Corrosion resistance and biocompatibility of a new porous surface for titanium implants


Alterations of the commercially pure titanium (cpTi) surface may be undertaken to improve its biological properties. The aim of this study was to investigate the biocompatibility of cpTi when submitted to a new, porous titanium, surface treatment (porous Ti). Five types of surface treatments, namely sintered microspheres porous titanium (porous Ti), titanium plasma spray (TPS), hydroxyapatite (HA), sandblasted and acid etched (SBAE), and resorbable blast medium, sandblasted with hydroxyapatite (RBM) were made. In the experimental methods, the corrosion potentials were measured over time, and then a linear sweep voltammetric analysis measured the polarization resistances and corrosion currents. For biocompatibility evaluation, MG63 osteoblast-like cells were used. Cell morphology, cell proliferation, total protein content, and alkaline phosphatase (ALP) activity were evaluated after 2 h, and after 2, 4 and 7 d. Porous Ti and SBAE showed a better corrosion resistance, with a weak corrosion current and a high polarization resistance, than the other surfaces. Cell attachment, cell morphology, cell proliferation, and ALP synthesis were influenced by the surface treatments, with a significant increase observed of the activity of osteoblast cells on the porous coating (porous Ti). Based on these results, it is suggested that the porous Ti surface has a significantly better biocompatibility than the other surface treatments and an excellent electrochemical performance.

Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application (1). This means that the tissue of the patient that comes into contact with the material does not experience any toxic, irritating, inflammatory, allergic, mutagenic or carcinogenetic action (2, 3). As the oral environment causes the biodegradation of metals, it can be presumed that the patient, to a certain extent, is exposed to the products of the corrosion process (3). The greater the corrosion of an alloy, the greater the proportion of its elements that will be released, and the risk of unwanted reactions in the oral tissues may also be increased. These unwanted reactions include unpleasant tastes, irritation, allergy or other reactions. Thus, corrosion influences the biocompatibility. The biocompatibility of dental alloys is primarily related to their corrosion behavior (2).

Commercially pure titanium (cpTi) is largely used as an implant material mainly because of its high in vitro and in vivo biocompatibility (4). This material allows direct bone-to-implant contact that has also been called ‘osteointegration’ (4–7). In an attempt to improve the quantity and quality of the bone–implant interface, surface treatments such as sandblasting, acid etching or plasma spraying are undertaken to induce chemical modifications associated with alterations of the surface topography (8–10). It has been shown that some methods of implant surface preparation can significantly affect the properties of the surface and subsequently the biological responses and rates of the cell attachment that occur at the surface (11–13). It is well known that surface topography has an effect on physical surface properties and plays a role in the corrosion process, which then will influence biological properties and, in particular, the response of osteoblast cells (14–16).

The aim of the present work was to evaluate if a new titanium porous coating (porous Ti) deposited on titanium implants can increase the corrosion resistance and the biocompatibility of biomedical implants. The factors which are believed to represent an improvement over four other currently used surface preparations are the corrosion-resistant behavior and the cytocompatibility. In order to confirm this hypothesis, electrochemical and cell culture tests were performed. This study is a preliminary in vitro analysis, carried out before using this new surface coating in a clinical situation.

Material and methods
Sample preparation
All specimens were supplied by Bio-Vac España Company (Valencia, Spain). Discs of cpTi were obtained from commercial bar stock that had a diameter of 10 mm and were
cut to a thickness of 1 mm. Discs were then submitted to the treatments described below.

**Porous Ti**: A porous layer was created by sintering cpTi particles and consisted of particle layers bonded to each other and to the substrate. The treatment produced pore sizes in the range of 80–250 μm, a volume of porosity of 35–40%, and a surface region with a three-dimensional interconnected porosity.

**Titanium plasma spray (TPS)**: Following the guidelines for coating with plasma spray (17) the plasma-sprayed implant was produced by an application of a titanium plasma projected to cpTi.

**Hydroxyapatite (HA)**: The HA coating was produced by an application of a HA plasma spray to cpTi. The ratio of Ca/P was 1.67 and the density was 99% of the theoretical density for solid HA.

**Sandblasted and acid etched (SBAE)**: The rough surface was prepared by sandblasting using an aluminum oxide blast (Al₂O₃, 75–150 μm) and etched using an acid solution comprising 90% HNO₃ and 10% HF, followed by alcohol cleaning (99.5%).

**Resorbable blast medium, sandblasted with hydroxyapatite (RBM)**: The surface was prepared by sandblasting, using an HA-containing blast media, and passivated with acid (30% HNO₃), followed by alcohol cleaning (99.5%). Prior to all experiments, the samples were sterilized by γ-irradiation (2500 Gy).

**Surface characterization**

The surface of the samples was examined at a magnification of ×150 before analysis using scanning electron microscopy (SEM) (Hitachi S800; Hitachi, Tokyo, Japan) at 5 kV.

**Roughness measurements**

Surface roughness was measured by profilometry using a Talsysurf 10 (Rank Taylor Hoson, Leicester, UK). Average surface roughness (Ra) measurements were taken at 10 different locations on two samples of each surface. This profilometer system consists of a needle (10 μm in diameter) which scans a 10 mm length of the surface. Results were expressed as Ra, which is the arithmetic mean of the height variation on the roughness profiles.

**Corrosion evaluation**

**Test solution**: The reference electrolyte was Fusayama–Meyer artificial saliva (18). All the ingredients were supplied by Sigma Chemical Company (St Louis, MO, USA). The composition of Fusayama–Meyer artificial saliva, which closely resembles natural saliva, is: KCl (0.4 g l⁻¹), NaCl (0.4 g l⁻¹), CaCl₂·2H₂O (0.506 g l⁻¹), NaH₂PO₄·2H₂O (0.690 g l⁻¹), Na₂S·9H₂O (0.005 g l⁻¹), and urea (1 g l⁻¹). The pH was measured with an Xe601 glass electrode (Radiometer Analytical, Villeurbanne, France) connected to a pHM 220 pH meter (Radiometer Analytical). The pH of this reference saliva, corresponding to our first test solution, was 5.3.

**Electrochemical setup**: A glass electrochemical cell (Rouaire, Courtaboeuf, France) was used. The thermostat (Bio-block, Illkirch, France) was set at 37 ± 0.1°C. Three openings in the cover of this cell allowed insertion of the following different electrodes (Radiometer Analytical): a TC 100 saturated calomel electrode (SCE); a platinum electrode; and an EDI 101 rotating disk electrode as the working electrode. The corrosion tests were conducted under open air to be as close as possible to clinical conditions. The entire three-electrode assembly was placed in a Faraday cage to limit noise disturbance and then connected to an EG & G PAR 273A type computer-controlled potentiostat (Perkin-Elmer, Evry, France). The speed of the rotating disc electrode was 500 r.p.m.

**Corrosion resistance analysis**: The corrosion potential (Ecorr) was measured for a period of 24 h. Voltammetry was then performed at a scanning rate of 1 mV s⁻¹ in a potential range of −800 to −1200 mV per SCE. The polarization curves and the Stern-Geary relationship were used to determine the corrosion current density (icorr) and the polarization resistance [Rcorr = (ΔE/ΔI)] at the end of the corrosion potential exposure. The polarization resistance values were given in kΩ cm⁻², and the corrosion current density values were given in μA cm⁻². In accordance with the international standard, ISO 10271 (19), the experiments were performed four times for each surface treatment in the test solution. The Ecorr, icorr, and Rcorr values were the mean values of the four experiments.

**Cell culture**

MG63 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). These osteoblast-like cells were used for the experiments largely because they were obtained from a human osteosarcoma and are well characterized. They display numerous osteoblastic traits that are typical of a relatively immature activity. However, the culture conditions under which MG63 cells will mineralize their matrix have not been defined (20–22).

The specimens were placed in each well of a multiwell tissue culture plate (Becton Dickinson, Franklin Lakes, NJ, USA). For all experiments, the culture medium used was Dulbecco’s modified Eagle’s medium (DMEM) (Eurobio, Les Ulis, France) supplemented with 10% fetal bovine serum (Valbiatech, Paris, France), L-glutamine (Eurobio), 50 μg ml⁻¹ streptomycin/100 IU ml⁻¹ penicillin (Roche, Meylan, France), 250 μg ml⁻¹ fungizone (Invitrogen, Cergy-Pontoise, France) and 50 μg ml⁻¹ freshly prepared ascorbic acid (Sigma Aldrich, St Quentin Falavier, France). After reaching confluence, the cells were harvested in a 0.025% trypsin/ethylene-diaminetetraacetic acid (EDTA) solution (Eurobio) and counted with an electronic counter cell (Beckman Coulter, Fullerton, UK). They were then seeded at 10⁵ cells per cm² on various substrates and cultured in the DMEM. The culture medium was removed every 2 d and replaced with fresh medium.

**Cell morphology**

The morphology of the cells was analyzed after 2 h, and after 2, 4 and 7 d of culture. Test and control cultures were fixed routinely in 0.1 M sodium cacodylate buffer containing 2% glutaraldehyde, postfixed in 0.2 M sodium cacodylate buffer containing 1% osmium tetroxide, and then dehydrated through a graded alcohol series (30%, 50%, 70%, absolute
alcohol). After the ethanol-dehydration step, cell cultures were immersed in a mixture of 100% ethanol and freon (1 : 1, v/v) and then in pure freon. The slips were then mounted on copper stubs with silver paint and coated with gold-palladium. The samples were examined with a Hitachi S800 SEM (Hitachi) at an accelerating voltage of 5 kV.

**Cell viability**

To evaluate viability, cells were cultured on samples for 2 h, and for 2, 4 and 7 d. The method applied was derived from Mossman (23). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test provided a measure of the integrity and activity of mitochondria in the cells that could be linked to the viability and number of cells in the culture. The tetrazolium salt, MTT, was converted into a dark-blue insoluble formazan product by mitochondrial succinic dehydrogenase of viable cells. A filtered MTT solution (5 mg ml\(^{-1}\)) (Sigma Aldrich) was added to each well, and cells were incubated at 37°C for 4 h. After complete removal of the culture medium, ethanol and dimethylsulfoxide (DMSO) (Amresco, Solon, OH, USA) (1 : 1, v/v) were added to each well to release the colored product into the solution. Cell viability was assessed by measuring the optical density of the colored product in the solution using a spectrometer (Microplate Reader EL 308; Bio-Teck Instrument, Winooski, VT, USA) set at 570 nm with a 96-well microplate reader (BD Biosciences, Pont de Claix, France). The blank reference was taken from wells without cells.

**Total protein content**

After 2 h, and 2, 4 and 7 d, the culture medium was removed and the samples were washed three times with phosphate-buffered saline (PBS) (Eurobio), treated with aqueous Triton (0.05%), and stored frozen at −20°C until assayed. After defrosting, the protein concentration was measured in these samples in accordance with the method described by Bradford (24), using the Bio-Rad protein assay (Bio-Rad, Hemel Hempstead, UK) with an albumin standard. The absorbance was measured spectrophotometrically at 600 nm and the total protein content (μg ml\(^{-1}\)) was determined.

**Alkaline phosphatase activity**

The treatment described above to evaluate total protein content was used to determine alkaline phosphatase (ALP) activity. After 2 h, and after 2, 4 and 7 d, the ALP activity was determined using \(p\)-nitrophenylphosphate as the substrate, in accordance with the method described by Lowry et al. (25). Supernatants used for calculating total protein content were assayed for ALP activity. The mixture comprised 0.5 M 2-amino-2-methyl-1-propanol, 5 mM \(p\)-nitrophenylphosphate and 5 mM magnesium chloride. The enzymatic reaction was stopped by adding 100 μl of 0.5 M NaOH. Enzyme activity was quantified by absorbance measurements at 410 nm using a 96-well microplate reader and calculated according to a series of \(p\)-nitrophenol standards. For each material, the ratio of enzyme activity (expressed in nanomoles of \(p\)-nitrophenol per min) to the mass of protein (expressed in μg) was determined.

**Statistical analysis**

Test mean values and standard deviations were computed for roughness, viability, total protein content, and ALP synthesis. Analysis of variance (ANOVA) and the Students’ t-test were used to assess the significance level of the differences between the experimental groups (\(n = 20\)). All statistical analyses were performed using commercial software (StatView for Windows, Version 5.0; SAS Institute, Cary, NC, USA). \(P\)-values of < 0.05 were considered statistically significant.

**Results**

**Surface characterization**

Figure 1 shows SEM images of surfaces. The porous Ti SEM observation showed a coating with a porous aspect. The titanium balls were interconnected between them on several plans. The balls had an irregular diameter, ranging between 50 and 100 μm. Zones of strong separate compactness (very interconnected balls) and zones of low compactness (variable pore dimensions) (Fig. 1A) can be distinguished. The TPS coating was characterized by many granularities (50 μm) out of titanium separated by flat zones (Fig. 1B). The HA coating had a granulous aspect, with small-size HA clusters (< 50 μm) (Fig. 1C). The SBAE preparation had an aspect characteristic of the surface treatment obtained by subtraction. It did not appear very porous, with a two-dimensional surface aspect (Fig. 1D). The RBM coating had a two-dimensional aspect characterized by a matter tangle in the plan with some specific hollows (Fig. 1E).

**Surface roughness**

Figure 2 shows the surface roughness of different specimens. The profilometer roughness measurements confirm, in a quantitative manner, the SEM observations. The porous Ti coating was significantly rougher compared to that of the other surfaces (\(P < 0.001\)). The TPS coating also had a roughness higher than that of the other surface types.

It was not possible to detect significant differences in roughness among HA, SBAE and RBM treatments (\(P > 0.005\)).

**Corrosion behavior**

We were unable to perform an analysis of corrosion resistance on the HA sample. Indeed, the HA layer was insulating and, consequently, it was impossible to raise a potential difference.

**Corrosion potential measurements of materials tested**

The corrosion potential of the different alloys was measured during a 24-h period in the Fusayama solution. The values obtained are shown in Table 1. Following an initial increase, the open circuit potential (OCP) stabilized (~40 to 20 mV per SCE) for all the surface conditions after 24 h.

**Polarization curves**

The values of the potentials of corrosion, the currents of corrosion, and the resistances of polarization are...
shown in Table 1. These values are the averages of those obtained for four samples. The current density and the polarization resistance values obtained indicate that the porous Ti coating and the SBAE treatment had the best electrochemical properties (Fig. 3). The RBM and TPS surfaces showed less corrosion resistance.

**Cytocompatibility**

**Cell morphology:** In order to visualize cellular spreading in an optimal way, two enlargements were used for each surface. Micrographs of each surface are shown in Fig. 4 after 2 h (J0) and after 7 d (J7) of culture. After 2 h of culture (J0), osteoblasts adhered to all supports by means of thin cytoplasmic digitations or filopodia. After 7 d of culture (J7), on each surface preparation, osteoblast-like MG63 were relatively confluent, presenting an elongated morphology. Cellular spreading was good on the porous Ti coating but not as extensive on the HA coating.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Porous Ti</th>
<th>TPS</th>
<th>SBAE</th>
<th>RBM</th>
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<tr>
<td>E&lt;sub&gt;corrosion&lt;/sub&gt; (mV/SCE)</td>
<td>9.00 ± 2</td>
<td>22.00 ± 2</td>
<td>2.00 ± 3</td>
<td>−28.00 ± 4</td>
</tr>
<tr>
<td>Rp (kΩ cm&lt;sup&gt;−2&lt;/sup&gt;)</td>
<td>603.00 ± 3</td>
<td>36.00 ± 4</td>
<td>616.00 ± 7</td>
<td>474.00 ± 4</td>
</tr>
<tr>
<td>i&lt;sub&gt;corrosion&lt;/sub&gt; (µA cm&lt;sup&gt;−2&lt;/sup&gt;)</td>
<td>0.37 ± 0.03</td>
<td>1.12 ± 0.12</td>
<td>0.32 ± 0.04</td>
<td>0.42 ± 0.08</td>
</tr>
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</table>

SCE, saturated calomel electrode; porous Ti, titanium porous coating; RBM, resorbable blast medium, sandblasted with hydroxyapatite; SBAE, sandblasted and acid etched; TPS, titanium plasma spray.
Cell viability: The density of MG63 cells was measured over different time-periods (2 h, and 2, 4 and 7 d) by using the MTT assay, as shown in Fig. 5.

After 2 h of adhesion (J0), there were no significant differences in cell density between the porous Ti coating and the TPS surface ($P > 0.05$). The adhesion of MG63 cells appeared to be significantly higher on the porous Ti coating than on the HA, SBAE, and RBM surfaces ($P < 0.05$). There was no significant difference in adhesion among all the other surface treatments ($P > 0.05$).

After 2 d of culture (J2), there was no significant difference in cell viability among various surface treatments ($P > 0.05$).

After 4 d of culture (J4), a noticeably higher difference of cell viability on the porous Ti coating was observed compared with TPS, HA, and RBM surfaces ($P < 0.001$) and a significant difference with the SBAE surface ($P < 0.05$). There was no significant difference in cell viability ($P > 0.05$) between SBAE and RBM surfaces. Cells were less viable on TPS and HA surfaces than on the porous Ti, SBAE, and RBM surfaces ($P < 0.001$).

After 7 d of culture (J7), the viability of MG63 cells was much higher on the porous Ti coating than on the other surfaces ($P < 0.001$). There was no significant difference in cell viability among TPS, SBAE and RBM surfaces ($P > 0.05$). The viability was very weak on the HA coating compared with the other surface treatments. A highly significant difference was observed between the HA coating and other surface preparations ($P < 0.001$).

In summary, various surface treatments influence cellular viability, with a stronger viability from 4 d of culture for the new coating proposed (porous Ti coating) and a weaker viability for the HA coating.

**Total protein content**

Results concerning the amount of protein are shown in Fig. 6.

After 2 h of adhesion (J0), cellular MG63 activity was much higher on the porous Ti coating than on the HA coating, as indicated by the MTT assay. This trend continued through the 4- and 7-d time points, with the porous Ti coating consistently showing higher protein content than the other surface treatments.

**Fig. 3.** Potentiodynamic polarization curves in Fusayama saliva. HA, hydroxyapatite; porous Ti, titanium porous coating; RBM, resorbable blast medium, sandblasted with hydroxyapatite; SBAE, sandblasted and acid etched; TPS, titanium plasma spray.

**Fig. 4.** Scanning electron microscopy (SEM) images of cells cultured on surface-modified titanium (Ti) specimens after 2 h and 7 d of culture. (A) Titanium porous coating (porous Ti); (B) titanium plasma spray (TPS); (C) hydroxyapatite (HA); (D) sandblasted and acid etched (SBAE); and (E) resorbable blast medium, sandblasted with hydroxyapatite (RBM).
coating \( (P < 0.01) \), and significantly higher than on the TPS and SBAE surfaces \( (P < 0.05) \). There was no significant difference among all the other surface treatments \( (P > 0.05) \).

After 2 d of culture (J2), the cellular MG63 activity was significantly lower for the HA coating than for the porous Ti coating \( (P < 0.05) \). There was no significant difference among the other surfaces \( (P > 0.05) \).

After 4 d of culture (J4), a strongly significant MG63 activity was detected on the porous Ti coating compared with the HA coating \( (P < 0.001) \), which was higher than on TPS, SBAE, and RBM surfaces \( (P < 0.01) \). There was no significant difference regarding cellular activity compared with the other surfaces \( (P > 0.05) \).

After 7 d of culture (J7), the MG63 activity was much higher on the porous Ti coating than on the HA and SBAE surface preparations \( (P < 0.001) \), significantly higher \( (P < 0.01) \) than on the TPS surface and not significantly different from the RBM surface \( (P > 0.05) \).

The activity of MG63 cells was much lower for the HA coating than for all other surface treatments \( (P < 0.001) \).

Between J0 and J7, the activity of MG63 cells showed a marked increase on all surfaces \( (P < 0.001) \).

To summarize, the synthesis of proteins was influenced by the type of surface treatment, with a higher MG63 cell activity on porous Ti and RBM surfaces.

**ALP activity**

Fig. 7 shows the ALP activity of the osteoblasts after 2 h, and after 2, 4 and 7 d of culture.

After 2 h of adhesion (J0), there was no significant difference among porous Ti, TPS, SBAE, and RBM surface preparations \( (P > 0.05) \). Osteoblasts cultured on the HA coating showed an ALP activity that was significantly stronger than when cultured on the RBM surface \( (P < 0.001) \), significantly stronger than when cultured on the porous Ti and TPS surfaces \( (P < 0.01) \), and not significantly different from the SBAE surface \( (P > 0.05) \).

After 2 d of culture (J2), there was no difference in the ALP activity of osteoblasts cultured on each surface coating \( (P > 0.05) \).

After 4 d of culture (J4), the ALP activity was noticeably higher for osteoblasts cultured on the porous Ti coating than on any of the other surfaces \( (P < 0.001) \). There was no significant difference in the ALP activity of osteoblasts cultured on any of the other surfaces \( (P > 0.05) \).

After 7 d of culture (J7), the ALP activity was significantly higher in osteoblasts cultured on the porous Ti coating than when cultured on any of the other surfaces \( (P < 0.001) \). The ALP activity was much lower in osteoblasts cultured on the HA coating than all the other preparations \( (P < 0.001) \). There was no significant difference of the ALP activity between all other surface treatments \( (P > 0.05) \).

Between J0 and J7, the ALP activity was significantly higher in osteoblasts cultured on the porous Ti coating \( (P < 0.001) \), significantly lower in osteoblasts cultured on SBAE and RBM surfaces \( (P < 0.05) \), not significantly different in osteoblasts cultured on the TPS surface \( (P > 0.05) \) and significantly lower in osteoblasts cultured on the HA coating \( (P < 0.001) \).

To summarize, from day 4 of culture, various surface treatments influenced the ALP activity, with a better activity for the porous Ti coating and a weak activity for the HA coating.
Discussion

The aim of the present work was to evaluate the corrosion behavior and the biocompatibility of a new porous titanium coating (porous Ti).

In this study, we found three significant roughness ranges: 11.9 ± 4.1 μm for porous Ti; 4.4 ± 0.37 for TPS; and 1.17 ± 0.31 to 1.97 ± 0.14 for HA, SBAE, and RBM. For SBAE surfaces, Szmukler et al. (7) observed a roughness value of 0.90–1.48 μm and Xavier et al. (26) reported a roughness value of ≈1.79 μm. There was no significant difference with the results that were obtained for the same surface (SBAE). Similarly, for TPS treatment, the value of 5.65 μm reported by Yang & Ong (27), was not significantly different from that reported in the present study. However for HA surfaces, Yang & Ong (27) reported a roughness value of 4.96 μm. For the same surface we reported a roughness value of 1.17 μm. It was established that differences in roughness between different surfaces come from the size of particles (27, 28).

At the time of the study of electrochemistry, we were unable to carry out a study of corrosion resistance with a titanium implant covered with HA because this coating is non-conducting. As suggested by Timothy et al. (29), this insulation indicates that the coating is dense with an absence of porosity.

For all the surfaces, the open circuit potential shows an initial increase, which is followed by stabilization. A protective passive film formed rapidly on the metal surfaces in the artificial saliva and remained stable during the entire immersion period. It was reported by Cat et al. and Koike et al. (30, 31), that a protective passive film is caused by titanium oxidation. Polarization curves were measured to classify the values of resistances of polarization and the corrosion currents. The corrosion current values are low and homogeneous (between 0.37 μA cm⁻² and 1.12 μA cm⁻²). No difference in corrosion resistance is measurable among RBM, porous Ti, and SBAE surfaces. The TPS surface shows the highest corrosion current (1.12 ± 0.12 μA cm⁻²) and consequently the poorest corrosion resistance. In a similar medium, Cat et al. (30) obtained a corrosion current of 0.75 μA cm⁻² for a sand-blasted surface, a value comparable to that which we found (0.32 ± 0.04 μA cm⁻²) for an SBAE surface. Reclariu et al. (32) found a current of corrosion of 0.7 μA cm⁻² for a TPS surface, whereas we found a current of 1.12 ± 0.12 μA cm⁻². The small difference between these two values could come from the higher chloride content in saliva that we used. As already stated, Cabrini et al. (33) showed that surface treatments such as sand-blasting, TPS or HA coating result in an increase in corrosion compared with unroughened surfaces. The increase in the values of corrosion could come either from the increase in the surface area exposed to the aggressive environment (28, 33) or from the porosity of the coating (34, 35). However, the results obtained do not allow differences in corrosion resistance to be attributed to the increase in surface or porosity. Indeed, the corrosion current is higher for the TPS surface than for the porous Ti surface, which has a larger area of contact and a high porosity. These differences could be a result of the difference in accessibility to oxygen, which is the main factor causing corrosion.

However, in any case, all surfaces showed excellent corrosion resistance, with the best resistance observed for the SBAE and porous Ti surfaces.

The results of SEM observations showed that cells adopted three different morphologies during adhesion. First, cells are spherical and adhere themselves to the substrate by way of thin filopodes. Second, when the cells are well fixed, they remain round and emit flattened cytoplasmic prolongations. Third, when the cells spread, they take on a much more flattened form. There is a similar morphology of osteoblasts on the surfaces. These results are in agreement with those of Wirth et al. (36) who performed a similar study on nickel-titanium implants of various roughnesses. To quantify the number of live cells on each surface treatment, we carried out an evaluation of the cellular viability by using an MTT test. This test makes it possible to quantify the mitochondrial activity and, as a consequence, to measure survival or the cellular proliferation (24). This test showed that, regardless of the surface preparation studied, there is proliferation of osteoblastic MG63 cells. Our results showed a higher proliferation of osteoblastic MG63 cells on the porous titanium coating than on other surface treatments after 7 d of culture. As Lincks et al. (21) showed, it is possible that the high proliferation of MG63 cells is influenced by the porosity or the roughness of surfaces. However, in this study the results obtained did not show a difference in proliferation between TPS and SBAE surfaces, whose levels of roughness are significantly different. These results agree with those of Xavier et al. (26), who showed that there is no difference in the proliferation of cells of 14-d cultures between a sand-blasted and an acid-etched surface and a blasting coating by titanium. After 7 d of culture, the HA coating showed the poorest cellular proliferation. This agrees with the results of Deligianni et al. (37), who showed that the proliferation of osteoblastic cells is higher on HA coatings of high roughness than on smoother HA coatings.

Various surface preparations influence cellular proliferation, with a higher proliferation as from 4 d of culture for the new coating proposed (porous Ti) and a lower proliferation for the HA coating.

Protein synthesis by the osteoblasts seems to be influenced by the surface treatment. Indeed, the cellular activity is significantly higher on porous Ti and RBM surfaces than on TPS, HA, and SBAE surfaces. Martin et al. (38) and Castellani et al. (3) showed, on titanium implants, that roughness influences the cellular activity, with a higher activity observed on rough surfaces. These observations are not in agreement with those of Montanaro et al. (15), who found that roughness did not have an influence on the cellular activity of HA coatings of several different levels of roughnesses. Our results suggest that roughness cannot be the only factor influencing the synthesis of proteins. Indeed, HA, SBAE, and RBM surfaces have a similar roughness, but a significantly different cellular activity. It is possible that the composition of the material, as well as the parameters of
the mode of treatment of the surface, also have an influence on the protein synthesis of MG63 cells.

The synthesis of proteins is influenced by the type of preparations, with a higher activity of the osteoblastic cells on the porous Ti coating and RBM preparation than other surface treatments.

Alkaline phosphatase is an early marker of osteoblast differentiation and relates to the matrix mineralized production. After 7 d of culture, the activity of ALP is significantly higher on the rougher surface (porous Ti). As Lincks et al. (21) suggested, the roughness of the surface would have an influence on the activity of ALP. However, the ALP activity is not significantly different between the TPS coating and the SBAE and RBM surfaces, which are smoother. The roughness of the surface is not the only parameter influencing the cellular activity. The synthesis of ALP is also influenced by the chemical composition of the surface (21, 38). Our results showed that the activity of ALP increased between 2 h and 7 d of culture on the porous Ti coating and decreased on the HA coating. These observations agree with those of Biggerelle M, Noël B, Iost A, Hardouin P. Effect of grooved titanium substratum on human osteoblastic cell growth. J Biomed Mater Res 2002; 60: 529–540.

Various surface preparations influenced the ALP activity, with an increased synthesis as from day 4 of culture for the new coating proposed (porous Ti).

In conclusion, evaluation of the corrosion behavior made it possible to show that all surface treatments tested have an excellent corrosion resistance. The cell biocompatibility results proved that all surface treatments, independently of their surface roughness and of the chemical composition of their surface preparation, are biocompatible, as they allowed the attachment, the proliferation, and the differentiation of cells. However, in vitro results showed that the new Ti coating may provide surface property advantages for a favorable bone response. The study results suggest that this new porous coating may optimize the integration and enhance the clinical function of the implant. However, in vitro testing is only a preliminary screening for biocompatibility. Therefore, the clinical relevance of the enhanced porous layer must be evaluated in controlled clinical studies.

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References


