary and secondary antibodies were purchased from Santa Cruz Biotechnology.

2.8. Gelatin zymography

Gelatin zymography was performed by using a modification of the procedure by Cimpan et al. [24]. Briefly, aliquots (20 μg protein) of conditioned culture medium, maintained in contact for 72 h with the sub-confluent cultures, were subjected to SDS-PAGE in 7.5% (w/v) polyacrylamide gels impregnated with 1 mg mL⁻¹ gelatin under non-reducing conditions. After electrophoresis, gels were washed two times for 30 min each in a buffer containing 50 mM Tris–HCl (pH 7.6), 50 mM NaCl, 10 mM CaCl₂, 0.05% Brij 35 and 2.5% Triton X-100 and then incubated for 18 h at 37 °C with the same buffer excluding Triton X-100. After incubation, the gels were stained with 0.1% Coomassie Brilliant Blue R-250 and then destained in acetic acid: methanol: water (1:2:7). Matrix metalloproteinase (MMP) specific gelatinolytic activities were detected as clear bands against a background of blue stained, intact gelatin impregnated acrylamide gel.

2.9. Statistical analysis

Statistical analysis of surface roughness and the LDH assay was performed with GraphPrism software using one-way ANOVA with Bonferroni’s multiple comparison tests.
Table 1
A summary of TiO₂ films properties.

<table>
<thead>
<tr>
<th>Specimen code (nm)</th>
<th>Deposition time (min)</th>
<th>Thickness (nm)</th>
<th>Transversal morphology</th>
<th>Roughness Ra (µm)</th>
<th>Contact angle (°)</th>
<th>Phases&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1200</td>
<td>No</td>
<td>200</td>
<td>Compact</td>
<td>0.35 (0.02)</td>
<td>60</td>
<td>Amorphous</td>
</tr>
<tr>
<td>T-9</td>
<td>90</td>
<td>600</td>
<td>Compact</td>
<td>0.71 (0.01)</td>
<td>55</td>
<td>a-R</td>
</tr>
<tr>
<td>T-12</td>
<td>250</td>
<td>1000</td>
<td>Compact undercoat + thin columnar overlay</td>
<td>0.75 (0.04)</td>
<td>9</td>
<td>a-R</td>
</tr>
<tr>
<td>T-14</td>
<td>360</td>
<td>1000</td>
<td>Columnar</td>
<td>0.92 (0.14)</td>
<td>8</td>
<td>A-R</td>
</tr>
</tbody>
</table>

<sup>a</sup> Standard deviations are given in parentheses. Mean values are statistically significantly different P<0.01 (T9 versus T1200) and P<0.001 (T12 versus T1200; T14 versus T1200).

<sup>b</sup> A (a) = majority (minority) of anatase; R (r) = majority (minority) of rutile.

3. Results and discussion

3.1. Characterization of TiO₂ films

It is well known that host tissues specifically interact with the outermost layer of an implant [25]. Thus, when a Ti implant is inserted into the human body, the surrounding tissues come into direct contact with the protective and bioactive oxide layer, mainly represented by TiO₂. Considering that the oxide layer which forms naturally on titanium is only a few nanometers thick and presents imperfections, we used the LP-MOCVD technique to deposit TiO₂ layers on flat Ti substrates with the purpose of

![Fig. 1](image-url)
improving the interfacial properties between the gingival tissue and the implant. These samples were called T9, T12 and T14 and they are presented in Table 1 along with their preparative and structural characteristics. T1200 is a native-oxide covered Ti specimen (Fig. 1a) which was polished with grade 1200 SiC paper prior to use as a control in cell culture experiments.

SEM micrographs evidenced different film morphologies from small (T9: Fig. 1b) to large (T12: Fig. 1c, and T14: Fig. 1d) grained deposits, depending on the deposition time: the longer the time, the thicker the film and the larger the crystal size. Big crystal clusters grow here and there over the surface increase the surface roughness. Even on rough surfaces the films proved conformal, i.e. they fit in polishing grooves.

Fig. 1e and f shows the cross sectional morphology of samples T9 and T14. The 1 μm thick deposit on T14 shows a columnar structure, whereas the 0.2 μm thick deposit on T9 shows a compact structure. This is consistent with a previous study showing that under the growth conditions listed in Section 2.1, the TiO₂ deposit adopts a compact structure as long as the thickness is less than 0.4 μm or so, and that a columnar structure forms upon increasing the thickness [26]. T12 is 0.6 μm thick. Its cross sectional morphology (not shown on Fig. 1) is made of a compact undercoat covered with a thin columnar overlay. The measured water contact angles (Table 1) are consistent with the observed morphologies. The high hydrophilic character of samples T12 (contact angle = 9°) and T14 (8°) comes from the micro-porous surface generated by inter columnar spacing. Conversely, the compact structure of T9 results in a far less hydrophilic surface with a water contact angle of 55°.

From GIXRD patterns, the films grown on T9, T12 and T14 were mixtures of crystallized anatase and rutile (Fig. 2). The respective amounts of anatase and rutile could not be measured quantitatively from the θ–θ patterns, either because intensities were too weak due to the thinness of deposit (T9) or because rutile proved (2 0 0) textured (T12 and T14). This is why Table 1 shows but qualitative evaluations.

3.2. Cell viability and cell morphology

An important objective of this study was to determine if the thin films of TiO₂ produced by LP-MOCVD technique affected cellular survival. To test this parameter, the LDH activity in the culture media was quantified. LDH is a soluble cytosolic enzyme, present in all cell types, and rapidly released into the cell culture medium upon damage of the plasma membrane. Therefore, it is the most widely used marker of cell membrane integrity and serves as a general means to assess cytotoxicity. HGF-1 cells in contact with TiO₂-coated and native-oxide covered Ti surfaces proved to display low LDH release in culture medium suggesting that none of these surfaces exerted cytotoxic effects. Moreover, no significant differences between the samples were noticed (Table 2).

In culture, the morphology indicates the health status of the cells. As shown in Fig. 3, at 72 h post-seeding, HGF-1 cells were fully spread, elongated and polygonal shaped, with cytoplasmic extensions and filopodia, indicating good cell attachment to the surface. Cell body of the gingival fibroblasts cultured on MOCVD-coated surfaces (Fig. 3b–d) became increasingly more voluminous with increased roughness. This could due to a sparse adsorption of proteins from culture medium on the surface of oxide crystals. These protein aggregates represent interaction sites to cells that, in this situation, extend across large areas adopting a more elongated and large morphology. On the four surfaces, an intense labeling of actin cytoskeleton was observed in the entire cytoplasm and bright fluorescent filiform stress fibers were visible.

3.3. Adhesive cell-biomaterial interactions mediated through fibronectin matrix

Cell adhesion to a biomaterial surface is, in many cases, a special form of cell–ECM interaction, since the biomaterial surface is always covered by proteins that become adsorbed to this from either serum within cell culture media, or body fluid within the in vivo environment. Some of these proteins with which the cells interact, such as fibronectin and vitronectin, represent very known ligands for the integrin family of cell adhesion receptors [27]. Integrin-mediated cell adhesion is associated with signaling events and modulation of gene expression that ultimately determine the following cellular events: proliferation, differentiation and survival [28]. Inadequate or inappropriate cell–ECM adhesions can lead to anoikis, a special type of adhesion driven apoptosis [29].

Fibronectin is an abundant component of the ECM consisting of repeating units of amino acids, which form domains that enable the molecule to interact with many cell types through both integrin and non-integrin receptors. It is encoded by a single gene, but alternative splicing of pre-mRNA allows formation of multiple isoforms that regulate adhesion-dependent survival signaling [30] and play a critical role in cell proliferation and differentiation [28]. FN binds primarily to the α5β1 integrins, through the arginine-glycine-aspartic acid (RGD) cell-binding site forming a prototypic integrin–ligand pair [31]. This receptor–ligand pair mediates FN fibril formation and governs ECM assembly, which is essential to cell function. During assembly, FN is initially organized into fine cell-associated fibrils and, through continued accumulation of FN by synthetic and secretory cell activities, these fibrils are organized into a dense and stable extracellular fibrillar network. Therefore, fibronectin organization could be a useful tool to determine the biocompatibility of dental implants. After 5 days of culture, we found out a different distribution pattern of this protein over analyzed surfaces (Fig. 4). Thus, removal and reorganization of FN

![Fig. 2. GIXRD patterns of the TiO₂ films deposited on Ti substrate.](image-url)
from the material surfaces into ECM like structures occurred on moderately wettable surfaces T1200 (Fig. 4a) and T9 (Fig. 4b). However, fibrillar FN network was better expressed on T9 than on the non-CVD-coated T1200 sample. The latter shows less fibrillar structures and veil-like FN zones indicating a non-uniformous removal/reorganization of FN adsorbed to and secreted on the material surface. On the more hydrophilic surfaces (T12 and T14), the background was majoritary FN immunostained and very few...
fibrils assembled (Fig. 4c and d). This suggests that the cells were essentially unable to reorganize FN into an ECM-like structure on the material surface.

Our results confirm the influence of surface wettability on the interactions of the cellular substrate with proteins and further on cell behavior. It is a general trend that mammalian cells interact better with hydrophilic (wettable) surfaces than with hydrophobic (non-wettable) surfaces [32–34]. In fact, the wettability of the surface may affect cell attachment either directly, since the attachment phase as an initial process involves physical-chemical interactions between cells and surfaces including ionic forces or indirectly through alterations in the adsorption process of conditioning molecules, such as proteins. On the contrary, other authors reported that cells adhere better on moderately hydrophobic surfaces with water contact angles of around $70^\circ$ [35–37]. These conflicting results may be due to the use of materials characterized by different surface topographic parameters such as roughness and micro-texture, and especially different surface chemistry that affect the material wetting behavior. In the present study, the most suitable TiO$_2$-coated substrate, in terms of FN fibril assembly that becomes incorporated into a stable ECM is T9. This sample is characterized by a moderately wettable (water contact angle $= 55^\circ$) and rough ($R_a = 0.71$ μm) MOCVD-deposited TiO$_2$ film.

3.4. Assessment of cell proliferation status

To definitively assess the biocompatibility of the native-oxide covered and TiO$_2$-coated Ti substrates, we addressed the relationship between the properties of analyzed surfaces and cell proliferation. A comparative approach of cell cycle analysis by BrdU incorporation into chromosomal DNA of the S-phase cells (Fig. 5) and PCNA Western blotting assay (Fig. 6) provided valuable information about the proliferation status of HGF-1 cells grown in contact with the specimens.

As shown in Fig. 5, the highest density of fluorescent nuclei was observed on the MOCVD-treated T9 sample (Fig. 5b). A lower number of S-phase cells were remarked on the non-treated disc, T1200 (Fig. 5a) with relatively smooth ($R_a = 0.35$ μm) and moderately wettable (contact angle $= 60^\circ$) surface. Samples T12 (Fig. 5c) and T14 (Fig. 5d) having highly hydrophilic TiO$_2$ deposits and different surface roughness displayed much less labeled cells. Most importantly, Western blot analysis of PCNA demonstrates that the intensity of the band corresponding to PCNA protein (∼36 kDa) decreases in the following order: T9 > T1200 = T12 > T14 (Fig. 6).

Taking together, BrdU immunocytochemistry and Western blot data, we conclude that the proliferation capacity of HGF-1 cells decreases in the following order: T9 > T1200 > T12 > T14. Therefore, the cell proliferation appears to run in parallel with the quality of FN-mediated cell adhesion and is sensitive to surface wettability. Comparison of cell proliferation potential between samples with quasi-similar water contact angle values but of different roughness, namely T1200/T9 on one hand and T12/T14 on the other hand, demonstrated that surface roughness could represent a secondary determinant of cell behavior. Thus, HGF-1 cells have proved to prefer a moderately rough surface, the optimal $R_a$ value being around 0.70 (the case of T9 and T12 samples). In the literature, cellular adhesion and cell proliferation have been shown to be sensitive to surface roughness [18,38,39]. For example, in their study on the effect of surface topography and composition on cellular response.

![Fig. 5](image1.png)

**Fig. 5.** Immunofluorescent detection of proliferative cells after BrdU incorporation showing the cells that undergo DNA synthesis, as indicated by presence of green bright nuclei, on: (a) native-oxide covered Ti surface, and MOCVD-TiO$_2$ coated specimens: (b) T9, (c) T12, and (d) T14.

![Fig. 6](image2.png)

**Fig. 6.** Western blotting detection of PCNA expression in lysates of the cells grown in contact with native-oxide covered Ti surface (lane 2), and MOCVD-TiO$_2$ coated specimens: T9 (lane 3), T12 (lane 4), and T14 (lane 5). MW = high molecular weight marker (lane 1).
of human gingival fibroblasts grown on NiTi substrates, Ponsnonet et al. [39] demonstrated that there might exist a roughness threshold (between 0.08 and 1 μm) over which cell proliferation became difficult and that the lowest cell proliferation occurred on the NiTi surfaces of the highest roughness.

3.5. MMP gelatinolytic activities expressed in cell culture media

It is recognized that cell–biomaterial interface in vitro differs substantially from the periimplant environment in vivo where implant failure can be attributed to several factors such as biological, microbiological and biomechanical [40]. However, the exact molecular mechanisms of implant failure are not well known. The loosening of implants used in dentistry and orthopedic surgery have been shown to involve proteolytic cascades and MMPs [41–43]. MMPs are a group of structurally related but genetically distinct endopeptidases that have the capacity to degrade all ECM and basement membrane components [44]. These enzymes are secreted especially by osteoclasts and gingival inflammatory infiltrate and, at high concentrations, they contribute to the resorption/osteolysis and to soft tissue/dental ligament destruction [45].

Considering that interaction with inadequate substrates could induce the cells to synthesize MMPs and eventually detach [28], we evaluated the gelatinolytic activities specific for the MMPs secreted in the conditioned culture media by HGF-1 cells maintained in contact with the analyzed specimens for 72 h. As shown in Fig. 7, in all analyzed media, the gelatinolytic activities were detected at 72 kDa, 96 kDa and ~190 kDa corresponding to proMMP-2, proMMP-9 and MMP-dimers respectively. In addition, a barely visible additional gelatinolytic band, corresponding to the active MMP-2 form (68 kDa), was present in each profile. These activities disappeared in the presence of 10 mM 1,10-phenantroline (data not shown). MMP-2 represents a MMP member which is constitutively expressed by many cell types, being associated with physiological tissue remodeling at low levels of expression, while MMP-9 is mainly involved in inflammatory conditions.

The gelatinolytic activities corresponding to proMMP-9 and especially proMMP-2 were lower for the MOCVD-coated surfaces than for the native-oxide covered surface. These results suggest that in vivo integration of native-oxide covered Ti implants would be more problematic than that of TiO2 MOCVD-coated Ti implants. Taking into account that the intensities of the gelatinolytic bands corresponding to proMMP-2 and proMMP-9 decreased in the following order: T14>T12>T9, we can suppose that the thickness and crystal size of the TiO2 coatings obtained by MOCVD represent the main surface characteristic of implant material influencing cellular capacity to degrade ECM. In addition, we cannot currently rule out the possibility that other film structural parameters such as roughness, transversal morphology, allotropic composition and wettability contribute to the proteolytic events at material–tissue interface. Therefore, we intend to go deeply into this study and complete it with studies on other MMP members known to be involved in implant failure.

4. Conclusions

In this study, we have confirmed that the LP-MOCVD technique is well adapted to modifying the surface of materials used in dental implantology. The present in vitro findings indicate that this technique can provide TiO2 covered titanium implants with superior qualities in terms of FN organization into fibrillar structures, proliferation and host tissue integration capacity. According to our experimental data, the major factors that proved to govern these important biological events for cell–material interactions, are the wettability and, secondarily, the roughness of the oxide surface. The most suitable substrate for the cellular model used in this study is characterized by a moderately rough and wettable TiO2 film.

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References