Development and characterization of advanced thin coatings on nanostructured titanium for biomedical applications

by

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A dissertation submitted by in partial fulfillment of the requirements for the
degree of Doctor of Philosophy in

Materials Science and Engineering

Universidad Carlos III de Madrid

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Expected: January-February 2020

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Dedicatoria

Como decía una antigua amiga, somos el resultado de todas las experiencias que vivimos. A mí siempre me gusta añadir que gran parte de esos momentos los forman las personas que nos acompañan. Sin esas personas no seríamos lo que somos ni tendríamos momentos que compartir. Esta tesis doctoral va dedicada a todas aquellas personas que de alguna manera u otra, siempre para bien, han contribuido.

La primera dedicatoria, y la más especial, es sin duda para esas dos personas que me lo han dado todo. Me han dado todo su amor y sacrificio para que hoy pueda estar aquí: mi madre y mi padre, Serafín y María Victoria. Jamás podré recompensaros por todo lo que habéis hecho por mí. Os quiero.

“Yo soy Hugo Mora Sánchez, Sánchez Mora soy”

A las familias. A los Mora por sus cosas y a los Sánchez por sus tantas otras cosas más. Gracias por estar siempre ahí.

“Yo no soy de Madrid, yo soy de ValleKas”

A los del barrio. A los que nunca fallan: Pozo, Leila, Pocholo, Ana, Goncho y Fran. Sois el punto de apoyo cuando las cosas se tuercen. Brindemos por los que están y por los que estuvieron.

“Yo soy un oso panda, y vengo de buenos aires...”

La verdad es que no me podría imaginar una vida sin el pueblo, sin sus fechas señaladas, y por supuesto, sin sus gentes. Para toda la peña de los delincuentes: David, Angel, Amanda, Paloma, Periko, Joan, Toni, Sergi, Brian, Irene, Celia, Cristina, Jenni, Patri, Alicia, Silvia. Acordaros que a la una en Eugenio.

“Aprende o muere”

Para los que fueron compañeros de carrera y ahora son amigos: Morejudo y Julian. Y para los que sufrimos en el master: Sergio, Marina y Ruben. De Madrid al cielo, que para eso somos aeronáuticos.

“Yo ’right mate?”

Bristol fue un largo paréntesis y una de las mejores experiencias que he vivido. Me alegro de haber podido conocer a tan buena gente. Los Spanishs King de Bristol: Roque, Rober, Ana, Miriam, Pedro, Alejandra, Aida, Laura, Quique, Juancar, Marina, Los peruanos, Osvaldo, Juliette, Ale, Marta, Cristina y Andrea. Yo ya cumplí con todos los retos de LittleBoy.

“¿Cuándo acabas tú la tesis?”

Esta larga y dura etapa, con todos sus buenos y sus malos momentos, termina por fin. Han sido cuatro años de risas en los cafés y en las comidas. De viajes. De discusiones interminables de política y que se yo. De ayudarnos los unos a los otros en los momentos difíciles. En definitiva dudo que a cualquier lado al que vaya tenga la suerte de encontrar gente como vosotros: Miguel, Ángel, Jaime, Marcos, Pabliño, Ceci, Cemre, Bárbara, Nerea, Alfonso, Verónica, Alex, Xaquín, Anna, Akshya, Gilbert. Siempre nos quedará Lavapiés.

ACKNOWLEDGEMENTS

In first place I would like to express my gratitude to my Thesis advisors Ilchat Sabirov and Jon Molina Aldareguia from the IMDEA Materials Institute and Endzhe Matykina from the Complutense University of Madrid for their priceless support and guidance during all the stages of the Thesis. In second place I have to thank the IMDEA Materials Insitute with a special mention to Miguel A. Monclus and to Miguel Castillo for their countless hours of help during the microstructural and mechanical characterizations carried out in the Thesis. I am truly grateful to the corrosion protection research group from Complutense University of Madrid, including Endzhe Matykina and Raul Arrabal, for their support and for facilitating me the use of their equipment and facilities to carry out the fabrication and characterization of the PEO coatings.

I would like also to thank Ludek Dluhos from Timplant Company in the Czech Republic and Professors Václav Babuška and Daniel Hrušák from Charles University in Prague, Czech Republic, for the design and supply of the chemically etched samples and the subsequent cell adhesion and proliferation assessments that led to a joint article. I thank Prof. Ruslan Z. Valiev from Ufa State Aviation Technical University for the supply of the nanostructured Titanium within the framework of the ViNaT European Project (ref. nº. 295322).

Finally I would like to thank Daniel Cermeño Pampliega from IdiPaz for performing the cellular assessment on the PEO coatings.

PUBLISHED AND SUBMITTED CONTENT

During the course of the Thesis, a review on the application of Ultra-fine grained commercially pure Titanium for biomedical applications was published. This publication was included in the present Thesis as part of the state of the art of processing of commercially pure Titanium through severe plastic deformation procedures, Section 2.1:

- H. Mora-Sanchez, I. Sabirov, M. A. Monclus, E. Matykina & J. M. Molina-Aldareguia (2016) Ultra-fine grained pure Titanium for biomedical applications, *Materials Technology*, 31:13, 756-771, DOI: 10.1080/10667857.2016.1238131

From the experimental works performed on the chemically etched nanostructured Titanium and the subsequent osteoblast adhesion and proliferation assessment, which comprises Sections 3.1.2 and 3.4.1 and the results presented in Chapter 4, an article was prepared which is pending for its submission to a relevant journal:

- H. Mora-Sanchez, L. Dluhos, V. Babuska, D. Hrusak, I. Sabirov, M. A. Monclus, R. Z. Valiev & J. M. Molina-Aldareguia. In vitro evaluation of osteoblast attachment and proliferation on UFG Ti surface designed via chemical etching.

Finally, the outcomes of the mechanical characterization of the PEO coatings have been presented at various international conferences:

- H. Mora-Sanchez, I. Sabirov, M. A. Monclus, E. Matykina, R. Arrabal, J.M. Molina-Aldareguia. Study of microstructure and mechanical behaviour of bioactive coatings on the surface of ultrafine grained pure titanium. *Nanobruecken 2019 Nanomechanical Testing Conference*. February 19-21, 2019, Berlin, Germany.
- H. Mora-Sanchez, I. Sabirov, M.A. Monclus, E. Matykina, R. Arrabal, J.M. Molina-Aldareguia. Microstructure and mechanical characterization of thin bioactive PEO coatings fabricated on UFG CP Ti. *Junior EUROMAT 2018*, July 9-12, 2018, Budapest, Hungary.
- H. Mora-Sanchez, I. Sabirov, M. A. Monclus, E. Matykina, R. Arrabal, J.M. Molina-Aldareguia. Performance of thin bioactive PEO coatings deposited on UFG CP Ti. *International Conference Imaginenano2018*, March 13-15, 2018, Bilbao, Spain.

To all those great scientists and colleagues

“There is plenty of room at the bottom”

Richard Feynman

To all those brave PhD students that are close to the end of the Thesis

“Shame on all the cowards who threw in the towel”

Nerviozzo, el Duo Kie

To all those brave PhD students that have just started the Thesis

“Run, you fools!”

Gandalf the Grey, J. R. R. Tolkien

Resumen

El titanio comercialmente puro nanoestructurado, o de grano fino (UFG CP Ti, por sus siglas en inglés), ha surgido como uno de los mejores candidatos para su aplicación en la fabricación de implantes debido a su biocompatibilidad, superior a la del resto de materiales metálicos utilizados en el campo de la biomedicina, y por las mejoras en las propiedades mecánicas que confiere microestructura de grano fino que se obtiene mediante procesos de deformación plástica severa (SPD). Sin embargo, el carácter bioinerte del Titanio puro hace necesaria la búsqueda de métodos para mejorar la osteoconductividad de su superficie para asegurar una rápida y exitosa integración con el hueso humano tras la implantación. Ha sido demostrado que la adhesión y proliferación celular y las capacidades osteoconductivas del Titanio puro de grano grueso y grano fino, se pueden mejorar siguiendo estrategias de modificación superficial que incluyen la utilización de métodos físicos y/o químicos para aumentar la rugosidad de la superficie y la fabricación de recubrimientos bioactivos. En particular, la técnica oxidación electrolítica asistida por plasma (PEO) resulta ser erige como una de los métodos más prometedores para la fabricación de recubrimientos finos de TiO_2 con altos contenidos en Ca y P. Este tipo de recubrimientos proporcionan a la superficie del titanio una combinación óptima de topografía y composición química para aplicaciones que requieren propiedades osteoconductivas. Sin embargo, existe un cierto vacío de información acerca del tratamiento mediante PEO del Titanio nanoestructurado en comparación con el amplio número de publicaciones científicas sobre recubrimientos de tipo PEO sobre Titanio de grano grueso. La presente tesis doctoral se centra en la modificación superficial del Titanio de grano grueso para mejorar su osteoconductividad. El efecto sobre la adhesión y proliferación celular de la topografía superficial producida mediante diversos ataques químicos del Titanio nanoestructurado fue estudiado durante esta tesis. Se observó que la proliferación celular dependía de las características de la topografía superficial. La parte principal de la Tesis consistió en la fabricación de recubrimientos de tipo PEO en Titanio comercialmente puro tanto de grano grueso como nanoestructurado usando tres tipos distintos de electrolito, uno de ellos con el objetivo de producir recubrimientos ricos en Ca y P. Este estudio perseguía dos objetivos: en primer lugar, comparar la resistencia a la corrosión, la interacción celular y las propiedades mecánicas conseguidas con los tres electrolitos; en segundo lugar, comparar la aplicabilidad del tratamiento PEO y los recubrimientos obtenidos en Titanio de grano grueso y nanoestructurado. Se observó que la microestructura de grano fino afectaba al ritmo de crecimiento de los recubrimientos, la generación de descargas de cada electrolito y la microestructura de las capas en contacto con el sustrato. Este último efecto jugó, al mismo tiempo, un papel fundamental en el comportamiento ante la corrosión de los recubrimientos. El comportamiento mecánico de los recubrimientos dependió enormemente de la microestructura de las capas externas de los recubrimientos, por lo que solo se encontraron diferencias en cuanto al espesor. Las células cultivadas sobre el recubrimiento rico en Ca y P fabricado a 120 s sobre el Ti nanoestructurado demostró la mejor proliferación y actividad metabólica, indicando el potencial de este recubrimiento para presentar propiedades osteoinductivas y osteoconductivas. Con el fin de mejorar las propiedades mecánicas de los recubrimientos, se propone un método alternativo a los métodos tradicionales de endurecimiento de recubrimientos de tipo PEO.

Abstract

UltraFine-Grained Commercially Pure Titanium (UFG CP Ti) has arisen as one of the best prospects for the fabrication of implantable appliances due to the superior biocompatibility of CP Ti with respect to the other metallic systems used in the biomedical field, and to the enhanced mechanical properties provided by the refined microstructure obtained through Severe Plastic Deformation (SPD) processes. However, the bioinert character of CP Ti makes necessary to improve the osteoconductivity of the surface in order to ensure a rapid and successful osseointegration. Surface modification approaches including physical and/or chemical roughening and the fabrication of bioactive coatings have been shown to improve the cell adhesion and proliferation and osteoconductivity of CG and UFG CP Ti. In particular, the Plasma Electrolytic Oxidation (PEO) technique is one of the most promising approaches to fabricate thin TiO_2 -based Ca- and P-containing coatings. These type of coatings provide a suitable combination of surface topography and chemistry for applications that require osteoconductive properties. However, there is a relative lack of knowledge regarding the PEO treatment of UFG CP Ti in comparison with the significant body of research on the PEO treatment of CG CP Ti.

The present Thesis is focused on the surface modification of UFG CP Ti to improve its osteoconductivity. The effect of the surface topography of chemically etched UFG CP Ti on the cell adhesion and proliferation was assessed. It was observed that the cell proliferation was dependent on the characteristics of the surface topography. The main body of the Thesis consisted on the fabrication of PEO coatings on both CG and UFG CP Ti using three different electrolytes, one of them aimed to produce Ca- and P-containing coatings. The main objective of this study was twofold: First, to compare the resulting corrosion resistance, cell-surface interaction, and mechanical properties of the three PEO treatments; and second, to compare the applicability of the PEO treatment and the coatings obtained on both CG and UFG CP Ti. The UFG microstructure was found to influence the coatings growth rate, the discharge generation of each treatment and the microstructure of the layers in intimate contact with the substrate. At the same time, the latter was found to cause significant differences in the corrosion behaviour between the CG CP Ti-coated and the UFG CP Ti-coated samples. The mechanical performance of the coatings was strongly dependent on the microstructure of the outer layers of the coatings. The Ca- and P-containing coating fabricated for 120 s on the UFG CP Ti presented the highest cellular proliferation rate and metabolic activity showing potential for osteoinductive and osteoconductive capabilities. In order to improve the mechanical performance of the coatings an alternative approach to the common methods for hardening of PEO coatings was proposed.

Table of contents

1	Introduction.....	1
1.1	Biocompatibility of metallic biomaterials.....	1
1.2	Ultrafine-grained commercially pure Titanium.....	2
1.3	Surface treatments for osteoconductivity enhancement of Ti-based biomaterials.....	3
1.4	Outline and objectives of the Thesis.....	4
2	State of the art	6
2.1	Severe plastic deformation processing of CP Ti.....	6
2.1.1	Processing description.....	6
2.1.2	Microstructure	7
2.1.3	Advanced properties	8
2.2	Fabrication of coatings via Plasma Electrolytic Oxidation	15
2.2.1	Characteristic of PEO treatment	15
2.2.2	PEO treatment of CG and UFG CP Ti for biomedical applications.....	19
3	Materials and experimental procedures	21
3.1	Materials and processing.....	21
3.1.1	Processing of CG CP Ti and UFG CP Ti.....	21
3.1.2	Chemical etching	22
3.1.3	PEO synthesis	23
3.2	Microstructural characterization.....	25
3.2.1	Scanning electron microscopy.....	25
3.2.2	Atomic force microscopy	25
3.2.3	Focused ion beam	26
3.2.4	Energy dispersive X-ray spectroscopy	27
3.2.5	X-Ray diffraction.....	27
3.2.6	Raman spectroscopy	28
3.2.7	Transmission electron microscopy.....	29
3.3	Mechanical characterization.....	31

3.3.1	Hardness and Young's modulus measurement: Nanoindentation	31
3.3.2	Tribology: Nanoscratching of PEO coatings	33
3.4	Functional properties	34
3.4.1	Electrochemical behaviour: corrosion resistance.....	34
3.4.2	Cellular assessment	36
4	Surface modification of UFG CP Ti via chemical etching	39
4.1	Surface characterization.....	39
4.2	Cell proliferation assessment	43
4.3	Discussion	45
4.3.1	Surface morphology modification	45
4.3.2	Effect of water addition.....	45
4.3.3	Increment of treatment time	45
4.3.4	Cell proliferation results.....	46
4.4	Conclusions	47
5	Microstructural characterization of the PEO coatings	48
5.1	Plasma electrolytic oxidation process	48
5.2	Surface and cross-section morphology of the coatings.....	50
5.2.1	Coatings fabricated in the C electrolyte	51
5.2.2	Coatings fabricated in the S1 electrolyte.....	59
5.2.3	Coatings fabricated in the S2 electrolyte.....	61
5.3	Compositional analysis of the coatings via EDX	63
5.4	Phase composition of the coatings via XRD and Raman analyses	68
5.5	Transmission electron microscopy of the PEO coatings	72
5.5.1	Inner layer	72
5.5.2	Outer compact layer (OCL).....	74
5.6	Discussion	81
5.6.1	Coatings formation.....	81
5.6.2	Effect of grain refinement of the substrate in the fabrication of PEO coatings ...	83

5.7	Conclusions	85
6	Mechanical characterization of PEO coatings	86
6.1	Nanoindentation on the surface	86
6.2	Nanoindentation on the cross-section of the coatings.....	91
6.3	Nanoscratching.....	96
6.3.1	Progressive loading scratch tests	96
6.3.2	Constant load reciprocating scratching	101
6.4	Discussion	103
6.4.1	Mechanical properties of nc-TiO ₂ and am-TiO ₂	103
6.4.2	Mechanical performance during scratch testing	103
6.4.3	Approaches to improve the mechanical performance of the PEO coatings	104
6.5	Conclusions	106
7	Corrosion characterization of PEO coatings	108
7.1	Open circuit potential evolution	108
7.2	DC polarization response	109
7.3	EIS response.....	110
7.3.1	Substrates response	111
7.3.2	PEO coatings response	114
7.4	Discussion	121
7.5	Conclusions	122
8	Cell assessment on PEO coatings.....	123
8.1	Qualitative examination of hMSCs morphology	123
8.2	Proliferation of hMSCs	123
8.3	Metabolic activity of hMSCs	123
8.4	hMSCs morphology and cytoskeleton distributions by SEM	125
8.5	Conclusions	126
9	General conclusions on PEO coatings and future work.....	127
9.1	Conclusions on the PEO treatment of CG and UFG CP Ti.....	127

9.2	Future work	128
10	References.....	129

List of abbreviations and symbols

List of abbreviations

UFG CP Ti	UltraFine-Grained	Commercially Pure Ti	ACP	Amorphous Calcium Phosphate
SPD	Severe Plastic Deformation		α-TCP	α -TriCalcium Phosphate
CG CP Ti	Coarse-Grained	Commercially Pure Ti	CSD	Coherently Scattering Domains
ALD	Atomic Layer Deposition		SEM	Scanning Electron Microscopy
PEO	Plasma Electrolytic Oxidation		EBSD	Electron BackScattered Diffraction
DC	Direct Current		EDS/EDX	Energy Dispersive X-Ray Spectroscopy
AC	Alternate Current		FIB	Focused Ion Beam
EIS	Electrochemical	Impedance Spectroscopy	FEGSEM	Field Emission Gun
ECAP	Equal Channel Angular Pressing		AFM	Atomic Force Microscope
ECAP-C	-Conform scheme		SPM	Scanning Probe Microscopy
HAGB	High Angle Grain Boundary		XRD	X-Ray Diffraction
LAGB	Low Angle Grain Boundary		FWHM	Full Width at Half Maximum
LCF	Low Cycle Fatigue		TEM	Transmission Electron Microscopy
hMSCs	human Mesenchymal Stem Cells		STEM	Scanning TEM
hFOB	human Fetal Osteoblast cells		HAADF	High Angle Annular Dark Field
SMAT	Surface Mechanical Attrition Treatment		CSR	Constant Strain Rate
CVD	Chemical Vapour Deposition		OCP	Open Circuit Potential
HA	Hydroxyapatite		ECM	Extra Cellular Matrix
EDTA	Ethylenediamine Tetraacetic Acid		nc-TiO₂	nanocrystalline TiO ₂
			am-TiO₂	amorphous TiO ₂
			OCL	Outer Compact Layer

List of symbols

σ_Y	Yield Strength	ν	Poisson's Ratio
σ_{UTS}	Ultimate Tensile Strength	R_e	Elastic Region Radius
ϵ_f	Elongation to failure	R_p	Elastic Region Radius
E	Young's Modulus	W	Wear Rate
H	Hardness	V_w	Wear Volume
V_B	Breakdown Voltage	L	Distance
t_{on}	Pulse-on time	E_{corr}	Corrosion Potential
E_p	Pulse Energy	j	Current Density
V_p	Pulse Voltage	j_{corr}	Corrosion Current Density
I_p	Pulse Current	j_{pass}	Passivation Current Density
f	Frequency	V	Voltage
S_a	Average Surface Roughness	V_{rms}	Root Mean Square Voltage
S_{sq}	Root Mean Square Surface Roughness	Φ_{eq}	Equivalent Sphere Diameter
S_{ku}	Surface Kurtosis	χ_A	Anatase Fraction
S_{sk}	Surface Skewness	χ_C	Crystalline Fraction
P	Load		
h	Depth		
S_t	Experimental Stiffness		
h_c	Contact Depth		
E_r	Reduced Elastic Modulus		
A_c	Contact Area		
h_r	Residual Depth		
h_e	Elastic Depth		

List of Figures

Figure 2-1. Schematic representation of principles of: a) conventional ECAP; and, b) ECAP-Conform.	7
Figure 2-2. Transversal bright (left) and dark (right) field images of CP Ti after ECAP-C processing for 6 passes and drawing.	7
Figure 2-3. a) Stress-strain curves for CP Ti before and after SPD processing tested in transversal and longitudinal directions; b) Pole figures for basal (0001) and prismatic planes (10-10) for the transversal section of the SPD processed CP Ti [61].	8
Figure 2-4. a) The S-N curves from fatigue testing of CP Ti (grade 4): (1) in the coarse-grained condition, (2) after 6 passes of ECAP-C and drawing with $\varepsilon=75\%$, and (3) after 10 passes of ECAP-C and drawing with $\varepsilon=75\%$ [89]; b) Maximum load applied in fatigue testing plotted as a function of the number of cycles to failure for dental implants machined from the coarse-grained CP Ti (grade 1) and this material processed by ECAP [92].	10
Figure 2-5. a) Evolution of the open circuit potential of the HF-pickled (open symbols) and HF/HNO ₃ -pickled (closed symbols) CG CP Ti, prismatic grains of the UFG CP Ti (nano Ti t.) and basal grains of the UFG CP Ti (nano Ti c.) during 24 h immersion in glucose- free Hank's solution; b) Effect of D-glucose on the open circuit potential during 24 h immersion of CG CP Ti, prismatic grains in the UFG CP Ti (nano Ti t.) and basal grains in the UFG CP Ti (nano Ti c.) in Hank's solution. Closed symbols - with D-glucose, open symbols - D-glucose-free. [106].	12
Figure 2-6. Anode plasma electrolysis processes taking place at: a) electrolyte-anode interface, and b) growing oxide [152].	15
Figure 2-7. SEM characterisation of the surface (lower part) and cross section (upper part) of coatings fabricated in an alkaline electrolyte on a 6061 aluminium alloy at 1000 Hz, duty cycle 20 % and increasing current density 5-25 A/dm ² [17].	17
Figure 2-8. Discharge types. a) types A, B and C as described by Hussein et. al. [24], and b) internal discharges type E and D as described by Cheng et. al. [30].	18
Figure 3-1. a) ECAP-C processing method, d) TEM bright field image and diffraction pattern (inset) of the CP Ti after ECAP-C 6 passes and subsequent drawing [65], c) pole figures, and d) schematic of the macrotexture developed [106].	22
Figure 3-2. Schematic of the electrochemical set-up for the PEO coatings fabrication: 1) Electrolytic cell, 2) thermostat, 3) cathode, 4) power supply, 5) computer, and 6) anode.	24
Figure 3-3. Schematic representation of X-Ray diffraction.	28

Figure 3-4. Structural analysis of ALD TiO ₂ thin films by micro-Raman spectroscopy [213].	29
Figure 3-5. Load vs. displacement curve [216].	31
Figure 3-6. Electrochemical set-up for DC potentiodynamic and EIS tests: 1) working electrode, 2) Ag/AgCl reference electrode, 3) graphite auxiliary electrode, 4) potentiostat, 5) thermostat, and 6) computer.	35
Figure 4-1. SEM images of the studied surfaces: a) 1A; b) 1B; c) 2A; d) 2B; e) 3A; f) 3B; and g) Non-etched.	40
Figure 4-2. AFM surface topography analysis. <i>A</i> : 3D images of samples: a) Non-etched; b) 1A; c) 2B; and d) 3B. <i>B</i> : Surface profiles extracted from the AFM maps of the studied samples. Symbols mark the features mentioned for each surface. The horizontal axis coincides with the lateral dimension of the maps 80 μm .	41
Figure 4-3. EDX map analyses. Left: Sample 2B. Right: Sample 3B. Top images: SEM image of the area analysed. Bottom images: EDX map of the region; Color-bar expresses the at. %. White circles identify the regions of higher Fe content. Red crosses mark EDX spot measurements performed.	42
Figure 4-4. <i>A</i> : Results of the cell proliferation tests. The data is given as a percentage with respect to the positive control. <i>B</i> : Fluorescent optical images of cell adhered to the tested surfaces at different culture times and scales: a) Sample 1A; b-d) Sample 3B. Photographs a) and b) were taken 6 hours after plating. Photographs c) and d) after 24 h.	43
Figure 4-5. Schematic representation of cell spreading.	44
Figure 5-1. Root mean square voltage (V_{RMS}) and current density (j) versus time curves for the C, S1 and S2 treatments on UFG CP Ti.	48
Figure 5-2. Thicknesses of the PEO coatings as measured by Eddy-current.	50
Figure 5-3. 3D AFM images of the: a) nC-6, b) nC-12, c) nS1-12, and d) nS2-12 coatings.	51
Figure 5-4. SEM images of the C coatings on both CG (a and c) and UFG (b and d) after 60 (a and b) and 120 s (c and d) treatment time.	52
Figure 5-5. Average area (μm^2) of pores in the PEO coatings.	52
Figure 5-6. Total population of pores in the PEO coatings.	53
Figure 5-7. Surface porosity (%) of the PEO coatings.	53
Figure 5-8. High magnification image of the nC-12 coating showing fine cracks on the surface. Fine pores, blisters and brighter particles were usually found surrounding them.	54
Figure 5-9. Average surface roughness (S_a) of the PEO coatings as measured by AFM.	54
Figure 5-10. SEM images of the cross-sections of the nC-6 coating obtained by FIB: a) top-view before FIB sectioning (top) and cross-section (bottom), the dashed line marks the actual location of the cross-section; b)	

higher magnification image of the area marked in image a.	55
Figure 5-11. SEM images of the cross-sections of coatings cC-12 (a) and nC-12 (b) coatings obtained by FIB.	56
Figure 5-12. a) Top-view of the nC-12 coating area analysed in the tomography. The slicing-imaging direction is marked by the arrow from front to back. b) Population of pores according to their volume. Volume bin size 0.025 μm^3 . c-d) 3D reconstruction of the free volume (porosity) obtained using the Avizo software. c) Total free volume, d) Porosity group one, e) porosity group two, and f) porosity group three.	57
Figure 5-13. Evolution of the ratio between the covered area by the free volume and the total area analysed across through the thickness.	59
Figure 5-14. SEM images of the S1 coatings on both CG (a and c) and UFG (b and d) after 60 (a and b) and 120 s (c and d) treatment time.	60
Figure 5-15. SEM images of the cross-sections of coatings cS1-6 (a) and nS1-12 (b) coatings obtained by FIB.	61
Figure 5-16. SEM images of the S2 coatings on both CG (a and c) and UFG (b and d) after 60 (a and b) and 120 s (c and d) treatment time.	62
Figure 5-17. SEM images of the cross-sections of coatings nS2-6 (a) and cS2-12 (b) coatings obtained by FIB.	63
Figure 5-18. EDX analysis of the nC-12 coating. Top image: SEM image of the area analysed EDX local (areas 1 to 3, Table 5-3). Bottom images: element maps in atomic percent corresponding to the area marked in image a.	65
Figure 5-19. SEM image of the cross-section of the coating nC-12. The elemental composition measured at areas from 4 through 7 is presented in Table 5-3.	67
Figure 5-20. Element maps in atomic percent corresponding to the area marked in image a.	67
Figure 5-21. element maps in atomic percent corresponding to the area marked in image a.	68
Figure 5-22. XRD spectra of the coatings fabricated in the C electrolyte on the CG (left) and the UFG (right) substrates. Ti: CP Titanium. An: TiO_2 -anatase. Ru: TiO_2 -rutile.	68
Figure 5-23. XRD spectra of the coatings fabricated in the S1 electrolyte on the CG (left) and the UFG (right) substrates. Ti: CP Titanium. An: TiO_2 -anatase. Ru: TiO_2 -rutile.	69
Figure 5-24. XRD spectra of the coatings fabricated in the S2 electrolyte on the CG (left) and the UFG (right) substrates. Ti: CP Titanium. An: TiO_2 -anatase. Ru: TiO_2 -rutile.	69
Figure 5-25. XRD background subtraction: a and b) Ti substrate diffractogram, c and d) PEO-coated sample. Images a and c represent the fitting of the spectrum with a 5 point background. Images b and d show the	

resultant spectra after subtraction.....	70
Figure 5-26. Calculated crystalline fraction X_C of all the PEO coatings.	71
Figure 5-27. Raman spectra of the samples nC-12, nS1-12 and nS2-12. Solid lines correspond to the Raman spectra taken at discharge-free areas while dashed lines correspond to the Raman spectra taken in the vicinities of discharges. An: TiO ₂ -anatase. Ru: TiO ₂ -rutile. Inset shows the Raman spectra in the 0-300 cm ⁻¹ Raman shift range.	72
Figure 5-28. Bright field TEM images at the inner layers of the cC-12 (<i>a</i> and <i>b</i>) and nC-12 (<i>c</i> and <i>d</i>) specimens.	73
Figure 5-29. Bright field TEM images at the inner layers of the nS1-12 (<i>a-c</i>) and nC-12 (<i>d</i>) specimens.	73
Figure 5-30. TEM of the cC-12 specimen: <i>a</i>) Bright field overview of the lamella, <i>b</i>) HAADF mode for EDX analysis, <i>c-e</i>) Ti, Ca and P maps (at. %), respectively.....	75
Figure 5-31. TEM of the nC-12 specimen: <i>a</i>) Bright field overview of the lamella, <i>b</i>) Bright field image of a nc-TiO ₂ am-TiO ₂ within the OCL, <i>c-e</i>) Ti, Ca and P maps (at. %), respectively, taken at the area marked in image <i>a</i>	76
Figure 5-32. TEM of the cC-12 specimen: <i>a</i>) Bright field overview of the lamella, <i>b</i>) Bright field image of the OCL, <i>c</i>) HAADF mode for EDX analysis, <i>d</i> and <i>e</i>) Ti, and P maps (at. %), respectively, taken at the area presented in image <i>c</i>	77
Figure 5-33. TEM of the nS2-12 specimen: <i>a</i>) Bright field overview of the lamella, <i>b</i> and <i>c</i>) Bright field images of the OCL.....	78
Figure 5-34. Segregation of Ca and P at the nc-TiO ₂ grain boundaries. Ti, Ca and P maps (at. %).	80
Figure 5-35. Diffraction pattern on sample nC-12.....	81
Figure 6-1. Hardness (H) vs. contact depth to thickness ratio (h_C/t_0) of the PEO coatings fabricated on both substrates in the three electrolytes for 120 s.....	87
Figure 6-2. Young's modulus (E) vs. contact depth to thickness ratio (h_C/t_0) of the PEO coatings fabricated on both substrates in the three electrolytes for 120 s.....	87
Figure 6-3. Representative print of the indentations on the surface of the nC-12 coating: <i>a</i>) SEM image; <i>b</i>) AFM image of the print in <i>a</i> ; and <i>c</i> , FIB milled cross-section of the print in <i>a</i> . Dashed line in image <i>a</i> shows the location of the cross-section.	88
Figure 6-4. Representative print of the indentations on the surface of the nS1-12 coating: <i>a</i> , SEM image; <i>b</i> , AFM image of the print in <i>a</i> ; and <i>c</i> , FIB milled cross-section of the print in <i>a</i> . Dashed line in image <i>a</i> shows the location of the cross-section.	89
Figure 6-5. Representative print of the indentations on the surface of the nS2-12 coating: <i>a</i> , SEM image; <i>b</i> ,	

AFM image of the print in <i>a</i> ; and <i>c</i> , FIB milled cross-section of the print in <i>a</i> . Dashed line in image <i>a</i> shows the location of the cross-section.	89
Figure 6-6. Young's modulus (<i>E</i>) versus Hardness (<i>H</i>) of the nC-12, nS1-12 and nS2-12 coatings. Linear fit equations are presented in Table 1.	92
Figure 6-7. Schematic of the plastic and elastic deformation volumes under the indenter. <i>a</i> , predominant presence of nc-TiO ₂ . <i>b</i> , predominant presence of am-TiO ₂	93
Figure 6-8. <i>H</i> and <i>E</i> modulus of the nC-12 coating against the Ca at. % measured at each indentation.	94
Figure 6-9. TEM BF images of the lamellas <i>a</i> , <i>b</i> , <i>c</i> and <i>d</i> , under the indentations indicated by the arrows. ...	95
Figure 6-10. SEM images of ramping scratches at 1 mN/um up to a normal load of 500 mN on the nC-12 coating: <i>a</i>) overview of a full scratch, <i>b</i>) Lc1, tensile cracks at low load, <i>c</i>) Lc1', cohesive damage of the OCL, <i>d</i>) Lc2, detachment of the OCL, and <i>e</i>) Lc3, final cut-through	97
Figure 6-11. <i>b-d</i>) Representative secondary electron images of loading scratches on samples nS1-12, cS2-12 and nS2-12, respectively. <i>a</i> , <i>e</i> and <i>f</i>) Raman spectra at adjacent coating and failure regions of the loading scratches in samples cC-12, cS1-12, and cS2-12, respectively.	99
Figure 6-12. Evolution of the friction coefficient during the loading scratches. Upper panel: nC-12. Lower panel: nS1-12 and nS2-12 coatings. Approximated positions of the critical loads are indicated.	100
Figure 6-13. Critical loads at the moment of the coatings' detachment.	100
Figure 6-14. SEM images of the wear tracks after the reciprocating constant normal force tests before (<i>a</i> , <i>c</i> and <i>e</i>) and after failure (<i>b</i> , <i>d</i> and <i>f</i>) at 100 mN. Images <i>a</i> and <i>b</i> correspond to the nC-12 coating after 20 and 30 cycles, respectively. Images <i>c</i> and <i>d</i> to the n S1-12 coating after 40 and 50 cycles, respectively. Images <i>e</i> and <i>f</i> to the cS2-12 coating after 40 and 50 cycles, respectively.	101
Figure 6-15. Wear rates vs. number of cycles. Dashed lines correspond to the wear rates obtained on both the CG and UFG substrates at 100 mN for 10 cycles. Open symbols indicate the failure of the coatings.	102
Figure 7-1. Evolution of the Open Circuit Potential (OCP) of the CG and UFG CP Ti substrates and PEO coatings during 1 hour of immersion in Hank's + D-glucose solution.	108
Figure 7-2. Direct current polarization curves of the CG and UFG CP Ti substrates and PEO coatings during 1 hour of immersion in Hank's + D-glucose solution.	109
Figure 7-3. Total impedance $ Z $ of the CG and UFG CP Ti substrates and PEO coatings during 1 hour of immersion in Hank's + D-glucose solution.	111
Figure 7-4. Complex plot (<i>a</i>) and Bode plots (<i>b</i> and <i>c</i>) of the CG CP Ti in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.	112
Figure 7-5. Complex plot (<i>a</i>) and Bode plots (<i>b</i> and <i>c</i>) of the UFG CP Ti in Hank's + D-glucose solution for	

the immersion times from 1 h to 14 days.	112
Figure 7-6. Randles equivalent circuit for the fitting of the EIS response of the CG and UFG CP Ti.	113
Figure 7-7. Complex plot (a) and Bode plots (b and c) of the cC-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.....	115
Figure 7-8. Complex plot (a) and Bode plots (b and c) of the nC-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.....	116
Figure 7-9. Complex plot (a) and Bode plots (b and c) of the cS1-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.....	116
Figure 7-10. Complex plot (a) and Bode plots (b and c) of the nS1-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.....	118
Figure 7-11. Randles equivalent circuit for the fitting of the EIS response of the cC-12, nC-12, cS1-12 and nS1-12 coatings.	118
Figure 7-12. Complex plot (a) and Bode plots (b and c) of the cS2-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.....	118
Figure 7-13. Complex plot (a) and Bode plots (b and c) of the nS2-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.....	119
Figure 7-14. Randles equivalent circuit for the fitting of the EIS response of the cS2-12 and nS2-12 coatings.	119
Figure 8-1. Cell proliferation of hMSCs cultured with CG and UFG CP Ti and PEO-coated samples at 48, 96 and 144 hours. Data are presented as mean $\pm$ standard deviation of at least three independent experiments. Differences were analyzed with $p < 0.05$ being considered statistically significant.....	124
Figure 8-2. Metabolic activity of the hMSCs cells on the control, bare CG and UFG CP Ti, and PEO-coated samples after 48 hours of culture. Data is expressed as mean $\pm$ standard deviation with $p < 0.05$ with the blank and test controls.....	124
Figure 8-3. SEM images taken in backscattered electrons mode of the surfaces after 72 hours in co-culture with hMSCs. a, CG CP Ti; b, UFG CP Ti; c, cC-12; d, nC-12; e, nS1-12; and f, nS2-12.	125

List of Tables

Table 1-1. Mechanical properties of the traditional metallic biomaterials. E, Young's/elastic modulus; σ_Y , yield strength; σ_{UTS} , ultimate tensile strength; and ϵ_f , elongation to failure.	3
Table 2-1. Comparison of tensile mechanical properties in some biomedical metallic materials, conventional Ti-based materials and UFG CP Ti. E, Young's/elastic modulus; σ_Y , yield strength; σ_{UTS} , ultimate tensile strength; and ϵ_f , elongation to failure.	9
Table 2-2. Fatigue limit comparison of CP Ti grade 4 in the conventional coarse-grained condition and after ECAP-based SPD processing followed by thermo-mechanical treatment.	11
Table 3-1. Impurity content in wt. % of the as-received CP Ti grade 4.	21
Table 3-2. Yield strength, ultimate tensile strength and elongation to failure of the as-received CG CP Ti, and their evolution during applied processing[22].	21
Table 3-3. Summary of samples and acid etching treatments: solution composition and treatment time.	23
Table 3-4. Composition, pH and conductivity of the electrolytes.	24
Table 3-5. Composition of the Hank's médium for electrochemical tests.	35
Table 4-1. Roughness measurements performed on samples from AFM maps.	39
Table 4-2. Cell density, major axis, Aspect Ratio and ratio between surface covered and total image area, after six hours. Data taken from images a and b in Figure 4 B (samples 1A and 3B, respectively).	44
Table 5-1. Breakdown voltage (V_B), Voltage at 60 s (V_{60}), final forming voltage (V_F), concentration, pH and conductivity of the electrolytes.	49
Table 5-2. Content of chemical elements measured by EDX. The presented data showed corresponds to the average of over 20 measurements on the surface of the samples.	64
Table 5-3. Content of chemical elements measured by EDX on the nC-12 sample. The average elemental content of the sample is also given for comparison.	66
Table 5-4. Elemental composition at the areas depicted in Figure 5-30 corresponding to the cC-12 specimen.	74
Table 5-5. Elemental composition at the areas depicted in Figure 5-31 corresponding to the nC-12 specimen.	78
Table 5-6. Elemental composition at the areas depicted in Figure 5-32 corresponding to the nS1-12 specimen.	79
Table 5-7. Elemental composition at the areas depicted in Figure 5-33 corresponding to the nS2-12 specimen.	80

Table 5-8. Elemental composition at the areas depicted in Figure 5-34 corresponding to the nC-12 specimen.	80
Table 6-1. Comparison of data from earlier reports on the mechanical properties of PEO coatings on CP Ti and its alloys. Hardness and Young's modulus values correspond to the data for contact depths below the 10 % of the coating thickness. Notice that the data presented correspond to nanoindentation and nanoscratching testing on the surface of the coatings with Berkovich tip unless specified otherwise: HV, Vickers hardness; $\ddagger$, micro testing Rtip ~ 200 μ m; $\dagger$, Ball-on-disk; and, $\downarrow$, Pin-on-disk.	90
Table 6-2 . Average (Mean), standard deviation (Std. Dev.), maximum (Max.) and minimum (Min.) of the E, H and H/Er ratio for the three samples (first four rows). The 'Fit' row presents the equations of the linear fits in Figure 6-6. Radius of the plastic (Rp) and elastic (Re) zones, respectively.	92
Table 6-3. The E and H and Ca and P contents measured at the indents of the lamellas a, b, c and d.	94
Table 6-4. Critical loads in N of the ramping scratches on the cC-12 and nC-12 samples.	98
Table 7-1. Open Circuit Potential (OCP), corrosion potential (E_{corr}) and passivation current density (j_{pass})..	108
Table 7-2. Equivalent circuit parameters for the CG and UFG CP Ti for 1h, 3d, 7d and 14d of immersion in Hank's + D-glucose solution.	113
Table 7-3. Equivalent circuit parameters for the cC-12, nC-12, cS1-12 and nS1-12 coatings 1h, 3d, 7d and 14d of immersion in Hank's + D-glucose solution.	117
Table 7-4. Equivalent circuit parameters for the cS2-12 and nS2-12 coatings 1h, 3d, 7d and 14d of immersion in Hank's + D-glucose solution.	120

1 Introduction

This PhD Thesis is focused on surface design of ultrafine-grained commercially pure titanium (UFG CP Ti) to improve its osteoconductivity for orthodontic applications. The idea of surface design is based on chemical treatments and deposition of osteoconductive coatings. The experimental work performed involved a thorough microstructural and mechanical characterization of coatings formed on UFG CP Ti by plasma electrolytic oxidation (PEO) as well as the assessment of their corrosion resistance properties in simulated physiological conditions and their interaction with cells via in-situ tests. Ultimately, the experiments performed in this work are dedicated to study certain biocompatibility considerations: corrosion resistance, cell-surface interactions, and mechanical integrity and compatibility; which are briefly discussed in the following section 1.1. The motivation and objectives of the Thesis are described in subsequent sections 1.2 - 1.4.

1.1 Biocompatibility of metallic biomaterials

Bio-materials are *implantable* materials which are intended to be in intimate contact with human tissues and fluids and are intrinsically *biocompatible*. The term biocompatible, or *biocompatibility*, applies to any medical device that can be used in intimate contact with living tissues *without causing any harmful effect* such as inflammatory reactions or allergies. Hence, the biocompatibility of a metallic biomaterial concerns the chemical and physical impact of the material on the human body [1].

The main concerns regarding the chemical performance of a metallic biomaterial in a physiological environment are its *toxicity* and its protection against *corrosion*. Indeed, those elements, which could be toxic, will lead to the rejection of the implant or to a disease. An excellent corrosion protection is essential to prevent the loss of material and the excessive release of metallic ions.

Exceptional mechanical properties (i.e. yield strength (σ_Y), ultimate tensile strength (σ_{UTS}), elongation to failure (ϵ_f), etc.) are necessary to ensure the long-term success of a biomedical device without a critical failure. Nonetheless, in bone healing or replacement applications, a low *Young's modulus* (E) is desired. The elastic modulus of bone is generally between 10 and 40 GPa [2]. A higher elastic modulus, such as those of the typically used metals (> 100 GPa), may lead to the so called “stress shielding” effect by which the adjacent bone can undergo atrophy since most of the loads are absorbed by the implant [1]. The human body is frequently under cyclic loading conditions such as in the case of walking and chewing. Hence, the implanted materials must have a large *fatigue limit* in the *high cycle regime* [3]. In the case of a total hip replacement, the loads applied during walking may be several times the patient's weight as the prosthesis is brought out of the vertical plane at each step while the whole patient's weight pivots on the one leg. Friction must be taken into consideration during cyclic loading as a fretting fatigue scenario may take place leading to the earlier damage of the implant contact surfaces [3]. The *hardness* (H) and *wear resistance* of the surface are important properties to minimize the generation of debris due to friction between the implant and bone. The debris may lead to abrasive wear, inflammatory reactions around the implant and even cytotoxicity [4], apart from the

release of metallic ions to the surrounding tissues.

The last, but certainly not the least, aspect about the biocompatibility of biomaterials is their *interaction with cells and tissues*. *Bioactive* materials are those with the ability to produce a biochemical bond to a specific tissue such as bone [1], [5]. The *osseointegration* of an implant takes place when there is a biomechanical bond between the surrounding healing bone and the surface of the implant [6]. The capability of promoting the formation of new bone on the surface of the material is called *osteoconductivity* and involves the adhesion and proliferation of mesenchymal stem cells, their differentiation into osteoblastic cells (i.e. *osteoinduction*) and the generation of new bone (i.e. *osteogenesis*) [5]. Hence, osseointegration of an implant implies the osteoconductivity of the implant surface, but the latter doesn't necessarily prove the former. Specific surface properties (i.e. chemistry, wettability, etc.) can enhance the osteoconductivity of the implant. On the contrary, a poor surface may as well lead to cytotoxicity (i.e. cell or tissue death).

It is important to bear in mind that the requirements for biocompatibility are complex and depend on the application. For example, the level and characteristics of the mechanical loads applied to an orthodontic implant are not the same as the ones applied to a total hip replacement. Similarly, the pH and composition of the fluids surrounding both implants may also differ.

1.2 Ultrafine-grained commercially pure Titanium

UltraFine-Grained Commercially Pure Titanium (UFG CP Ti) has arisen as one of the best prospects for the fabrication of implantable appliances due to the superior biocompatibility of CP Ti with respect to the other metallic systems used in the biomedical field, and enhanced mechanical properties thanks to the refined microstructure through Severe Plastic Deformation (SPD) processes.

Metallic materials have been the prevalent choice for the fabrication of implantable medical devices since the 1860s, when Dr. J. Lister developed the aseptic surgical technique preventing the implanted region from infecting [7]. Metals have been largely used in orthopaedic implanted devices for both temporary (i.e. screws, pins and bone plates) and permanent (i.e. total joint replacements) applications due to their high strength, good formability, and excellent fatigue and fracture performance [1]. Complying with the biocompatibility considerations described in the previous section, the traditional metallic systems used in implantable devices are Fe-Cr-Ni alloys, Co-Cr-based alloys, and CP Ti and its alloys [8], [9]. Ti-based materials have interesting advantages with respect to the other metallic materials: 1) enhanced corrosion resistance; 2) lower Young's modulus; and 3) elemental bioinertness [10]. Ti-based materials have the ability to develop a highly stable TiO₂ passive layer on its surface on most media. This natural corrosion protection can be enhanced by surface treatments such as anodization. Ti-based materials are less likely to produce "stress-shielding" effect due to its lower elastic modulus (~ 110 GPa) [11], [12]. Ti is considered as a bioinert element, meaning that it does not produce any harmful nor beneficial reaction on the human body even in large doses [13].

Among the Ti-based materials CP Ti and the Ti6Al4V alloy are the dominating choices. While the Ti6Al4V alloy is the preferred material for load bearing applications such as total joint replacements, Coarse-Grained

(CG) CP Ti has been relegated to the fabrication of stents, wires and fixation devices due to its lower mechanical properties (i.e. σ_Y and σ_{UTS}) [10]. In spite of the excellent corrosion resistance and mechanical properties of the Ti6Al4V alloy, Al and V are toxic elements and represent a great risk in the case of excessive ion liberation or debris generation. Hence from the point of view of biocompatibility, non-alloyed CP Ti is the best candidate. The great interest in enhancing the mechanical properties of CP Ti led to the development of UFG microstructures via SPD processing [14]–[20]. Among the vast number of SPD methods applied to CP Ti, the combination of Equal Channel Angular Pressing-Conform scheme (ECAP-C) processing and a subsequent drawing provided an UFG CP Ti with mechanical properties that are competitive with those of the traditional metallic biomaterials (Table 1-1) while preserving the advantages of CG CP Ti in terms of chemical biocompatibility (i.e. corrosion and toxicity) [21]. This improvement in the mechanical performance of CP Ti offers the possibility to its use in permanent bone-replacement load-bearing applications such as orthodontic implants. Furthermore, in the case of orthodontic applications, this enhancement allows for miniaturization of the implant which makes the surgery less invasive and opens the possibility for implantation in narrower jawbones.

Table 1-1. Mechanical properties of the traditional metallic biomaterials. E, Young's/elastic modulus; σ_Y , yield strength; σ_{UTS} , ultimate tensile strength; and ϵ_f , elongation to failure.

Material	E (GPa)	σ_Y (MPa)	σ_{UTS} (MPa)	ϵ_f (%)
316L stainless steel	~ 200	200-700	500-1350	10-40
Co-based alloys	~ 240	500-1500	900-1800	10-50
CG Ti-6Al-4V	110	860	930	10-15
CG Ti grade 4	115	480	550	15
UFG Ti grade 4: ECAP-C + Drawing	115	1300	1350	9

The UFG microstructure of CP Ti has already shown enhanced cell adhesion and proliferation with respect to its CG counterpart [22]–[24]. This enhancement was related to a higher surface energy that facilitates the absorption of proteins that are necessary for the adhesion and proliferation of mesenchymal cells (i.e. wettability). Despite the modest enhancement, it is necessary to further improve the osteoconductivity of the surface in order to ensure the osseointegration and the short- and long-term success of UFG CP Ti appliances.

1.3 Surface treatments for osteoconductivity enhancement of Ti-based biomaterials

There is a large body of research on the osteoconductivity enhancement of Ti-based materials based on the modification of their surface [25], [26]. Several approaches have been applied including physical and/or chemical roughening and the fabrication of osteoconductive coatings: Plasma Spraying, Grit Blasting, Acid-etching, Anodization, Magnetron Sputtering, Atomic Layer Deposition (ALD), and Plasma Electrolytic Oxidation (PEO), among others. It has been shown that surface roughening at the macro-, micro-, and nano-

scale enhance respectively the mechanical interlocking between the implant and the bone ongrowth, the interlocking between the implant surface and the mineralized bone matrix, and the protein adsorption and cellular processes [6], [27]. Deposition of hydroxyapatite (HA, i.e. hard phase of bone) or Ca- and P-containing bioactive coatings have arisen as attractive approaches to provide chemical affinity to the surface of the biomaterial in the case of bone-contact applications [28], [29]. Among the mentioned surface modification methodologies, the PEO technique is one of the most promising since it is an electrochemical approach that allows for the fabrication of thin ($\sim 3\text{-}20\text{ }\mu\text{m}$) ceramic TiO_2 coatings on Ti-based materials with a suitable surface roughness, composition, porosity, strong adhesion to the substrate and enhanced corrosion protection [30]. One of the strongest capabilities of the PEO process is the ability to incorporate Ca- and P-containing species from the electrolyte increasing the chemical affinity towards bone.

There is a great deal of information on the PEO treatment of CG CP Ti. The benefits of this treatment have already been shown in terms of corrosion protection, mechanical properties and bioactivity [28], [29], [31]–[36]. In contrast, there are only a few works reporting the PEO treatment of UFG CP Ti and its effect on the mentioned biocompatibility aspects [37]–[41]. Hence, one of the main objectives of this work is to understand the effect of the grain refinement of CP Ti on the PEO treatment and on the properties of the fabricated coatings.

1.4 Outline and objectives of the Thesis

This PhD Thesis is focused on the biocompatibility of osteoconductive surface modification treatments on UFG CP Ti for orthodontic applications. Two different surface modification approaches are considered: 1) Chemical etching and 2) fabrication of PEO coatings.

Chapter 2 contains a literature review on the processing of CG and UFG CP Ti and on the application of surface modification approaches to CG and UFG CP Ti. In Chapter 3, the materials, experimental procedures and techniques used in this work are described. In Chapter 4, the effect of the surface topography of UFG CP Ti on the cell adhesion and proliferation is investigated. Different surface topographies were produced via chemical etching of the UFG CP Ti using various HF/HNO_3 -based solutions with varying etching parameters. The most promising surface topographies were selected for cell attachment and proliferation assessment.

The main body of the Thesis is focused on the formation of thin ($\leq 10\text{ }\mu\text{m}$) bioactive PEO coatings on the surface of CG and UFG CP Ti using three different treatments, as well as on their microstructural and mechanical characterization and the assessment of the corrosion and osteoconductive performance. Hence, this part is divided into four chapters dealing with the microstructural characterization of the coatings (Chapter 5), and the assessment of their mechanical properties (Chapter 6), corrosion resistance (Chapter 7), and cell-surface interactions (Chapter 8). Finally, the general conclusions of this work and future work are gathered in Chapter 9. The objective of these chapters is twofold: First, to compare the resulting corrosion resistance, cell-surface interaction, and mechanical properties of the three PEO treatments; second, to compare the applicability of the PEO treatment on the UFG CP Ti to the one on the CG CP Ti. Three electrolytes were selected: two simple phosphate-based electrolytes generally used in the anodization and PEO treatment of CP

Ti for the enhancement of the corrosion and wear resistance, and a Ca and P-containing complex electrolyte with the aim to produce an osteoconductive coating. The H, E, and tribological behavior of the coatings were assessed by means of instrumented nanoindentation and nanoscratching. Although the effect of the coatings on the fatigue performance was not investigated due to technical difficulties, the thickness of the coatings was kept $\leq 10\text{ }\mu\text{m}$ in order to minimize their effect on the fatigue performance of the substrates while improving its surface properties. The corrosion performance of the coatings was studied by means of direct current (DC) potentiodynamic polarization tests and alternate current (AC) electrochemical impedance spectroscopy (EIS) in simulated physiological conditions. Finally, the adhesion and proliferation of mesenchymal stem cells was assessed by *in vitro* tests.

2 State of the art

2.1 Severe plastic deformation processing of CP Ti

Both “bottom-up” and “top-down” approaches for processing of bulk UFG Ti have received attention in the research community. Vacuum sintering and the process of hydrogen sintering and phase transformation of TiH_2 powders are nice illustrations of the “bottom-up” approach [42]. The main shortcoming of this approach is possible porosity as well as contamination of powders during processing [43]. A well-known example of the “top-down” approach is grain refinement by severe plastic deformation (SPD) techniques [44]. A significant advantage of SPD techniques is their applicability to nearly all Ti-based materials. Nowadays, from a practical point of view, SPD of bulk billets appears to be the most promising processing route for manufacturing UFG CP Ti for biomedical applications.

2.1.1 *Processing description*

The most widely developed and used SPD technique is the so-called Equal Channel Angular Pressing (ECAP) method [45]–[48]. In ECAP, the material is pressed throughout a die that contains a bent channel forming a sharp angle (Figure 2-1 *a*). The shear strain is imposed in the thin region connecting the inner and outer corners [49]. The dimensions of both channels are the same and, thus, the dimensions of the initial billet are maintained after subsequent ECAP passes. This allows the repetition of the process until the desired total plastic strain is introduced into the material resulting in grain refinement. The die corner geometry has a strong influence on the stress field applied. ECAP dies with 90° and 120° corners are the most used configurations [50]–[55]. ECAP processing of Ti is typically carried out in the temperature range from room temperature to 450°C depending on the required microstructure. Typically low temperatures facilitate the formation of high angle grain boundaries (HAGBs) and optimum UFG microstructures, although the process becomes more difficult [46]. Progressive reduction of the temperature is also often used [56]. The processing speed rate does not have a very significant effect on the process [46]. Numerous combinations of processing parameters (processing temperature, intersection angle, speed/pressure rate, processing route) during processing of Ti are reported in the literature. Rotating the sample between consecutive passes allows to combine different strain directions, or strain paths (i.e. routes A, Ba, Bc, C, etc.) [47]. It was demonstrated that the route Bc (rotation by 90° around the long axis of the sample in the same direction after each pass) produced optimum microstructure [46], [56]–[58].

Figure 2-1. Schematic representation of principles of: a) conventional ECAP; and, b) ECAP-Conform.

To further facilitate the grain refinement and to improve the mechanical properties, traditional cold or hot working techniques (i.e. rolling, swaging or drawing) can be used for post-SPD processing [21], [59], [60] and to re-shape the final product [61], [62]. Conventional ECAP routes are not suitable for the processing of long rods in a continuum way. To solve this problem, a novel ECAP route based on the Conform scheme, the so-called ECAP-Conform (ECAP-C) [63], was developed (Figure 2-1, b). The ECAP-C allows fabrication of long rods reducing significantly wastage of material during processing and dramatically increasing processing efficiency, which is very attractive for industry. The earlier works were focused on ECAP-C processing of CP Ti for grain refinement [14] and the optimum temperature for the process was found to be 200°C [14], [21]. As in the case of conventional ECAP, the ECAP-C processed rods can be successfully combined with post processing steps (e.g. drawing or extrusion) [15], [64] and annealing treatments for further microstructural design and improvement of mechanical properties [65].

2.1.2 Microstructure

As it was already noted above, the final microstructure of the SPD processed CP Ti is strongly determined by the deformation path and processing parameters. We would like to lay the main emphasis on the microstructural features of the ECAP-Conform processed CP Ti followed by additional warm deformation processing (drawing), since by now this is the only commercialized technique for fabrication of UFG CP Ti for manufacturing of implants. There is a body of research on microstructure evolution during such complex SPD processing of CP Ti [14], [21].

Figure 2-2. Transversal bright (left) and dark (right) field images of CP Ti after ECAP-C processing for 6 passes and drawing.

The as-ECAP-C microstructure of CP Ti strongly depends on the number of passes. At the early stages of plastic deformation after 1-2 ECAP-C passes, the microstructure is characterized by high density of deformation twins and substructure. Further ECAP-C processing to 4 passes results in transformation of low angle grain boundaries (LAGBs) and HAGBs and formation of a homogeneous UFG microstructure with the average grain/subgrain size of ~ 230 nm. After 6-8 passes, HAGBs dominate in the microstructure, and the average grain size is reduced down to ~ 200 nm (Figure 2-2). Dislocation density and the levels of elastic microdistortions of the crystal lattice tend to gradually increase with number of ECAP-Conform passes up to $1.17 \cdot 10^{15} \text{ m}^{-2}$ and 0.0025 after 8 passes, respectively. During ECAP-C a crystallographic texture is developed, which can be described as a combination of orientations of pyramidal and prismatic components. The subsequent drawing at 200 °C, as the final processing operation, results in further refinement of the grain structure down to ~ 150 nm and formation of a very strong (10 $\bar{1}$ 0) fibre crystallographic texture with basal planes parallel to the rod axis (Figure 2-3 b) [60].

2.1.3 Advanced properties

2.1.3.1 Strength and ductility

Ideally, a metallic material for manufacturing of implants and prosthesis should have a high level of strength and a low elastic modulus. The latter property is important in order to reduce the so-called stress-shielding effect [10], [66], [67]. It is seen in Table 2-1 that UFG CP Ti fulfils this combination of properties better than stainless steels and Co-based alloys competitors and it is similar to the Ti-6Al-4V alloy. However, the recently developed Ti β -phase alloys have even lower E (~ 70 GPa) due to the addition of alloying elements [66], [68], [69].

Figure 2-3. a) Stress-strain curves for CP Ti before and after SPD processing tested in transversal and longitudinal directions; b) Pole figures for basal (0001) and prismatic planes (10-10) for the transversal section of the SPD processed CP Ti [60].

Following the well-known Hall-Petch effect, the finer the grains, the higher is the yield strength due to grain size hardening effect [70]–[72]. This is true at low temperatures ($< 0.5 T_m$). It is seen from Table 2-1, the UFG CP Ti can demonstrate an extraordinary high yield strength up to 1300 MPa and ultimate tensile strength up to 1350 MPa at room temperature. Dislocation hardening is also an effective strengthening mechanism in UFG

CP Ti [73]. Other factors affecting mechanical behaviour of UFG CP Ti include fraction of HAGBs as well as grain boundary conditions (equilibrium/non-equilibrium) [74]. As was already pointed out in previous sections, microstructure-properties relations can be controlled by the tuning of processing parameters [21], [64], [75]. The purity of Ti can also play a role as demonstrated in [76], [77], where both yield and ultimate tensile strength were higher for CP Ti grade 4 with higher impurity content than for CP Ti grade 2.

Analysis of Table 2-1 also shows that UFG CP Ti produced via complex SPD processing routes (i.e. combination of several processing techniques) typically demonstrates higher mechanical strength due to finer grains. Nevertheless, it should be noted that mechanical strength of the UFG CP Ti is determined by the interplay of its microstructure and crystallographic texture. Although route Bc was presented to be the best processing for optimum microstructure in all sections of the processed samples, Ba and C routes result in slightly higher mechanical properties due to elongated grains in the longitudinal direction [56]. In [60], it was shown that UFG CP Ti can demonstrate significant anisotropy of tensile mechanical properties related to the strong crystallographic texture, i.e. the orientation of basal and prismatic planes with respect to the applied stress. The yield stress in the longitudinal direction (for which the applied load is perpendicular to the the c-axis and two prismatic planes) was twice that of the transversal direction (for which the c-axis was randomly inclined with respect to the applied load). The ultimate tensile strength however, was very similar in both cases due to work hardening along prismatic slip systems in the transversal direction. Anisotropy of mechanical strength in compressive deformation was also reported in [78] (Figure 2-2 a).

Table 2-1. Comparison of tensile mechanical properties in some biomedical metallic materials, conventional Ti-based materials and UFG CP Ti. E, Young's/elastic modulus; σ_Y , yield strength; σ_{UTS} , ultimate tensile strength; and ϵ_f , elongation to failure.

material/processing	E (GPa)	σ_Y (MPa)	σ_{UTS} (MPa)	ϵ_f (%)	REF
CG Ti grade 4 (α phase)	115	480	550	15	[79]
CG Ti-6Al-4V (α phase)	110	860	930	10-15	[79]
316L stainless steel (ASTM F138)	~200	200-700	500-1350	10-40	[10]
Co-based alloys	~240	500-1500	900-1800	10-50	[10]
UFG Ti grade 2: ECAP Bc (8)	-	750	882	13,8	[80]
UFG Ti grade 4: ECAP-C (8)	-	990	1100	11	[64]
UFG Ti grade 4: ECAP-C (6) + Drawn	-	1300	1350	9	[15]

From Table 2-1, it is also seen that SPD reduces the ductility of the material with increasing total strain inserted into material during processing. Ductility can be enhanced by the application of annealing treatments in order to recover non-equilibrium grain boundaries [59], [65], without a significant growth of the ultrafine grains and reduction of mechanical strength. Gunderov et al. [21] concluded that the increase in the true strain at failure in UFG CP Ti processed by ECAP-C combined with drawing, when compared with coarse-grained CP Ti, was

due to an increase in the crack(s) formation resistance of the material [80].

2.1.3.2 Fatigue performance

Besides static loads, the human skeleton is continuously subjected to cyclic loading at any part of the body. For instance, walking involves, among others, legs and hip, and chewing the mandible. So biomedical implants are also subjected to cyclic loading, and their fatigue performance is a special subject for their design. It has been demonstrated that nanostructuring of CP Ti typically leads to higher fatigue lifetimes in both low cycle fatigue (LCF) and high cycle fatigue (HCF) [81], [82] (Figure 2-4 a). This improvement is due to the overall strength enhancement compared with the coarse-grained counterpart in the case of LCF [83] and due to the improvement of the crack nucleation resistance under HCF [21], [80]. The higher crack nucleation resistance in the UFG CP Ti was related to the formation of high energy HAGBs [84]. This grain boundary state, on the other hand, could lead to an undesired ‘scenario’ if their energy is so high that intergranular cracking occurs [85].

Unlike UFG fcc metals (i.e. Cu or Al, [81], [86]), when comparing fatigue behaviour during plastic strain-controlled tests, pure hcp Ti does not present any degradation of fatigue properties [87]. In the HCF regime, UFG CP Ti obtained by ECAP may present fatigue limits of ~ 350 MPa ($N_f > 10^6$) [88], ~ 380 MPa ($N_f > 4 \times 10^4$) [87], ~ 480 MPa [89], [90], depending on the microstructure developed in the material and its purity. These values are still lower than the fatigue limit of the well-known Ti6Al4V ELI alloy (515 MPa).

Figure 2-4. a) The S-N curves from fatigue testing of CP Ti (grade 4): (1) in the coarse-grained condition, (2) after 6 passes of ECAP-C and drawing with $\epsilon=75\%$, and (3) after 10 passes of ECAP-C and drawing with $\epsilon=75\%$ [88]; b) Maximum load applied in fatigue testing plotted as a function of the number of cycles to failure for dental implants machined from the coarse-grained CP Ti (grade 1) and this material processed by ECAP [91].

However, fatigue limit of UFG CP Ti can be further improved by additional thermal treatments (Table 2-2). These thermal treatments are useful for some recovery of non-equilibrium HAGBs to prevent early crack initiation [92].

Table 2-2. Fatigue limit comparison of CP Ti grade 4 in the conventional coarse-grained condition and after ECAP-based SPD processing followed by thermo-mechanical treatment.

First step		Second step		Third step	Fatigue Limit (MPa)	REF
Coarse-grained, grade 4		-		-	380	[82]
ECAP Bc	4 passes	Drawing, 60%		Annealing, 350°C for 1,5 h	590	[92]
ECAP Bc	4 passes	Warm Rolling, 350 °C, 70%		-	640	[92]
ECAP-C	10 passes	Drawing, 200 °C, 75%		-	610	[82]

The initiation of a crack strongly depends also on the presence of stress raisers. Special attention is usually paid to the surface state regarding mainly defects (i.e. pits and scratches) and/or excessive roughness [93]. Biomedical devices often present an enhanced surface roughness in order to improve their osseointegration during their service life. One must bear in mind this important fact, as it might dramatically decrease its fatigue life, even though the fatigue properties of the bulk material are the high-class of the market. As an example, it is reported that the fatigue limit of CP Ti (grade 4) is reduced by 12% after a surface treatment that is meant to increase the surface roughness [94]. Moreover, consideration must also be taken regarding the shape of the appliance and its worst-case working condition, as it is studied, for instance, for dental implants [95]–[97].

The fatigue properties of dental implants are dramatically reduced also due to their thread root, which induce preferential points for crack nucleation. Special certification standards are available for this type of devices (ISO 14801:2007). There are no many examples of dental implants made of UFG Ti, and at least not of their fatigue characterization. Figueiredo et al. [91] studied the fatigue properties of dental implants manufactured from UFG (grade 1) Ti following the ISO 14801 standard (ISO 14801:2007). The fatigue life of implants made from UFG CP Ti was higher by one order of magnitude (10^5 to 10^6 life cycles) at the same stress levels with respect to that of the coarse-grained Ti implants (Figure 2-4 b). Medvedev et al. [98] reported results of biological-simulated bending strain-controlled fatigue testing of dental implants made of UFG Ti (processed by ECAP-C and drawing). Although the tests were not standardized, they demonstrated that the fatigue strength of UFG Ti was not affected by a biological environment.

2.1.3.3 Corrosion resistance

Corrosion resistance is one of the most important properties of metals used for biomedical implants. There is a significant body of research on the effect of grain size on corrosion resistance of metallic materials, and the most comprehensive overviews are presented in Refs. [99], [100]. Most of the research on corrosion behavior of UFG CP Ti was focused on its performance in aggressive environments. Balyanov et al. [101] were the first to demonstrate that UFG CP Ti produced by ECAP had lower corrosion rates and higher corrosion resistance than its coarse-grained counterpart in H_2SO_4 and HCl solutions. Further annealing treatment of UFG CP Ti may lead even to a better corrosion resistance due to reduction of dislocation density and relieve of residual stresses [102]. No attention to the role of crystallographic texture in corrosion behavior of the UFG CP Ti was

paid in the earlier works, whereas later studies clearly demonstrated that the effect of crystallographic texture might be even more significant than that of grain size [103]. It was shown that basal planes have higher corrosion resistance in NaCl based solutions [103], [104].

Figure 2-5. a) Evolution of the open circuit potential of the HF-pickled (open symbols) and HF/HNO₃-pickled (closed symbols) CG CP Ti, prismatic grains of the UFG CP Ti (nano Ti t.) and basal grains of the UFG CP Ti (nano Ti c.) during 24 h immersion in glucose- free Hank's solution; b) Effect of D-glucose on the open circuit potential during 24 h immersion of CG CP Ti, prismatic grains in the UFG CP Ti (nano Ti t.) and basal grains in the UFG CP Ti (nano Ti c.) in Hank's solution. Closed symbols - with D-glucose, open symbols - D-glucose-free. [105].

As it is well known, the corrosion behaviour of metallic materials is strongly determined by environment. However, only a few reports on corrosion behaviour of UFG CP Ti in physiological solutions are available in the current literature [104]–[107]. Gurao et al. [104] indicated that grain size and crystallographic texture have a major effect on corrosion performance in simulated body fluid, although the material studied in that work presented quite a large variation in grain size (~ 0.2 – $10\ \mu\text{m}$) and lacked a very strong texture. Recently, Matykina et al. [105] studied the corrosion behaviour of CP Ti produced by ECAP-Conform combined with drawing, which showed a small grain size (150–200 nm) and a very strong crystallographic fibre texture with the $\langle 10\bar{1}0 \rangle$ direction and the (0001) basal planes parallel to the rod axis. Significant electrochemical anisotropy

of the material in the Hank's solution was found (represented as a large difference between the OCPs (Open Circuit Potential) of basal and prismatic planes in Figure 2-5 *a*, which manifested itself at different dissolution rates in the grains with basal (4 nm/year) and prismatic (1.2 nm/year) orientation. This anisotropy was further enhanced in the presence of D-glucose resulting in a corrosion potential difference between orientations with basal and prismatic grains of about 200 mV (Figure 2-5 *b*). It was also shown that the anisotropy strongly depends on the surface treatment of the sample (Figure 2-5 *a*), and it is significantly more pronounced in case of pickling in HF acid, which results in different surface roughness of the prismatic and basal grains. The electrochemical anisotropy can be reduced by pickling in HF/HNO₃-solution producing a homogeneous morphology with a lower surface roughness and similar electrochemical behaviour of prismatic and basal grains that results in uniform dissolution rate of the implant surface [105].

2.1.3.4 *Biocompatibility*

Other important functional properties of biomedical metallic materials include biocompatibility and bioactivity. From the biocompatibility perspective, CP Ti has proven as an ideal biomaterial due to its negligible harmful effect when Ti ions are released into human tissues [10], [66], [67]. Biocompatibility is enhanced by the excellent corrosion resistance of Ti due to the highly stable passive TiO₂ layer that forms naturally on the surface [1], [83]. However, CP Ti is bionert (i.e. has very low bioactivity) and does not bond directly to the bone, which can lead to small shifting and loosening of the implant during service life [67]. Nanostructuring of CP Ti has been shown to increase cell adhesion and proliferation in a wide variety of cell cultures [108]–[114]. For example, SPD processed UFG CP Ti showed higher cell adhesion (after 16 h) and proliferation values (up to 5 days) in mouse fibroblast cell culture 3T3 [108]. In another study, better wettability (i.e. lower contact angle) of UFG CP Ti samples compared to the CG material was reported [115]. Higher cell adhesion and proliferation was related to a larger volume fraction of grain boundaries, facilitating the adhesion of osteoblast cells. Crystallographic texture can also significantly affect the biocompatibility of the UFG CP Ti. Hoseini et al. [116] performed cell attachment tests using MC3T3 pre-osteoblast cells on CP Ti with varying grain size and crystallographic texture. The number of attached cells was found to be higher on the samples having more (0002) basal planes on the surface.

There are two main approaches to improve bioactivity of the surface of UFG CP Ti. First, it has been suggested that surface topography of the material plays a major role in cell colonization since the surface topography dramatically affects cell response [24], [117]. There are numerous studies on hierarchical modification of the surface of the nanostructured materials by combination of sandblasting, acid etching and alkali treatments. These surface modifications improved cytocompatibility and apatite-forming ability of Ti-based materials [118]. In [119], attachment and proliferation of osteoblast-like cells MG63 on CP Ti was slightly enhanced and the ability of the cells to form apatite was also improved via producing hierarchical porous surface. Nanoscale conical groove-like structures were revealed on the samples with higher bioactivity in [120]. While the cell density on the surface of CG CP Ti was about 53 % after 72 h, the surface cell occupation on the UFG CP Ti with much higher surface roughness was 87.2 % [117]. Significant effect of surface nanotopography on cell

adhesion and spreading was also observed during tests using human mesenchymal stem cells (hMSCs) attachment and spreading tests [121]. The results proved the positive effect of the grain refinement on the adhesion and spreading in the initial phases, being the biovolume up to 2.5 times greater on the surface of UFG CP Ti than on the coarse-grained counterpart, which was related to the richness of the surface morphology of the UFG CP Ti, with its more densely spaced and more numerous peaks at the nanoscale. However, the exact mechanism underlying this enhancement has not been understood yet. It should be noted that the biocompatibility of CP Ti can be improved by surface processing treatments that produce nanocrystalline surfaces, such as surface mechanical attrition treatment (SMAT) [111], [122].

The second approach to promote the formation of a bone-like layer on the implant surface and improve mechanical and tribological characteristics is the deposition of bioactive coatings [67]. This can be done via various chemical, electrochemical and physico-chemical methods. For instance PEO [37] and ALD [123], can be mentioned among those applied to UFG CP Ti. TiO_2 is well known for its high stability in most media and good biocompatibility [124], and it has shown HA forming ability in *in vitro* tests after soaking in simulated body fluid [125], opening the possibility to promote the osseointegration of Ti-based implants while enhancing the surface hardness and the corrosion protection. In addition, TiO_2 also possess interesting semiconductive, photocatalytic and optical properties which have made it a very attractive material for microelectronic devices [126], [127].

ALD has attracted a lot of attention from the research community and several industries due to its ability to produce thin coatings (from few tens of nm to few μm) with atomic level control of composition and thickness over large areas, complex shapes and nanoscale structures (i.e. nanopores and nanotubes) [128], [129]. Thin ALD-deposited TiO_2 coatings have been investigated for a wide range of applications in the biomedical field [130]: self-sterilizing devices and bacteria inactivation [131], [132]; drug and gene delivery [131], [133]; biosensors [134]–[136]; imaging applications [133]; and, filtering and molecular separation [137]. Furthermore, the HA forming ability of ALD-deposited TiO_2 has been also demonstrated [125], [138]. In a first step, Ca^{2+} ions deposit on the surface of TiO_2 forming amorphous calcium rich phosphates (ACP) [139]. Eventually, negatively charged HA crystallizes. Crystallization of HA was facilitated with crystalline anatase rather than with rutile due to a better lattice match [138]. Regarding the application to UFG CP Ti, Nazarov and co-workers [140] modified the surface of SPD-processed CP Ti with the combination of chemical etching (piranha solution) and a 20 nm thick ALD TiO_2 coating showing that the oxide coating enhanced the adhesion of osteoblasts cells with respect to both the untreated and the etched Ti surfaces in *in vitro* tests. In a more recent work [141], the authors demonstrated in *in vivo* tests that the ALD treated surface presented higher bone formation and a higher level of osseointegration through removal torque tests than both the untreated and the etched Ti screws. Fully crystalline ALD anatase films (~ 100 nm) deposited on silicon substrate at 300 °C by Lyytinen and co-workers [142] using TiCl_4 and H_2O as precursors showed hardness (H) and elastic modulus (E) values of ~ 9.7 GPa and ~ 165 GPa, respectively, by instrumented nanoindentation. The E of 200 nm titania films measured by Borgese et. al. [143] using the X-ray diffraction Debye Ring Analysis for Stress

measurement (DRAST) method [144] was of ~ 151 GPa.

The fabrication of hydroxyapatite (HA, i.e. hard phase of bone) or Ca- and P-containing coatings on CG CP Ti via PEO process has gained great interest during the last decade [145]–[148]. Although there are numerous works on the PEO treatment of CG CP Ti for biomedical applications, the number of research works devoted to the PEO treatment of UFG CP Ti is rather limited [37]–[39], [41], [149], [150]. The characteristics of the PEO process and its application to CG and UFG CP Ti are reviewed in the following section.

2.2 Fabrication of coatings via Plasma Electrolytic Oxidation

2.2.1 Characteristic of PEO treatment

The PEO process is a high voltage electrochemical surface modification technique commonly used for the fabrication of ceramic oxide coatings on aluminium, magnesium and titanium with the assistance of plasma micro-arc discharges [151]. PEO coatings are characterized by a strong adhesion to the substrate, hardness and wear resistance, and by a high corrosion protection [152]–[155]. Henceforth, PEO coatings are commonly applied for load bearing and corrosion protection applications. However, one of the most appealing characteristic of the PEO process for a wide range of applications is the possibility of modifying both the surface topography and the elemental composition by the addition of electrolyte elements via the plasma chemical reactions during discharges [29]. One of the most interesting applications of PEO coatings is the fabrication of calcium phosphates-based coatings for biomedical applications, especially in the case of CP Titanium and its alloys [156]–[160]. PEO coatings also offer interesting optical and photo-catalytic properties [161], [162] and low thermal conductivity which make them good candidates for thermal control applications such as the white and black Keronite coatings used in space missions [163], [164].

Figure 2-6. Anode plasma electrolysis processes taking place at: a) electrolyte-anode interface, and b) growing oxide [151].

Similarly to conventional anodizing, the work piece to be PEO-treated is immersed in an electrolytic cell as the anode. However, in contrast to anodization, the applied voltage is significantly increased until the so called breakdown voltage (V_B) is reached when sustained sparking on the surface is visible (U_5 in Figure 2-6). According to the model presented by Yerokhin et. al. [151] (Figure 2-6 *b*), an anodic oxide film grows following the Faraday and Ohm's laws on the substrate surface at relatively low voltages (U_1 - U_4 in Figure 2-6 *b*, i.e. Al - Al_2O_3 , Ti - TiO_2). This film is dissolved ($U_4 \sim$ corrosion potential) and subsequently repassivated as the voltage increases. The repassivation is followed by the dielectric breakdown of the oxide which allows for a faster grow of the film (U_5). At this point, the cell voltage increases at a lower rate and it might be accompanied by visible sparking on the surface of the oxide. However, the breakdown of TiO_2 might not be accompanied by sparking but by oxygen evolution. At higher voltages (U_6), larger discharges or micro-arcs appear. Finally discharges are sustained all over the surface being powerful enough to penetrate through the coating U_7 . The formation of new oxide/coating in PEO takes place by the action of discharges across the thickness of the forming coating rather than by continuous flow of ions as in anodizing [165]. While the second model (*b* in Figure 2-6 represents the processes taking place in the oxide film), the first model in [151] (Figure 2-6 *a*) represents the processes that take place on the anode-electrolyte interface, which involve the formation of a gaseous envelope and sparking within it (U_1 - U_2), followed by the formation of a gaseous vapour plasma envelope ($> U_2$). This model does not consider any impact on the anode material.

The discharges, have a local character ($\sim 10 \mu m$) and produce a plasma column across the thickness where both substrate and electrolyte ions combine with oxygen via plasma chemical reactions. However, the high temperatures and pressures that accompany the discharge affect also the adjacent coating (20-50 μm). It has been estimated that temperatures at the central core of the discharge could be as high as ~ 16000 K and in the range of 3000-4000 K at the adjacent coating [166]. The action of a discharge is terminated when the affected area becomes insulated by the newly formed oxide, while the discharging process takes place over other regions of the coating simultaneously. While the coating thickens with the treatment time, the morphology and phase composition of the coatings are continuously modified.

The typical morphology of PEO coatings consist on rough porous surfaces and complex internal porosities that are created by the main discharges, by secondary discharges and by gas evolution (Figure 2-7). Further, the common through-thickness morphology consist on: a thin (from hundreds of nm to few μm) inner nanocrystalline and nanoporous layer in intimate contact with the substrate, assigned to the oxidation of the substrate; and a thicker (from few to tens of μm) outer layer of compact oxide and micro porosity. PEO coatings are also characterised by the coexistence of crystalline phases and amorphous material within the outer layer. Indeed, the composition of these phases depends on the substrate (i.e. main oxide, Ti - TiO_2) and the electrolyte composition. However, the discharge characteristics in terms of temperatures attained and cooling process play also a major role in the sintering of the possible phases. The morphology, elemental and phase composition and growth rate of the coatings are ultimately determined by the discharge characteristics (i.e. duration, intensity and spatial distribution) and vice versa, the microstructural features influence the discharge

characteristics.

Figure 2-7. SEM characterisation of the surface (lower part) and cross section (upper part) of coatings fabricated in an alkaline electrolyte on a 6061 aluminium alloy at 1000 Hz, duty cycle 20 % and increasing current density 5-25 A/dm² [17].

Henceforth, the main factors affecting the PEO treatment of a given metal are the electrolyte used (i.e. composition, concentration, conductivity, solubility, etc.) [167]–[169] and the electrical signal applied during the process (i.e. voltage, current density, DC/AC mode, frequency, etc.) [170]–[172]. Other influencing factors are the stirring rate and temperature of the electrolyte.

The great interest in controlling the micro-discharge characteristics have led to thorough studies in order to understand the discharge initiation, lifetime and processes associated. Typical discharges taking place during PEO, termed as Type B discharges by Hussein and co-workers [173] (Figure 2-8 *a*), consist on a cascade of multiple single discharge events, where each single event may last from tens to hundreds of μ s, being the total time of the cascade of few to tens of ms [166], [174]. The process of a single discharge is now known to be as follows: *i*) An electron avalanche across the thickness occurs due to the dielectric breakdown of the TiO₂ at the coating-substrate interface. The electron avalanches increase the local temperature high enough to melt the surrounding coating. Subsequently, a discharge channel is formed through the thickness of the coating. *ii*) The substrate is rapidly consumed liberating ions into the channel. At the same time the strong electric field draws anionic components from the electrolyte into the channel. These events form a plasma column into the discharge channel. It is accepted that most of the new oxide is formed in this plasma column by plasma chemical reactions between metallic ions from the substrate and electrolyte-derived anions (including oxygen). *iii*) As the discharge shuts down, the plasma collapses condensing as a liquid. This new liquid is both deposited in the interior of the channel and expelled towards the surface. The oxide liquid solidifies rapidly by quenching at the contact with the cooler electrolyte.

Figure 2-8. Discharge types. a) types A, B and C as described by Hussein et. al. [24], and b) internal discharges type E and D as described by Cheng et. al. [30].

The production of oxide mass depend on the duration and intensity of the discharges which, at the same time, depend on the pulse-on time (t_{on}) and the pulse energy of the cell voltage signal (E_p), respectively [175]:

$$t_{on} = 1/f \cdot \text{duty cycle (\%)} \quad \text{Eq. (2.1)}$$

$$E_p = \int_0^{t_{on}} V_p I_p dt \quad \text{Eq. (2.2)}$$

Where f stands for frequency, and V_p and I_p are the voltage and current during the pulse. The duty cycle represents the portion of the pulse in the anodic polarization. According to Eq. (2.2), the intensity of the type B discharges would increase with the cell voltage, which increases with the treatment time forming larger discharge channels in the coatings. Following Eq. (2.1), the discharge characteristics can be controlled in pulsed DC and AC modes when the t_{on} approximates the total time of the discharge cascade, i.e. the discharge can be shut down if the t_{on} is shorter than the total cascade time. One must be aware that the cooling process is independent of the pulse time t_{on} . In turn, the allowed cooling time of a discharge affected region would increase as the duty cycle decreases. This offers the opportunity to control the sintering process of the possible phases between pulses. Additionally, secondary discharges might take place in following cycles as heated and

melted TiO_2 are more conductive [176].

Apart from type B discharges, other types of discharges have been observed. Together with type B, Hussein and co-workers [173] also found weaker type A and C discharges which take place at the surface of the coatings and below the surface within the outer layers of the coatings, respectively (Figure 2-8 a). The effect of these discharges is not as significant as in the case of type B and are only known to produce surface defects and porosity. According to extensive internal porosity and enhanced deposition of electrolyte-derived species in the inner part of PEO coatings, Cheng et. al. [177] proposed the type D and E discharges to occur within the electrolyte-filled internal porosity (Figure 2-8 b). Type D discharges were proposed to occur inside the cavities close to the inner layer. Type E, on the other hand, would take place through the outer compact coating above the cavities. Both types would cause the melting of the coating material and the generation of gas inside the cavities. The internal cavities could expand due to the gas pressure on the melted material and the oxide softened by heat. In addition, the type D and E discharges would be also responsible for the thickening of the inner layer (i.e. Ti substrate oxidation) and the outer compact layer, respectively. Henceforth, type D and E discharges would modify the internal structure of the coatings within the porosity generated by the type B discharges.

2.2.2 PEO treatment of CG and UFG CP Ti for biomedical applications

The industrial interest on CP Ti and its alloys motivated the study of their PEO processing in alkaline and acid electrolytes commonly used in conventional anodizing (i.e. Na_3PO_4 , H_2SO_3 , H_3PO_4 , etc.) to further improve the surface mechanical properties and the corrosion protection [162], [178]–[186]. The practical application of PEO process for fabrication of osteoconductive coatings on CG CP Ti gained interest during the last decade [145]–[148]. The first studies were performed on the synthesis of anodic titania films and the effect of electrolytes containing Ca and P species (i.e. sodium phosphates, calcium acetate ($\text{Ca}(\text{CH}_3\text{CO}_2)_2$), β -calcium glycerophosphate ($\beta\text{-C}_3\text{H}_5(\text{OH})_2\text{PO}_4\text{Ca}$), among others) [187]–[189]. The main objective of these treatments was to form coatings with a Ca to P ratio of 1.5-1.6 for the formation of amorphous calcium phosphate (ACP), α -tricalcium phosphate ($\alpha\text{-Ca}_3(\text{PO}_4)_2$, $\alpha\text{-TCP}$) and HA. In these electrolytes, Ca^{2+} forms negatively charged polyphosphate complexes (i.e. $\text{Me}^{\text{II}}_3\text{P}_6\text{O}_{18}\cdot\text{H}_2\text{O}$) that are drawn to the anode by electrophoresis. At the anode, the complexes undergo thermolysis and the insoluble products are deposited in the coating. Other works used other chelating agents such as ethylenediamine tetraacetic acid (EDTA) to form $[\text{CaY}]^{2-}$ complexes ($\text{Y}=[_2(\text{OOC})\text{NCH}_2\text{CH}_2\text{N}(\text{COO})_2]^{4-}$) to drive Ca ions to the coating where they combine with other species through plasma reactions [30], [176]. Various studies reported PEO oxide coatings including Ca- and P-containing phases such as CaTiO_3 , $\alpha\text{-TCP}$, $\beta\text{-Ca}_2\text{PO}_7$, CaCO_3 , CaO or HA [190]–[192]. The crystallization of these phases takes place at relatively high temperatures ($\sim 1000^\circ\text{C}$) and they are usually formed for treatment times ≥ 300 s. Post-thermal treatments have been applied to promote the crystallization of HA [193]–[195]. PEO TiO_2 -based Ca- and P-containing coatings provide an appropriate combination of favourable surface topography and composition to promote the cell proliferation and adhesion and the development of extracellular matrix in *in vitro* tests [176], [196]. Immersion in simulated body fluid (SBF) have also shown

the deposition of HA on the surface of the coatings [197]. Osseointegration has been proven to be accelerated in *in vivo* tests [198]. On the other hand, the successful application of these coatings in clinical tests have been demonstrated (i.e. TiUnite, NobelBiocare) [199], [200].

The number of research works devoted to the PEO treatment of UFG CP Ti is rather limited in comparison with the number of works on CG CP Ti [37]–[39], [41], [149], [150]. In comparison with PEO-coated CG CP Ti, PEO coatings fabricated on UFG CP Ti tend to show higher growth rates, larger amounts of electrolyte-derived species and larger fractions of high temperature phases such as rutile. However, significant differences in elemental and phase composition tend to appear for treatment times beyond 2 mins. For instance, Yao and co-workers [37] showed that the thickness of the coatings fabricated for 2 minutes on the CG and UFG CP Ti were of 6 and 10 μm , respectively. The coating fabricated for 20 mins in [37] had a thickness of 20 μm in the CG substrate while the thickness of the coating on the UFG substrate was found above 50 μm . Alsaran et. al. [38] reported that the Ca/P atomic ratio increase from 1.4 in the CG substrate to 1.7 in the UFG substrate after a 7.5 mins treatment. For the 5 mins treatment in [37] the atomic ratio increased to 1.58 on the UFG substrate in comparison to the atomic ratio on the CG substrate of 1.18. After 20 mins of treatment the ratio in the UFG substrate increased up to 1.96 and to 1.57 in the CG substrate. Regarding the phase composition, the coatings fabricated on the UFG CP Ti showed higher amounts of rutile and earlier crystallization of Ca/P-containing compounds such as HA and α -TCP in comparison with the PEO-coated CG CP Ti. These results indicate that the temperatures achieved on the UFG CP Ti were higher than on the CG CP Ti. On the other hand, Parfenov et. al. [3] noted a slightly lower fraction of crystalline phases in the UFG substrate in comparison to the CG substrate (29.5 % and 32.2 %, respectively) which might be associated to higher contents of electrolyte derived species that are known to deposit preferentially as amorphous material and to inhibit the crystallization [201]. This indicates that the sintering of crystalline phases during PEO is not trivial and that it may be extremely sensible to the synergy of the electrolyte composition, the processing parameters and the solidification properties.

The aforementioned works concluded that these differences could be attributed to the higher chemical activity of the surface of UFG CP Ti due to a larger fraction of high-energy grain boundaries and dislocations [202]. The defects on the surface of UFG CP Ti might act as nucleation sites for TiO_2 . Due to the greater number of nucleation sites, a homogeneous distribution of microdefects might be formed within the growing anodic film which could serve as more TiO_2 nucleation points, oxygen evolution sites and dielectric breakdown points. Accordingly, high density of microdefects would lead to a higher density of discharges [203], which would subsequently result in earlier stable sparking conditions [149] and thicker coatings [33], [175], [176], [204]. In terms of corrosion protection, coatings on UFG CP Ti have been observed to provide better corrosion protection [149] than coatings on CG CP Ti, whereas the latter presented a nobler response [150]. The H and E reported for PEO coatings independently of the substrate state might be found in the ranges 3-6 GPa and 70-140 GPa, respectively [186], [205]–[207].

3 Materials and experimental procedures

3.1 Materials and processing

3.1.1 *Processing of CG CP Ti and UFG CP Ti*

The base material used in this Thesis was a CG CP Ti (Grade 4) with an average grain size of about 25 μm . The impurities content in wt. % are presented in Table 3-1. The as-received material in the shape of hot-rolled rods of 12 mm in diameter were processed by a specific SPD process to obtain the UFG CP Ti at the Ufa State Aviation Technical University. The SPD process consisted in the ECAP method following the C scheme (Figure 3-1, *a*). The ECAP-C process was carried out at 200 °C throughout a die having an angle of 120°. This configuration facilitates the production of 11 x 11 mm² (in cross section) UFG Ti billets and the accumulation of ~0.7 strain per pass. Six ECAP-C passes were performed resulting in a total strain of 4.2. The ECAP-C processing was followed by drawing at 200 °C inducing an equivalent strain of 0.6 to produce a rod of 6 mm in diameter [21], [64].

This processing methodology led to the formation of a homogeneous UFG microstructure characterized by an average grain size of about 200 nm, a high dislocation density of $\sim 10^{15} \text{ m}^{-2}$, and a high fraction of high-angle grain boundaries. The size of coherently scattering domains (CSD) decreased to ~26 nm, while the level of elastic microdistortion of crystal lattices increased to $\sim 23 \cdot 10^{-4}$ (Figure 3-1, *b*) [21]. The process favoured the development of a fiber-like texture as demonstrated by the pole figures and as schematized in Figure 3-1 *c* and *d* respectively, with the basal and prismatic planes parallel and perpendicular to the rod/extrusion axis, respectively [105].

Table 3-1. Impurity content in wt. % of the as-received CP Ti grade 4.

Ti	C	Fe	N	H	O
Base	0.04	0.14	0.006	0.0015	0.36

Table 3-2. Yield strength, ultimate tensile strength and elongation to failure of the as-received CG CP Ti, and their evolution during applied processing[21].

Condition	σ_y (MPa)	σ_{UTS} (MPa)	ε_f (%)
as-received	625	760	29.3
ECAP-C 6 passes	973	1020	13.7
ECAP-C 6 passes + drawing	1190	1230	11

Gunderov et. al. [21] showed the beneficial effect of the ECAP process on the mechanical properties of the CP Ti, which were further enhanced after the subsequent drawing (Table 3-2). CG CP Ti samples for PEO coating fabrication were cut directly from the as-received rods in discs having about 3 mm in thickness and about 12 mm in diameter.

Figure 3-1. a) ECAP-C processing method, d) TEM bright field image and diffraction pattern (inset) of the CP Ti after ECAP-C 6 passes and subsequent drawing [64], c) pole figures, and d) schematic of the macrotexture developed [105].

The UFG CP Ti samples for chemical etching were cut directly from the SPD processed rods having the shape of small discs of about 3 mm in thickness with diameter around 5 mm. Two requirements were set for the fabrication of UFG CP Ti samples for further fabrication of the PEO coatings: *i*) guaranteed coated surface area of 1 cm² for future corrosion tests; and, *ii*) a flat surface and a counter parallel surface were required to ensure a correct testing of the coatings by means of nanoindentation. The SPD-processed rods were cut into 30 mm in length segments and parallel surfaces were milled until a thickness of about 3 mm was achieved.

3.1.2 Chemical etching

UFG CP Ti was treated by chemical etching using different HF/HNO₃-based acid mixtures and treatment times to produce seven surface conditions, as summarized in Table 3-3. Surface treatments were designed and performed by producer of dental implants Timplant (www.timplant.cz). The commercial concentrations of the acids were of 50 wt. % and 63 wt. % for HF and HNO₃, respectively. The first surface belongs to the as-prepared sample (i.e. before etching) that served as a reference for the following etching treatments. The

samples 1A and 1B were subjected to the treatment by chemical solutions having the same HF/HNO₃ molar ratio for the same treatment time. However, 45 ml of water were added in the case of the 1B etching solution. For the samples 2A and 2B, the same mixture was used but the treatment time was increased from 3 to 5 min, respectively. The samples 3A and 3B were subjected to etching treatments using HF:HNO₃:H₂O solutions developed by producer of dental implants, Timplant (www.timplant.cz).

Table 3-3. Summary of samples and acid etching treatments: solution composition and treatment time.

Sample	Treatment	
	Solution composition (volume ratio)	Time (min)
Non-etched	-	-
1A	1:4.5 - HF:HNO ₃	5
1B	1:4.5:4.5 - HF:HNO ₃ :H ₂ O	5
2A	2:1 - HF:HNO ₃	3
2B	2:1 - HF:HNO ₃	5
3A	Timplant I ¹	-
3B	Timplant II ¹	-

3.1.3 PEO synthesis

The plasma electrolytic oxidation technique was applied to both CG and UFG CP Ti. The electrochemical set-up is schematized in Figure 3-2. The treatments were carried out in a double-walled cell (1) connected to a water thermostat (2) to maintain the temperature of the electrolytes at 20 °C. A stainless-steel (AISI 316) plate was used as counter electrode (3, cathode). An AC EAC-S2000 power supply (ET Systems Electronic) was used to generate the voltage signal for the process (4). An external computer (5) was connected to the system in order to control the power source, to program the PEO treatment and to record the voltage and current signals using a LabView software. The sample to be coated was connected as the working electrode (6, anode). The electrolytes were continuously stirred during the process. As a standard surface preparation procedure for PEO coatings fabrication, CG and UFG CP Ti samples were ground down by 600 grade silicon carbide sandpaper. The electrical connection of the Ti samples was performed by copper-wired metallic clamps. The sample-clamp mounting was painted with an electrical-insulating Lacquer 45 resin (MacDermind plc.) exposing only the area to be coated. In the case of the CG samples this area was about 0.7 cm² and of 1-1.2 cm² for the UFG samples. The area of each sample was measured in order to take it into account for the setting of PEO current and for corrosion tests. A square waveform AC voltage signal was applied. The signal had a positive-to-negative voltage amplitude, V+/V-, of 490/-30 V and a frequency of 300 Hz. The current density was limited to a maximum of 0.3 A/cm². An initial ramp of 60 s was used to reach the pre-set V+/V- values. Coatings were fabricated for 60, 90 and 120 s. The composition, pH and conductivity of the three electrolytes used are presented in Table 3-4. 1 L of each solution was prepared using high purity grade chemicals. The surface of the materials was cleaned with ethanol before and rinsed with deionised water after the PEO

¹ Timplant Company know-how

treatment.

Figure 3-2. Schematic of the electrochemical set-up for the PEO coatings fabrication: 1) Electrolytic cell, 2) thermostat, 3) cathode, 4) power supply, 5) computer, and 6) anode.

In further text, the coatings are referred to using the following code: $c/n-C/S1/S2-6/9/12$; where c stands for *coarse-grained* (i.e. CG) and n for *nanostructured* (i.e. UFG); C, S1 and S2 correspond to the treatment; and 6, 9, and 12 to the treatment times 60, 90 and 120 seconds. For example, the nS2-9 refers to the coating using the electrolyte S2 on the UFG substrate for 90 seconds.

Table 3-4. Composition, pH and conductivity of the electrolytes.

Composition	Amount	pH	Conductivity (mS/cm ³)
C		11	15.55
$C_6H_{10}CaO_6 \cdot 5H_2O$	0.05 M		
$Na_2(EDTA) \cdot 2H_2O$	0.055 M		
$NaH_2PO_4 \cdot 2H_2O$	0.025 M		
NaOH	0.15 M		
S1		12	7.91
Na_3PO_4	0.026 M		
S2		1	55.1
H_3PO_4	1 M		

3.2 Microstructural characterization

3.2.1 *Scanning electron microscopy*

Scanning electron microscopy (SEM) is a well-known technique in the materials science field due to its huge potential to reveal microstructural features in the micro- and nanoscale by scanning the surface of a sample with a stable electron beam. The beam is focused through electromagnetic fields and lenses towards the surface. The electron beam excites a certain volume of the material near the surface. As a result of this interaction, low-energy secondary electrons, high-energy backscattered electrons and characteristic X-rays scape the affected region. Typical SEM images are a reconstruction of the collected secondary electrons. Backscattered electrons are an important source of information for the study of crystalline materials (i.e. Electron Backscatter Diffraction, EBSD). Elemental composition information is obtained by the analysis of the characteristic X-rays from the surface (i.e. Energy Dispersive X-Ray Spectroscopy, EDS or EDX) [208].

An important factor influencing the application of SEM is the electrical conductivity of the sample to be analysed. For instance, polymers and ceramic materials might require to be coated with a fine layer of gold of tens of nanometres, and it is paramount to ensure the conductivity of the sample to the microscope stage. Poor image definition, image drifting and surface static charging might arise if this requirement is not met. Another essential condition is that SEM has to be performed in vacuum to ensure the optimal generation of the electron beam and to avoid the interaction of the primary electrons and subsequent signals with any particle.

In this work, SEM was used not only for evaluation of the different surface morphologies encountered, but also as an essential tool in the post-analysis of nanoindentation and nanoscratching tests. Two SEM systems in combination with other techniques, such as EDX or Focused Ion Beam micro-machining (FIB), were used depending on the application. For examination of surfaces, a SEM EVO MA15 microscope (Carl Zeiss, Oberkochen, Germany) was used in combination with EDS. The SEM was operated at an accelerating voltage of 20 kV and currents between 291 pA and 1.7 nA. A double beam FIB-FEGSEM (Field Emission Gun) Helios NanoLab 600i (FEI company, Oregon, USA) was used at 5 kV and 0.69 nA for examination of nanoindentation prints and TEM lamella fabrication, while cross-section examination was performed at 30 kV and 1.4 nA.

3.2.2 *Atomic force microscopy*

The atomic force microscopy (AFM) is a scanning probe microscopy (SPM) technique, which uses the weak repulsive/attractive forces between the atoms of a probe tip and a surface to collect topographical information within the range from few tens of micrometres ($> 100\text{ }\mu\text{m}$) down to a few nanometres ($\sim 1\text{ nm}$) [209]. A fine tip at the free end of a cantilever deflects as it scans the surface. The deflection is measured by a laser beam pointed at the back of the cantilever generating a signal that is processed to form a 3D image. In this work, the atomic force microscope was operated in the non-contact mode, as it prevents from excessive degradation of the tip and sample. In this mode, the cantilever is mechanically oscillated over the surface slightly above its resonant frequency resulting in oscillating amplitudes below 10 nm. Close enough to the surface, weak attractive and repulsive forces induce a change in the phase of the vibration amplitude of the cantilever. This

shift is recorded and provides a feedback signal for imaging and to adjust the tip to sample distance. AFM is extremely useful to characterize surface features from tens of nanometers up to an upper limit of about 10 μm in height.

The atomic force microscope Park XE150 (Park Systems, California USA) was used in this work to characterize the surface roughness of the chemically etched UFG CP Ti samples and PEO coatings. The following surface roughness parameters were measured: S_a , average surface roughness; S_{sq} , root mean square surface roughness; S_{kw} , surface skewness; and S_{ku} , surface kurtosis. Typically, three 80 x 80 μm representative areas were scanned. Whenever possible, AFM was also used to obtain images of the wear tracks after nanoscratching to measure the corresponding wear volume.

3.2.3 *Focused ion beam*

A focused ion beam (FIB) can be used in tandem with a SEM system to perform site-specific deposition or erosion of materials. Instead of an electron beam, a beam of high-energy Gallium ions is focused onto the surface of a material as a micro-machining tool. Milling areas and depths are generally within the range of tens of microns down to hundreds of nanometers (i.e. 50 μm to 100 nm) [210]. It can be operated at different accelerating voltages and currents allowing for heavy sputtering of the target material (30 kV and 21 nA) or for precise fine polishing (5 kV and 15 pA). The erosive action of the beam is influenced by the interaction of the ions and the material to be eroded.

In this work, the focused ion beam was used to perform micro-machining operations with the following objectives:

- i. Unspoiled cross-section examination of PEO coatings: In order to have a detailed view of the coatings cross-section without damage the FIB was used at 30 kV and currents between 21 and 0.43 nA. The material at the region of interest is sequentially sliced off lowering the emission current at each step.
- ii. Preparation of site-specific lamellas for Transmission Electron Microscopy: TEM observation requires specimens with a thickness below 200 nm in order to ensure the transmission of the electron beam through the material. The FIB has the advantage of allowing to obtain TEM specimens at a specific site up to 50 μm wide and depth. The process consists on a sequence of steps in which a ~ 1 μm thick slice of the material is extracted from the sample and placed onto a copper grid designed for its handling into the TEM microscope. Once on the grid, the slice is thinned down to about 100 nm. During the slicing and extraction stages, the FIB is typically operated at 30 kV and at currents from 21 nA to 0.43 nA. The main objective during the thinning stage is to thin the slice down without damaging it. Hence, at 30 kV currents below 0.43 nA were used. When the thickness becomes close enough to 100 nm the FIB was operated at 5 kV and currents about 15 pA to obtain the finest result.
- iii. Tomography: In order to gain a better understanding of the large and complex porosity found in the PEO coatings, it was decided to perform a tomography study of one of the coatings. A 25 μm wide, 20 μm long, and 20 μm deep volume was sliced at a step size of 48 nm using the FIB at 30 kV and 0.43

nA. After each slicing a new cross-section of the coating was revealed and an SEM image was taken. All the images were segmented in order to obtain binary black and white images, where the areas of interest (i.e. pores or coating) would take one of the two colours. Once segmented, a 3D volume could be reconstructed using the Avizo software (Thermo Fisher Scientific). Statistical analysis of the porosity volume was obtained using this software (i.e. total porosity, extension, characteristic volumes, etc.). Top-to-bottom plane-by-plane analysis was performed reconstructing the volume in ImageJ software to obtain the pores covered area to total analysed area ratio.

3.2.4 *Energy dispersive X-ray spectroscopy*

Energy dispersive X-ray spectroscopy (EDX) is a characterization technique that uses the characteristic X-rays emitted from the surface under the electron beam of an SEM system to obtain information about the elemental composition of the material [208]. As explained in the section 2.1, the electron beam of an SEM penetrates into the surface for a few microns and causes the emission of secondary electrons and characteristic X-rays from the surface. Primary electrons penetrating the material interact with ground state electrons ejecting them from their energy levels and generating holes in the atomic structure. Electrons at higher-energy levels fill these holes. In doing so, the excess of energy is emitted in the form of an X-ray (i.e. K-alpha lines). A specialized energy dispersive detector can collect these characteristic X-rays of the order of keV and construct a spectrum where each intensity peak (or combination of peaks) corresponds to the characteristic X-ray energy of a given element. Qualitative and quantitative information can be extracted from these spectra about the elements presence and their concentration percentages (i.e. wt. % or at. %)

An Oxford INCA 330 microanalysis system attached to the SEM EVO MA15 was used in this work to quantify the elemental composition of the PEO coatings. The electron beam was used at 20 kV enough to excite the heaviest element found in the samples (Ti), and the current and processing time (generally 1.4 nA and 3-4, respectively) were adjusted to obtain a suitable combination of counts and dead time (> 11000 counts and a dead time in the range from 10 to 20 %, respectively). To quantify the spectra, the processing algorithm was calibrated with pure Co. The EDX detector in the FIB-FEGSEM Helios NanoLab 600i was used to identify the elemental composition around the indents after the nanoindentation of PEO coatings to correlate the hardness and Young's modulus measured by nanoindentation with the local composition.

3.2.5 *X-Ray diffraction*

X-ray diffraction (XRD) is a technique extensively used in materials science for the identification and quantification of the phases that might be present in crystalline materials, such as metals and ceramics, both in bulk and powder. Short wavelength X-rays photons (1 keV – 120 keV) interact with crystal structures resulting in both elastic and inelastic scattering (Thompson and Compton scattering, respectively). When the X-ray beam interacts with a crystal lattice at a given orientation fulfilling the Bragg's law (Eq. (3.1)) a strong diffraction (peak) takes place due to constructive elastic scattering of the X-rays (Figure 3-3).

In Eq. (3.1): d stands for the inter-planar distance of the lattice, θ corresponds to the incident angle, n is an

integer representing the order of the diffraction, and λ the wavelength of the X-rays. Hence each strong diffraction or peak corresponds to a single set of planes of a lattice. A single phase generates few diffractions according to its lattice parameters at different incident/diffraction angles. The resulting XRD spectrum is a combination of characteristic peaks of a single phase, which typically have constant relative intensity ratios with respect to the predominant diffraction. Cross-checking the spectrum with a data base one can identify the target material. For instance, the main diffractions of the CG CP Ti using a Cu cathode are: 35° ($d = 2.55 \text{ \AA}$, (010)), 38° ($d = 2.35 \text{ \AA}$, (002)), 40° ($d = 2.25 \text{ \AA}$, (011)), and 63° ($d = 1.47 \text{ \AA}$, (110)); corresponding to the hexagonal closely packed (hcp) α phase of CP Ti with the following lattice parameters: a , $2.95 \text{ \AA}$; and c , $4.69 \text{ \AA}$. When several phases are present in the material, the resulting spectrum will be composed by several combinations of diffractions that can be identified by cross-checking with a data base. The amount of each phase can be quantified by fitting the spectrum using, for example, the Rietveld method.

$$2d \sin \theta = n\lambda \quad \text{Eq. (3.1).}$$

Figure 3-3. Schematic representation of X-Ray diffraction.

The X-ray sources typically used are Cu or Co cathodes which emit X-rays with wavelengths of $1.54 \text{ \AA}$ and $1.78 \text{ \AA}$, respectively. Depending on the application, different measuring set ups can be used such as the generally used Bragg-Brentano mode or the Grazing Angle X-ray Diffraction typically used for study of thin films. During this Thesis substrates and coatings were analysed using the Bragg-Brentano configuration using a Cu cathode with a scan range from 20° to 80° and a step size of 0.0026° .

3.2.6 Raman spectroscopy

Raman spectroscopy is a fast and versatile tool that allows to obtain accurate information about the microstructure and electronic properties of a material by analysing the inelastic scattering of visible light photons by a molecule or a lattice (Raman Scattering) [211]. Extensively used in the field of carbon-based materials (i.e. carbon nanotubes, graphene, glass-like carbon, etc.) to evaluate the microstructure and quality, Raman spectroscopy can also be applied to metallic oxides such as those of titanium and copper. Raman spectroscopy offers three main advantages with respect to XRD:

- i. Contributions from both crystalline and amorphous phases can be accounted for.
- ii. Site-specific measurements with a laser spot radius of 10 μm .
- iii. Fast measurements: < 60 s.

An incident laser beam is focused on the material. While most of the incident photons undergo elastic scatter, a small part excites the atomic bonds of the material exchanging energy with it due to inelastic scattering. The crystal bonds enter in a higher-energy vibrational state while the scattered photons lose a Δ of energy equal to the energy required to excite the crystal structure. This shift in energy is recorded and represented in the Raman spectrum in cm^{-1} as peaks or wide bands depending on the level of crystallinity. The energy exchange depends on the type of bond and the crystal structure. Analogous to the XRD spectra analysis, a combination of Raman shifts can be related to a given phase. Raman spectra of ALD TiO_2 films grown on Silicon are presented in Figure 3-4 [212]. The figure illustrates the capability of Raman to differentiate not only between TiO_2 phases (A stands for anatase and R for rutile), but crystalline and amorphous TiO_2 . Highly ordered crystal phases result in sharp peaks, while amorphous material usually produces wide low intensity bands.

Figure 3-4. Structural analysis of ALD TiO_2 thin films by micro-Raman spectroscopy [212].

A inVia confocal Raman microscope (Renishaw, Wotton-under-Edge United Kingdom) with a green laser (532 nm) at 10 % of its power during 1 s of exposing time for 3 accumulations. The measurement was centred at 946 cm^{-1} . Ten measurements per sample were performed at different sites. The degree of crystallinity was evaluated by the relative ratios between the total area of crystalline bands and the total area of amorphous wide bands and the width at half of the maximum (FWHM) of the peaks.

3.2.7 Transmission electron microscopy

Transmission electron microscopy (TEM) is a high resolution (< 0.25 nm) microscopy technique extensively used in the materials science field for the detailed study of the atomic arrangement in crystalline structures

[213][214]. A focused electron beam is accelerated at high energies (~ 200 kV, 0.0027 nm wavelength) through thin specimens (< 200 nm in thickness). Along the beam path, the incident electrons may be scattered (i.e. elastic and inelastic) by the atoms within the sample. In turn, a transmitted (non-scattered) and a diffracted (scattered) beam are produced behind the sample. Three main imaging modes are typically used depending on the position of an objective: bright-field; dark-field; and high resolution microscopy. In bright-field mode, the diffracted beam is blocked, while the transmitted beam is used for imaging using a small objective aperture. The result is a contrast image depending on the scattered portion of the incident beam. On the contrary, in the dark-field, the diffracted beam is used either by tilting the incident beam or by using an annular detector. As an example, porosity or defects, such as dislocations, which do not disturb the incident beam, would generate a bright point or region in the bright-field mode, whereas in the dark-field imaging, a dark region would be observed. Diffraction of the incident beam might be given by interaction with an atomic core or an electron cloud. The heavier the atom, the larger the scattering (both elastic and inelastic) and a darker image is obtained in the bright-field imaging mode. Much important is the interaction between the incident beam and crystalline structures. When the orientation of a lattice with respect to the incident beam fulfils the Bragg condition Eq. (3.1), a strong diffraction takes place and the crystal appears black in the image (in bright-field mode). From this interaction, diffraction patterns can be obtained to characterize the phases. Apart from the transmitted and diffracted beams, the incident electron beam in TEM generates the same signals that are generated in SEM: secondary electrons, backscattered electrons, and characteristic X-rays.

In this work, TEM was used to characterize the through thickness morphology, crystalline character and elemental composition of the PEO coatings. Due to the complex microstructure of the PEO coatings, the following goals were pursued:

- i. Evaluation of the coating/substrate interface
- ii. Distribution of crystalline and amorphous TiO_2
- iii. Distribution of P and Ca
- iv. Gain insights into the solidification and crystallization processes of TiO_2 during PEO:
 - Identification of TiO_2 phases formed and distribution
 - Characterize the crystal size, shape, and distribution
 - Influence of Ca and P in the TiO_2 solidification and crystallization.

The microscope used was a FEG S/TEM Talos F200X (FEI) equipped with an EDS detector. The microscope can operate in both TEM and STEM (Scanning) modes having lateral resolutions at 200 kV of less than 0.25 nm and 0.16 nm, respectively. Bright-field images were taken in TEM mode at 200 kV. Diffraction patterns were taken with objective apertures of 400 and 100 nm radius. Quantitative elemental analysis by EDS was performed using High Angle Annular Dark Field (HAADF) images in STEM mode. Elemental maps were taken to evaluate the elements distribution through the thickness at low magnification ($\sim \times 14\text{k}$) and at high magnification ($\sim \times 160\text{k}$) at crystalline regions to observe the distribution of Ca and P in these regions.

3.3 Mechanical characterization

3.3.1 Hardness and Young's modulus measurement: Nanoindentation

Instrumented nanoindentation is a depth-sensing indentation testing technique which allows for the determination of the hardness and the elastic modulus of small volumes ($\sim 7 \text{ um}^3$), as it operates at depths down to hundreds of nanometres ($\sim 100 \text{ nm}$) and loads of the order of mN ($\sim 1 \text{ mN}$) using a sharp tip (tip radius $< 200 \text{ nm}$) [215].

The main feature is the continuous recording of both vertical displacement and applied load during the indentation with resolutions of less than a nanometre and of few nN. With this data, it is possible to calculate the experimental H and E of a material following the methodology developed by Oliver and Pharr [216]. Figure 3-5 represents a typical load vs. displacement (P vs. h) loading-unloading curve and the experimental parameters that can be extracted from it. The experimental stiffness (S_t), can be estimated by fitting the linear part of the unloading curve (Eq. (3.2)). The contact depth h_c is then calculated (Eq. (3.3)). As the geometry of the tip is known, an area function (dependent on the tip geometry) is used to calculate the contact area A_c at the h_c (Eq. (3.4)). Hardness (H) is then calculated, as it is defined, dividing the maximum load by the contact area (Eq. (3.5)).

Figure 3-5. Load vs. displacement curve [215].

$$S_t = \frac{dP}{dh} \quad \text{Eq. (3.2)}$$

$$h_c = h_{max} - \epsilon \cdot \frac{P_{max}}{S_t} \quad \text{Eq. (3.3)}$$

$$\epsilon = 0.72^2$$

$$A_c = f(h_c) \quad \text{Eq. (3.4)}$$

$$H = \frac{P_{max}}{A(h_c)} \quad \text{Eq. (3.5)}$$

To calculate the Young's modulus of the material, a reduced modulus (E_R) must be defined to account for the effect of a non-rigid indenter on the load-displacement behaviour. The reduced modulus can be estimated through S_t and A_c (Eq. (3.6)) and it is related to the elastic modulus of the material (E) by Eq. (3.7):

² Value for a Berkovich indenter.

$$E_R = \frac{\sqrt{\pi}}{2} \cdot \frac{S_t}{\sqrt{A(h_c)}} \quad \text{Eq. (3.6)}$$

$$\frac{1}{E_R} = \frac{(1-\nu^2)}{E} + \frac{(1-\nu_i^2)}{E_i} \quad \text{Eq. (3.7)}$$

Being ν the Poisson's ratio and the subscript i referring to the indenter. As in the present work, most commercial indenter tips are usually made of diamond, hence: $E_i = 1050$ GPa and $\nu_i = 0.07$. The Poisson's ratio of the material used in this work was the corresponding to the anatase phase of TiO_2 : 0.27.

Instrumented nanoindentation is extremely sensible to the morphology of the tested material. Excessive roughness and/or tilting of the surface, or discontinuities within the testing volume might disturb the early stages of the test, falsify the final outcome, or introduce artefacts during the load application, respectively. When the scale of these features is comparable to the required indentation depth it is assumed that the testing condition is away from the ideal situation and the results are no longer valid. The morphology of the PEO coatings studied herein is marked by a heterogeneous character, surface roughness and significant inner porosity, which prohibited the mechanical testing of the coatings directly on their surface. In order to obtain reliable data, the nanoindentation tests were carried out on the cross-section of the coatings. In doing so, it was possible to get rid of the surface roughness and to select the indenting points away from pores and cavities to minimize their influence on the recorded data. Nevertheless, as most of the H and E data of PEO coatings reported in the literature was obtained by surface indentation, cyclic indentations up to a maximum load of 1 N were also performed.

Initially, 3 mm sections were cut from the coated samples and embedded in epoxy resin so their cross-section could be subjected to traditional metallographic preparation through successive grinding and polishing steps. This process failed to provide smooth surfaces, and it damaged the coating. In addition, it produced significant tilt across the coating with respect to the extremely low indentation depth selected. The IM4000Plus Ion Milling system (Hitachi) was finally used to produce unspoiled 1 mm wide flat and featureless cross-sections of the coatings. For the purpose of this Thesis, the system was used in the cross-section milling configuration.

Nanoindentation tests were performed on a Hysitron TI950 Triboindenter (Hysitron, Minneapolis, USA). A diamond Berkovich tip with a radius of 100 nm was used for the nanomechanical characterization of the coatings. The tests consisted on constant strain rate (CSR) loadings in displacement controlled mode to a maximum indentation depth of 150 nm. This indentation depth was selected to minimize the effect of medium-size pores and cavities by keeping the elastic fields as small as possible while producing enough plastic deformation to obtain reliable results. According to Chen and Bull estimation, a Berkovich tip at this depth, for our material (i.e. E/H ratio³), would produce a spherical elastic region with a radius (R_e) of $\sim 1.5 \mu\text{m}$ [217]. Hardness and elastic modulus of the coatings were obtained following the Oliver and Pharr method. The

³ Ratio estimated from nanoindentation results on samples prepared by traditional metallographic method.

indentation prints were inspected by SEM and AFM to confirm the contact area calculated and to account for any pile-up or sink-in. To minimize the thermal drift of the tip and correct the machine compliance, several cyclic indentations at 12 mN were performed on the UFG CP Ti substrate prior to the indentation of the coating.

In practice, the thickness and inner porosity of the coating limited the available coating volume (or area) to be tested. Thus, the locations for the indentations were selected by SEM, and located using the SPM tool of the TI950 system, which allowed precise positioning of the tip. The R_e estimation was used as the minimum distance with respect to pores and cavities. The EDS technique was used to measure the composition in 3 μm in diameter circular areas around the indents. In the case of the nC-12 coating, a second batch of indentations was performed selecting the indentation position according to the local content of Ca.

3.3.2 Tribology: Nanoscratching of PEO coatings

Scratch testing is one of the most popular techniques to assess the adhesion and wear of coated systems [218], [219]. A nanoscratching test consists on a nanoindenter stylus moving laterally on a surface while a normal load of the order of mN is applied. The lateral and normal displacements and loads during the tests are recorded. This data is analysed in combination with optical, SEM and/or AFM images of the scratches. Two scratching methodologies were used in this work:

- i. Progressive loading scratch tests: The indenter is drawn along a given length while the normal force is increased from zero up to a set maximum load. The main objective of these tests is to identify the failure modes that might take place: cracks, cohesive loosening, and delamination/adhesion failure, amongst others. The critical loads at which the failure events took place are subsequently reported.
- ii. Constant load reciprocating scratch tests: At a constant normal load, the stylus is drawn several times over the same length to evaluate the wear behaviour of the coated system. Two main parameters are to be defined at a given constant load: the number of cycles necessary to produce the wear failure of the coating and the wear rate. The wear failure of the coating is defined herein as the point at which the substrate is reached by wear or delamination at the track; or at which delamination or cohesive loosening takes place at the sides of the scratch. The wear rates (W) were calculated as follows:

$$W = \frac{V_w}{P \cdot L} \quad \text{Eq. (3.8)}$$

Being V_w is the wear volume, P the applied load and L the total distance.

Nanoscratching tests were performed under the same conditions over the uncoated CG and UFG CP Ti and over the coated substrates after the PEO C, S1 and S2 treatments at 120 s. A diamond conical tip with a tip radius of 10 μm was used. Loading scratches were performed from zero up to a maximum load of 500 mN with a loading rate of 1 mN/ μm and at a speed of 5 $\mu\text{m/s}$. The surface was scanned with the same tip before and after the test. Failure modes were identified by means of SEM. The distance of these failures to the initial point of the scratch were measured and compared with the load and depth profiles recorded.

Constant load reciprocating scratches were performed according to the critical loads registered during the loading scratches: 50, 100 and 150 mN. Scratches were run for 10, 20 and 30 cycles at each load along 70 μm at 2.3 $\mu\text{m/s}$. The wear volumes were measured by stereoscopy using SEM images of the remaining tracks at 0° and 5°. 3D models were reconstructed using the MeXAlicona software. The volume calculated consisted on the volume at the track and at the side.

3.4 Functional properties

3.4.1 *Electrochemical behaviour: corrosion resistance*

The electrochemical behaviour of the PEO coatings was evaluated by means of DC potentiodynamic tests and AC electrochemical impedance spectroscopy (EIS). Direct current potentiodynamic tests allow for the determination of the corrosion rate, the corrosion potential (E_{corr}) and current (j_{corr}), and the passive or active potential range of a material immersed in a specific medium. During the tests, the specimen is polarized over a voltage range with respect to the open circuit potential (OCP) of the material while the current density flowing through the system is recorded. The electrochemical behaviour of the material can be analysed by interpretation of the resulting potential vs. current curve. Corrosion takes place at the point at which both the anodic and cathodic parts of the curve converge. For Ti-based materials the E_{corr} and j_{corr} can be extrapolated from the cathodic branch of the polarization curve. For potentials higher than the E_{corr} (anodic part of the curve) the material undergoes oxidation. For some metals such as Ti, an j -constant segment can be found, meaning that no dissolution or slow dissolution (oxidation) of the material is taking place (j_{pass}). Hence the material is passive. Otherwise, the material is being dissolved (active).

EIS is an AC electrochemical technique used to study the kinetics and mechanisms involved during the corrosion process of a material. The tests consist on the application of a sinusoidal voltage signal of few mV (with respect to a reference voltage) to the working electrode scanning the total impedance of the system for a range of frequencies from $\sim 10^5$ Hz to 10^{-3} Hz. Barrier-like coatings or processes such as diffusion of active species under an AC voltage represent an impedance which induces a shift between the voltage and the current, in a similar way to a capacitor in a circuit. The shift or phase angle is a time dependent parameter which changes its magnitude with the frequency of the applied AC voltage. Each process taking place during the tests produces a characteristic time-dependent or frequency-dependent change in the impedance. The experimental impedance can be simulated by an equivalent circuit in which each resistor and/or capacitance element represent an electrochemical process. The capacitances and resistances of the equivalent circuit are calculated to fit the experimental data at every frequency.

3.4.1.1 *Electrolyte preparation and electrochemical equipment set-up*

Uncoated CG and UFG CP Ti substrates and PEO coatings fabricated on all the three electrolytes for 120 s on both substrates were subjected to polarization and EIS corrosion tests. The bare substrate samples were prepared following the same assembly as for the PEO treatment. The coated samples were not disassembled from the PEO treatment mount in order to keep the coated area as the testing area.

Both polarization and EIS tests were performed using a GillAC potentiostat (ACM instruments) and a double-walled electrochemical cell connected to a water thermostat to keep the temperature inside the cell during the tests at 37 °C. The same Ag/AgCl reference electrode (3 M KCl) and graphitic auxiliary electrode were used for all the measurements, being the specimen the working electrode. The set-up is schematized in the Figure 3-6: 1) working electrode/testing sample, 2) Ag/AgCl reference electrode, 3) graphite auxiliary electrode, 4) potentiostat, 5) double-walled cell and water thermostat, and 6) computer. The medium selected was a Hank's solution with D-glucose which is considered as an inexpensive good first approximation to a physiological medium for laboratory testing. The composition of the medium can be found in the Table 3-5. The OCP after 1 h of immersion was measured before the polarization experiments. Two samples per condition were used for both experiments.

Table 3-5. Composition of the Hank's medium for electrochemical tests.

Compound	Amount (g/l)
NaCl	8
KCl	0.4
CaCl ₂	0.139
MgSO ₄	0.097
Na ₂ HPO ₄	0.048
KH ₂ PO ₄	0.06
D-glucose	1
NaHCO ₃	0.35

Figure 3-6. Electrochemical set-up for DC potentiodynamic and EIS tests: 1) working electrode, 2) Ag/AgCl reference electrode, 3) graphite auxiliary electrode, 4) potentiostat, 5) thermostat, and 6) computer.

3.4.1.1 Polarization measurements

Potentiodynamic DC polarization curves were obtained from – 250 mV to 3000 mV with respect to the measured OCP at a sweep rate of 0.3 mV/s.

3.4.1.2 Electrochemical impedance spectroscopy

The samples were immersed in the specified Hank's medium for a total time span of 14 days. EIS measurements were taken after 1 hour, 3, 7 and 14 days. The medium was changed after each measurement and after 3 days of immersion in order to prevent proliferation of bacteria and changes in the solution pH. The OCP was measured for five minutes until it reached a stable value which was used as reference for the EIS measurement. The experimental impedance was fitted using the ZView software. The impedance parameters reported correspond to those producing a goodness of fit (ξ) below 0.01.

3.4.2 Cellular assessment

3.4.2.1 Chemically etched UFG CP Ti surfaces

The experimental works presented in this section was performed by Prof. Václav Babuška and Prof. Daniel Hrušák in the Charles University of Prague, Czech Republic.

3.4.2.1.1 Cell culture

Three treated samples were selected for cell proliferation tests based on the outcomes of the surface characterisation. The selection of the samples followed two criteria: *i*) Highest average surface roughness and *ii*) well-differentiated topographies. The non-etched sample was also subjected to testing for comparison.

A human fetal osteoblast cell line, hFOB 1.19 (ATCC, CRL11372™), established by Harris et al. [33], was grown in a 1:1 mixture of Ham's F12 Medium and Dulbecco's Modified Eagle's Medium with 2.5 mM L-glutamine (without phenol red) (Gibco, Life Technologies, Paisley, UK) supplemented with 10% (v/v) FBS and 0.3 mg/ml geneticin (G418, Serva Electrophoresis GmbH, Heidelberg, Deutschland). Cells were maintained at 34 °C under 5% CO₂ in a humidified incubator. The cell line used was obtained from ATCC (American type culture collection, Rockville, MD, USA) and cultured in accordance with ATCC recommendations. Culture media were refreshed as needed.

3.4.2.1.2 Cell viability and proliferation of hFOB cells

Cell proliferation after 48 hours from plating was assessed by MTT viability and proliferation assay (ScienCell™ Research Laboratories, Carlsbad, CA, USA) according to the manufacturer's instruction. This assay is based on the conversion of pale yellow tetrazolium MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to purple formazan crystals, which can be solubilised and then spectrophotometrically quantified.

The samples were placed into a 96-well plate (TPP, St. Louis, MO, USA). Cells harvested with trypsin solution from Petri dishes were resuspended in culture medium and seeded at a density of approximately 250,000

cells/ml onto the top of the discs of nanostructured titanium in 20 μ l volume. As positive control, cells grown directly on the 96-well tissue culture plate were used.

After 48 hours of incubation, cells were washed with phosphate-buffered saline (PBS) and incubated with 10 μ l MTT (25 mg/ml) solution at 37 °C. After 4 hours, 100 μ l of MTT solubilisation buffer (equal to the volume of original culture medium) was added to each well and the insoluble formazan formed was dissolved by pipetting up and down. The absorbance was measured at 570 nm (spectrophotometer Nano Drop 1000, Thermo Fisher Scientific, Waltham, MA, USA), subtracting the background absorbance determined at 690 nm.

3.4.2.1.3 Fluorescent microscopy

Cultured cells were stained with CellTracker™ Green 5-chloromethylfluorescein diacetate (CMFDA) (Molecular Probes, Inc., Eugene, Oregon, USA) according to the manufacturer's instruction. Briefly, cells were properly washed with PBS and incubated with 4 μ M CMTFDA working solution for 30 min at 37 °C. Then, the dye working solution was replaced with fresh, pre-warmed medium and the cells were incubated for another 30 minutes at 37 °C. Stained cells were analysed using an Olympus IX 70 fluorescent microscope equipped with Cell R system at 40x, 100x and 400x magnification. The initial cell attachment and the spreading of the cells on the substrate were examined after 6 h and 24 h, respectively. The area occupied by the cells was assessed by analysis of gained images by the programme ImageJ (W. S. Rasband, U. S. National Institutes of Health, Bethesda, Maryland, USA).

3.4.2.2 PEO coatings on CG and UFG CP Ti

The experimental works presented in this section was performed by Daniel Cermeño Pampliega in the facilities of IdiPaz, Madrid, Spain.

3.4.2.2.1 Cell culture and seeding

Human mesenchymal stem cells (hMSCs) were isolated from adipose tissue obtained from patients undergoing plastic surgeries at the University Hospital La Paz. The voluntary patients were provided with an informed consent form with enough information to make an informed decision about their donation of the disposable fat material. The personal data and genetic information of the donors was completely preserved through confidential data management protocols in accordance with General Data Protection Regulation. Biopsies were digested with 0.25 % Collagenase Type I, and minced with scalpels. Samples were incubated during 1 hour in a shaking water bath and maintained upright for 5 minutes to allow separation of the lipid layer from the aqueous layer. The top lipid layer was discarded followed by centrifugation at 300xg during 10 minutes. Cell pellets were resuspended in 10 ml of MesenCult Medium (Stem Cell Technologies, Inc. Vancouver, Canada), and nucleated cells were counted using 3 % Acid with Methylene Blue. In parallel, titanium pieces were sterilized with UV light. The mesenchymal stem cell portions were seeded either alone (controls) or with different surface-coated titanium implants and incubated in MesenCult medium and 1 % Penicilin Streptomycin at 37 °C and 5 % CO₂.

3.4.2.2.2 Cell proliferation of hMSCs

The proliferation of hMSCs cells cultured on the titanium surfaces was evaluated by measuring the cell number present on the material after 2, 4 and 6 (confluency) days of culture. The initial cell concentrations were 50000 cells/well on 24 well plates. Once the culture period was over, the cells were detached by trypsinization and counted with a Neubauer chamber. Total numbers of live cells were determined by Trypan Blue analysis. Three measurements on each group were performed per experiment.

3.4.2.2.3 Metabolic activity of hMSCs

Measuring changes cell viability is a fundamental method for assessing cell health, determining genotoxicity and evaluating metabolic interferences of titanium implants. The effects of PEO-coated titanium pieces on cell proliferation of hMSCs were determined by CyQUANT proliferation kit (ThermoFisher, Massachusetts USA). A total of 50.000 hMSCs/well were seeded onto titanium specimens into 24 well plates during 48 hours. On experimental day, XTT reagent component A was mixed with Electron Coupling reagent in a ratio 6:1 to prepare a working solution. Immediately after preparation, 140 μ L of working solution were added to each well containing 200 μ L of fresh hMSCs media. In addition, the same proportion of working solution plus fresh media was added to control (no titanium pieces) and blank (no cells) wells. The XTT-specific absorbance was measured at 450 nm. The 660 nm absorbance reading was used to eliminate the background signal contributed by cell debris or other non-specific absorbances. The specific absorbances of each sample was determined using the following formula:

$$\text{Specific absorbance} = (\text{Abs}_{450\text{nm}}(\text{test}) - \text{Abs}_{450\text{nm}}(\text{blank})) - \text{Abs}_{660\text{nm}}(\text{test}) \quad \text{Eq. (3.9)}$$

The specific absorbance signal from the treated cells was compared to the signal from the dilution series or untreated cells to determine the viable cells per well and how they react to the co-culture with titanium pieces.

3.4.2.2.4 Assessment of hMSCs morphology and adhesion by SEM

The effects of the bare substrates and the PEO-coated samples on enhancing human MSC adhesion were evaluated by SEM. A cellular concentration of 150.000 cells/well was tested on non-tissue culture treated 6 well plates to improve for adhesion of cells on the testing surfaces. Cells were co-cultured with 3 pieces of each surface condition per well, fed with MesenCult Medium (Stem Cell Technologies, Inc. Vancouver, Canada) and maintained at 37 °C 5 % CO₂ during 72 hours. On experimental day, cells were washed with D-PBS and fixed in paraformaldehyde at 4 % during 3 hours. Cells were dehydrated post-fixing by serial rinses of 50, 70, 90 and 100 % ethanol. In order to ensure the electrical conductivity of the surfaces given the dielectric nature of the PEO coatings the samples were gold-coated. Images were taken in both secondary electrons (SEs) and backscattered electrons (BSEs) modes in a double beam FIB-FEGSEM (Field Emission Gun) Helios NanoLab 600i (FEI) at 3 kV and 1.4 nA in order to obtain a superficial images.

4 Surface modification of UFG CP Ti via chemical etching

4.1 Surface characterization

SEM images of all treated samples are presented in Figure 4-1. The non-etched sample showed a ploughed surface produced by the grinding process after the cutting of the SPD processed rod (Figure 4-1 g). Solutions 1A and 1B produced deep pits within wide dimples (Figure 4-1 a, b). The surface outside these features was smooth. In the case of the 1A solution, the deep pits had an average diameter of 0.81 μm while the diameter of the dimples varied from 1 to 6.4 μm , being of 2.68 μm in average. The distribution of these features was homogeneous. The addition of water to the etching solution (solution 1B) reduced the diameters and depths of dimples and pits. In addition, the dimples had an elongated shape. It can be hypothesized that the addition of water lowered the efficiency of the acid mixture miscarrying the complete modification of the initial ploughed surface. Surface morphology was significantly modified after treatments 2A and 2B (Figure 4-1 c, d). Both surfaces presented low depth dimples of 2 to 6 μm in diameter and sharp ridges. The longer treated sample (surface 2B) also presented slightly longer sharp ridges and finer pits with diameters below 2 μm . The undulated surfaces generated by the solutions 3A and 3B were similar and characterized by sharp peaks and short ridges (Figure 4-1 e, f). While following the etching route 3A produced dissolution pits with diameters between 1 and 4 μm , the 3B solution resulted in a very promising surface with a homogeneous surface texture and reduced presence of corrosion pits. A characteristic feature of these two surfaces was the presence of white speckles all over the surface, whose population was larger on the surface 3B. These white speckles were also evident in samples 2A and 2B, although in this case their number and size was lower. They were not found after the treatments with the solutions 1A and 1B.

Table 4-1. Roughness measurements performed on samples from AFM maps.

Sample	Surface roughness parameters (μm)				Statistical descriptors (-)	
	S_a ⁴	S_{sq} ⁵	S_{pv} ⁶	S_z ⁷	S_{sk} ⁸	S_{ku} ⁹
Non-etched	0.12	0.15	1.34	1.23	-0.35	3.39
1A	0.20	0.26	2.67	2.50	-0.82	4.34
1B	0.10	0.13	1.10	1.01	-0.52	3.72
2A	0.17	0.23	2.14	1.88	-0.63	4.55
2B	0.23	0.29	2.86	2.51	-0.46	4.47
3A	0.18	0.24	2.59	2.18	-0.29	4.66
3B	0.19	0.24	1.99	1.80	0.24	3.53

⁴ Average surface roughness

⁵ Root mean square surface roughness

⁶ Peak-to-Valley

⁷ Ten point heights average

⁸ Surface Skewness

⁹ Surface Kurtosis

Figure 4-1. SEM images of the studied surfaces: a) 1A; b) 1B; c) 2A; d) 2B; e) 3A; f) 3B; and g) Non-etched

The roughness results extracted from the 80 x 80 μm AFM maps are presented in Table 4-1. It is seen that the etching routes 2B, 1A and 3B produced the highest roughness: 0.23, 0.2 and 0.19 μm , respectively. Furthermore, their surface topographies were well defined and distinct from each other (Figure 4-2 A, Table

4-1).

Figure 4-2. AFM surface topography analysis. *A*: 3D images of samples: a) Non-etched; b) 1A; c) 2B; and d) 3B. *B*: Surface profiles extracted from the AFM maps of the studied samples. Symbols mark the features mentioned for each surface. The horizontal axis coincides with the lateral dimension of the maps 80 μm

Therefore, these samples were selected for a more detailed analysis and also for characterization by the cell proliferation tests. Figure 4-2 *B* shows representative surface profiles extracted from the AFM maps of samples 2B, 1A and 3B. These profiles put in evidence the dominant features formed in each surface, which are marked by stars. The pits formed following etching route 1A had depths between 0.5 and 1 μm . Sharp peaks can be observed along the profile of solution 3B being of ~ 500 nm in height. They can certainly be attributed to the white speckles found during SEM inspection. It can be observed for both samples that these surface features

were not only homogeneously dispersed over the surface (as observed in the SEM images), but also their height distribution was homogeneous. In contrast, the protrusions (marked by a circle in the profile) and dimples of surface 2B were found to be sporadic, disrupting the main surface.

The skewness values (S_{sk}) obtained, showed that the surface of the sample 3B was slightly dominated by peaks ($S_{sk} > 0$), rather than by pits or valleys as in the case of solutions 1A and 2B, which had a preference to form valleys ($S_{sk} < 0$), as it was clearly seen in the SEM images and AFM profiles, (Figure 4-1 and Figure 4-2 *B*, respectively). The kurtosis of the surface describes the sharpness of the peaks and valleys, being rounded for those surfaces presenting $S_{ku} < 3$, and sharper, when the kurtosis value exceeded 3. In these cases, the S_{ku} parameter was above 3 in all studied specimens.

Figure 4-3. EDX map analyses. Left: Sample 2B. Right: Sample 3B. Top images: SEM image of the area analysed. Bottom images: EDX map of the region; Color-bar expresses the at. %. White circles identify the regions of higher Fe content. Red crosses mark EDX spot measurements performed

EDX measurements were performed on the three samples, with especial attention paid to the white speckles on samples 2B and 3B (Figure 4-1 *d* and *f*, respectively). Fluorine was found with the following average contents: 1.02 at. %, 3.1 at. % and 3.63 at. % for samples 1A, 2B and 3B, respectively. In the case of both 2B and 3B samples, the measurements showed the speckles to be Fe-rich particles with an Fe content of about 3 at. %. Regions with low Fe content below 0.2 at. % were also found. On the contrary, sample 1A did not present

any significant content of Fe on its surface.

Area mappings were also performed on samples 2B and 3B (Figure 4-3). They revealed Fe-rich (3 at. %) features partially embedded into the surface (marked by white arrows) and also few surface regions having a size of 1 to 2 μm and an average concentration of 7 at. % of Fe sub-superficial impurities (marked by red arrows). A similar area measurement performed on the non-etched sample also revealed the presence of Fe-rich regions. They were larger than in the case of the 2B and 3B samples, $\sim 3.3 \mu\text{m}$ in average.

Figure 4-4. A: Results of the cell proliferation tests. The data is given as a percentage with respect to the positive control. B: Fluorescent optical images of cell adhered to the tested surfaces at different culture times and scales: a) Sample 1A; b-d) Sample 3B. Photographs a) and b) were taken 6 hours after plating. Photographs c) and d) after 24 h.

4.2 Cell proliferation assessment

The results of the cell proliferation tests are presented in Figure 4-4 A. Two out of the three modified surfaces (3B and 1A) showed proliferation rates reasonably constant for all the measurements (34.6 and 38.0 %, respectively). Sample 2B did not show significant improvement with the exception of the second measurement,

in which the proliferation rate grew dramatically up to 34.6 % in average. It is worth to notice that the non-etched sample also presented an average 2.2 % higher proliferation rate with respect to the positive control.

Fluorescence photographs of the cultured cells in the most successful samples were taken, Figure 4-4 B. Cells were already attached to the treated surface of Ti after 6 hours of culture (images *a* and *b* in Figure 4-4 B). After 24 hours, the surface area occupied by cells in the 1A sample greatly increased up to 5 mm² (from 0.8 mm² after 6 hours).

In order to compare both samples (1A and 3B) the cell density, their dimension and shape (elliptical approximation) and the ratio between the surface covered by the cells and the total area of the image were calculated from the images *a* and *b* in Figure 4-4 B. This data is summarized in Table 4-2. The area covered was measured by creating a colour threshold after enhancing the brightness of the yellow-green points in the images (this procedure was performed using the software *ImageJ*). This area was then normalized with the total area of the image, last column in Table 4-2. It is seen from the images that in both cases, cells had a rounded shape with a maximum lateral dimension of about 18 μm . According to the cell density and area covered data, the sample 1A had a larger number of cells attached to the surface.

Table 4-2. Cell density, major axis, Aspect Ratio and ratio between surface covered and total image area, after six hours. Data taken from images *a* and *b* in Figure 4 B (samples 1A and 3B, respectively).

Sample	Cell density (Ncells/mm ²)	Major axis (μm)	AR	Surface covered/image
1A	0.63	18.29 ± 3.87	1.58	0.42
3B	0.31	18.82 ± 4.70	1.51	0.14

Images *c* and *d* in Figure 4-4 B correspond to the cell culture in sample 3B after plating for 24 hours. In contrast with the cells observed on images *a* and *b* in Figure 4-4 B, the cells have adopted a clear elongated shape. It can be related to the formation of lamellipodia protrusions. This fact puts in evidence the formation of F-actin bundles. This is triggered by internal protein signalling from the trans-membrane integrins, attached to the Extra-Cellular Matrix (ECM) by focal contacts, to the nucleus of the cell [220], Figure 4-5. The cell density is greatly increased even forming a multilayer arrangement. These values are in a good agreement with the results presented in Figure 4-4 A.

Figure 4-5. Schematic representation of cell spreading.

4.3 Discussion

4.3.1 *Surface morphology modification*

The analysis of the SEM images and AFM measurements indicated that different surface topographies were developed as the result of the variation of the acids' contents in the solution as well as treatment time. It can be suggested that short time etchings of 5 mins using HF/HNO₃ based mixtures led to the formation of bimodal surfaces consisting of micron-scale features dispersed in a nano-rough surface. The lateral dimensions of these features laid in the range from 1 to 10 μm . The depths and heights of the features were below 1 μm .

The acidic modification tended to the formation of numerous homogeneously distributed dimple-pit structures, smoother surfaces and elimination of iron impurities on the surface as HNO₃ acid was introduced in the mixtures. The nitric acid played a key role in the surface topography modification, especially in differentiating the dissolution features' morphology due to its interaction with iron impurities, at two different scales. Firstly, at lower scales (within 10 x 10 μm), the features evolved from rounded wide sharp dimples to wide smoothened dimples housing sharp deep pits. It was suggested in [105] that the nitric acid was responsible for the dissolution of the iron impurities, producing the characteristic deep pits in samples 1A and 1B. Secondly, at larger scales (above 10 x 10 μm), the passivating effect of HNO₃ on the intensive dissolution of the HF aid to smoothen the surface and contributed to homogenize the distribution of the micro-scale features produced.

4.3.2 *Effect of water addition*

Both samples 1A and 1B presented a smooth scalloped surface formed by dimples and dissolution pits. However, the addition of water into the chemical solution decreased the number, diameter and depth of the developed pits. Moreover, the initial ploughed surface was not completely modified by the water containing etchant.

4.3.3 *Increment of treatment time*

This combination of acids thoroughly modified the surface of titanium through the continuous generation of wide dimples with sharp ridges. The surface roughness increased after 5 minutes due to the remaining ridges from previous dimples. In addition, as the dissolution of the surface advanced, the number of undissolved Fe particles increased due to the lack of nitric acid.

As it was already mentioned, the use of the HF-based mixtures has always been limited to the surface preparation of Ti or to the inclusion of F ions [221]–[224]. In the latter case, the morphological modifications of the surface were minimal and within a range of tens or hundreds of nanometers. Other studies effectively combined HF etching with the subsequent HNO₃ passivation obtaining micro-scale roughness [225]. In contrast to the acid etchings used in this work, other authors selected acidic H₂SO₄/H₂O₂ and alkaline NH₄OH/H₂O₂ solutions for etching UFG CP Ti [226], [227]. Sponge-like structures with surface depths between 10 and 50 μm were obtained after 2 h etching with the acidic mixture and after 5 min etching with the alkaline one. In this study, we present surface morphologies that fall in the range from tens of micrometers to

hundreds of nanometers, with the expected beneficial effect of F ions inclusions after one step etching for 5 min.

4.3.4 Cell proliferation results

The surface modifications by etching have proven a great osteoconductivity enhancement of the UFG CP Titanium. The results also demonstrated the beneficial effect of the ultra-fine grained structure of the Ti, as the cell proliferation was improved by a modest 2.2 % in average, with respect to the control.

All the surfaces had similar roughness values around 0.2 μm . However, comparing the average of the three measurements for each etching route, we observed that two out of the three surfaces attained the highest proliferation rates being of 34.61 % and 38.04 % for samples 3B and 1A, respectively. These values represented an enhancement of the proliferation rate by factors of 16 and 17 compared to the average one of the non-etched surface. On the other hand, sample 2B had lower proliferation rates in two of the measurements, while one of them showed rates comparable with the other two samples. So the developed surface morphology might play a more important role than the roughness increment just by itself.

Qualitative analysis of the fluorescent optical images for the 1A and 3B samples after 6 hours of proliferation (images *a* and *b* in Figure 4-4 B, respectively) clearly shows the appearance of adherent cells from round to polygonal shape, though reliable statistical data cannot be extracted from those images. After twenty-four hours, the cell culture in sample 3B became a multilayer of elongated cells. This evidence proved the satisfactory evolution of the cells towards the spreading and migration stages. It can be hypothesized that the elongated shape corresponds to the lamellipodia produced by the stretching of the cytoskeleton during the motion of the cells with the aim of looking for new adhesive points [228]–[230]. This motion takes place after the cells get successfully attached to the surface. Cell attachment, spreading, motion and proliferation are driven by the availability of adhesive spots in the ECM absorbed on the surface.

Based on the proliferation results and the fluorescent images, it can be assumed that cells on surfaces 1A and 3B, were able to find the necessary adhesive proteins. Thus, these surfaces must influence the protein absorption onto the surface in a favourable way for the cells to attach and spread. This means that homogeneity in size and distribution of surface features with dimensions between 1 to 10 μm produced high proliferation rates. The only main difference found in the proliferation tests was the narrower standard deviations of sample 3B (below 3%), while the standard deviations in sample 1A were above the 3 %, even reaching 14.24 % in the first measurement.

It can be suggested that the larger deviations for sample 1A are due to variations in the size and depth of generated dimples and pits. It should be noted that the surface analysis (SEM, AFM, and EDX) was carried out only for one surface per etching route, and the surfaces subjected to the cellular tests were not analysed. Moreover, stronger variations were found for sample 2B. Large deviations were expected in this kind of tests. However, the differences in the proliferation tests of sample 2B seemed to be disproportional. In principle, the same etching route should produce similar surfaces and thus similar results. Among many others, one of the

possible explanations should be the dissimilarity of the UFG CP Ti surface, caused by variations in the dimples and pits pattern in different samples, which are consequently exposed by the highly sensitive cell culture method. Fe-rich particles present on the surface did not affect significantly the cell response. It might be hypothesised, that Fe in such a low quantity does not affect significantly the bioactivity of the surface.

Diluted HF solutions may be also used to induce the chemical modification of the surface upon inclusions of F complexes. The presence of fluorine ions in CG Ti treated by HF-based solutions has been shown to be very beneficial for the cell attachment, proliferation and differentiation processes [223]. Cooper and co-workers [221], found that as the at. % of F increases on the surface, the proliferation rates decreased. Our results correlate well with the latter finding since the fluorine contents in samples 1A and 3B were of 1.02 and 3.63 at. %, respectively, and the proliferation rate was higher in sample 1A than in sample 3B. The main concerns regarding the presence of the Fe-containing particles would be: *i*) they could be considered in practice as debris producing abrasive wear; *ii*) oxides derived from the corrosion products of iron. Apart from the likely dissolution of iron ions in the surrounding fluids, they might compromise the corrosion resistance of Ti.

4.4 Conclusions

The work presented in this Chapter was focused on the surface design by chemical etching of the UFG CP Ti to enable its bioactivity. It was demonstrated that etching treatments of the surface of the UFG CP Ti using HF-HNO₃-H₂O mixtures for 5 min can effectively modify the initially rough ploughed surface. The variation in content of chemicals in the mixture can lead to different surface topographies. The roughness of the surface at the nano-scale depends on the HF/HNO₃ molar ratios, whereas the main micro-scale features arise from the interaction of the HNO₃ with the Fe impurities of the bulk CP Ti. Bioactivity of the surface of the UFG CP Ti dramatically depends on its topography. The highest proliferation rates were observed on homogeneous surfaces having a homogeneous distribution of micro-scale features. Fe-rich particles did not influence the bioactivity of the surface.

5 Microstructural characterization of the PEO coatings

5.1 Plasma electrolytic oxidation process

In further text, the coatings are referred to using the following code: *c/n-C/S1/S2-6/9/12*; where *c* stands for *coarse-grained* (i.e. CG) and *n* for *nanostructured* (i.e. UFG); C, S1 and S2 correspond to the treatment; and 6, 9, and 12 to the treatment times 60, 90 and 120 seconds. For example, the nS2-9 refers to the coating using the electrolyte S2 on the UFG substrate for 90 seconds.

The evolution of the root mean square voltage (V_{RMS} (V)) and current density (j (mA/cm²)) with treatment time in the three electrolytes for 120 s are shown in Figure 5-1. The electrical responses showed high reproducibility on both substrates in all the electrolytes.

Figure 5-1. Root mean square voltage (V_{RMS}) and current density (j) versus time curves for the C, S1 and S2 treatments on UFG CP Ti.

A first stage was observed where all the treatments followed the pre-set initial slope of 5.78 V_{RMS}/s (8.17/-0.5 V/s) and the current densities remained constant. The growth of an anodic TiO_2 took place during this stage [192]. Homogeneous gas bubble generation, associated to oxygen evolution at flaws and nanocrystalline TiO_2 (nc- TiO_2) [231], was evident on all the surfaces. During the C treatment of both CG and UFG CP Ti, bubbles appeared at around 75 V_{RMS} . In the S1 and S2 electrolytes, bubbles were seen at ~ 75 and 100 V_{RMS} , respectively, for the CG substrate and at 45 and 55 V_{RMS} , respectively, for the UFG substrate. The voltages were determined with a timer at the moment the bubbles were visible. This stage was followed by the dielectric breakdown of the coatings and visible microdischarges, i.e. sparking on the surfaces. The breakdown voltage (V_B , Table 5-1) was determined from Figure 5-1 at the time microdischarges were visible on the surface of the samples. At this point the current densities reached the maximum allowed of 0.3 A/cm². Homogeneous

sparking in the S1 treatments (283 V_{RMS} , Table 5-1) was visible shortly after the first microdischarges (250 V_{RMS}), explaining the slower increase of the current density. After the initiation of the sparking, the cell voltage during both C and S2 treatments followed a slower increase rate of $\sim 3.29 V_{RMS}/s$ (4.66/-0.28 V/s) and $\sim 3 V_{RMS}/s$ (4.25/-0.26 V/s), respectively, while the S1 treatment followed an asymptotic rising towards a final constant forming voltage of 320 V_{RMS} (452/-28 V) at 80 s. At 60 s, when the system was set to operate freely at maximum current density, the C treatment had reached 260 V_{RMS} (367/-22.5 V) and its voltage growing rate decreased further to 0.7 V_{RMS}/s (0.94/-0.06 V/s) for the rest of the process achieving a final voltage of 300 V_{RMS} (423/-26 V) at 120 s. A higher constant forming voltage would have been reached in the C electrolyte for longer treatment times.

The S2 treatment showed an erratic behavior after 60 s: the voltage started to decrease, while the current increased dramatically above 0.3 A/cm² after 90 s. The sparks formed waves that travelled over the surface for short periods of time (~ 1 s). At longer treatment times, intense red sparks developed at the edges of the coated area. The current peaked after the initiation of the sparking (~ 35 s). During this segment, the power source was still forcing the voltage to increase until 60 s. The peaking of the current over the 0.3 A/cm² maximum may indicate a significant thickening of the coating at the sites of breakdown [232], increasing its resistance, for which the current through the system had to increase in order to keep the growing rate of the voltage. After 60 s, the voltage started to drop while the current density remained constant, which indicated a lower resistance that may be assigned to oxide material loss under sparking conditions [233]. However, the system was required to reach higher voltages (490/-30 V), for which the current needed to be increased but was limited. At some point around 90 s, the source was no longer able to hold the current density at 0.3 A/cm² and the voltage drop was accentuated.

Table 5-1. Breakdown voltage (V_B), Voltage at 60 s (V_{60}), final forming voltage (V_F), concentration, pH and conductivity of the electrolytes.

Treatment	V_B (V_{RMS})	V_B (V)	V_{60} (V)	V_F (V)	Concentration (M)	pH	Conductivity (mS/cm)
C	144	204/-12.5	367/-22.5	424/-26	0.05	11	15.55
S1	250-283	400/-24.5	415/-25.4	452/-28	0.026	12	7.91
S2	144	204/-12.5	353/-21.6	336/-21 ¹⁰	1	1	55.1

The values of the coating thickness as measured by Eddy-current are shown in Figure 5-2. The larger thickness measured at 60 s in the UFG CP Ti-coated samples indicated the effect of the substrate refinement on the growth rate of the coatings during both the anodic regime and the early stages of sparking. As a consequence, thicker coatings were obtained on the UFG CP Ti in the C and S1 electrolytes beyond 60 s of treatment. In the case of the C coatings, the thickness increased significantly between 60 to 90 s from ~ 5 to ~ 9 μm , respectively, on the CG substrate and from 8 to 10 μm on the UFG substrate. However, the values of the coating thickness

¹⁰ Voltage at 90 s before instabilities

remained constant on both substrate at 120 s. The S1 produced thicker coatings as the treatment time increased with an almost constant rate. The coatings on the CG substrate were significantly thinner. For instance, the nS1-6 coating had the same thickness as the cS1-12 coating, which was about 3 μm , whereas the nS1-12 coating was 5 μm thick.

Figure 5-2. Thicknesses of the PEO coatings as measured by Eddy-current.

The thickness of the S2 coatings were seen to decrease on both substrates with the treatment time suggesting that intense discharges during the S2 treatment resulted in the loss of coating material in agreement with the decrease of the cell voltage after 60 s (Figure 5-1). During the treatment on the CG substrate the thickness increased from ~ 3 μm for the first 60 s up to a maximum of 5.5 μm at 90 s. The thickness decreased to approximately 4.5 μm for 120 s. In the case of the UFG substrate, the thickness decreased with increasing treatment time from 6.5 μm at 60 s to 4 μm after 90 s and finally down to 3 μm after 120 s. Uniform well-developed PEO coatings have been obtained in acid electrolytes (i.e. H_3PO_4 , H_2SO_4 , etc.) at current densities below 100 mA/cm^2 and forming voltages between 50 to 350 V [182]–[184], [234]–[236]. On the other hand, Rokosz et. al. [237] reported the formation of bubble-like morphologies and the decrease of the coating thickness with the treatment time at 450 V. Henceforth, it could be said that the electrical parameters used in the present work in the S2 acid electrolyte led to coating destruction when the cell voltage was forced above 350 V (beyond 60 s, Table 5-1).

5.2 Surface and cross-section morphology of the coatings

The surface morphology of the PEO coatings was determined by the nature and spatial distribution of the sparks taking place during the process. To be more specific, the porous structure was created by the discharges. For identical electrical parameters applied, the discharge generation was strongly dependent on the electrolyte used and the species taking part. Hence, the morphology of the coatings differed with each treatment, as it is

seen from the AFM images in Figure 5-3. According to the SEM images of the cross-sections presented in the following section (figures 6-10, 6-11, 6-15 and 6-17), the formation of the coatings was due to powerful through-thickness discharges originated at the coating-substrate interface, in good agreement with the type B discharges described by Hussein et. al. [173]. Internal porosity within the coatings suggested the action of type D and E discharges [177]. The through-thickness morphology of the coatings consisted on a thin inner nanoporous layer ($< 1 \mu\text{m}$) in contact with the substrate and a thicker outer compact layer. No significant differences were found when comparing the morphology of the coatings on both substrates. With the treatment time the more powerful discharges were generated increasing the population and size of the pores. Although the differences were not very pronounced due to the short treatment times studied in this work.

Figure 5-3. 3D AFM images of the: a) nC-6, b) nC-12, c) nS1-12, and d) nS2-12 coatings.

5.2.1 Coatings fabricated in the C electrolyte

The C coatings were characterized by the presence of volcano-shaped pores (Figure 5-3 a). The surfaces of the coatings cC-6 and nC-6 were covered by these structures having an average pore size of $\sim 1 \mu\text{m}$ and a maximum pore size of $3 \mu\text{m}$ (Figure 5-4 a and b). The intensity and number of the discharges grew with the treatment time (Figure 5-5 and Figure 5-6), producing larger discharge channels with the inner diameter in the range between 3 and $6 \mu\text{m}$ (Figure 5-4 c and d). They were usually accompanied by nearby sub-micron and micron-size pores.

The coating thickened and the surface became undulated (Figure 5-3 b). No major differences were found between the coatings fabricated at 90 and 120 s except for a small increment in the maximum pore size leading to surface porosities of about 2 % in the cC-12 coating and just above 1.5 % in the nC-12 coating (Figure 5-7). The 90 and 120 s C coatings presented fine cracks on the surfaces having a length of 5-10 μm . The cracks could be the result of shrinking during the cooling or the solidification of dissimilar density volumes, i.e. at 25 °C the densities of anatase and rutile are 3.85 and 4.22 g/cm^3 , respectively. Fine pores, less than 100 nm in diameter, were found along and nearby the cracks, usually accompanied by blisters and brighter particles

(Figure 5-8). The average surface roughness of the coatings (R_a , Figure 5-9) decreased with the treatment time due to the change in the surface morphology (Figure 5-3 *a* and *b*).

Figure 5-4. SEM images of the C coatings on both CG (*a* and *c*) and UFG (*b* and *d*) after 60 (*a* and *b*) and 120 s (*c* and *d*) treatment time.

Figure 5-5. Average area (μm²) of pores in the PEO coatings.

Figure 5-6. Total population of pores in the PEO coatings.

Figure 5-7. Surface porosity (%) of the PEO coatings.

Figure 5-8. High magnification image of the nC-12 coating showing fine cracks on the surface. Fine pores, blisters and brighter particles were usually found surrounding them.

Figure 5-9. Average surface roughness (S_a) of the PEO coatings as measured by AFM.

The cross-section SEM images revealed that the largest surface pores consisted on through-thickness channels corresponding to the type B discharges described by Hussein et. al. [173] (Figure 5-10 and Figure 5-11). The 60 s coating had an irregular surface due to the pore lobes with the height of 3 to 5 μm , which was relevant to the average value of the coating thickness and average roughness (Figure 5-10 *a*). The adjacent coating thickened accordingly to the size of the discharge. At 120 s more powerful discharges took place producing bigger channels. Figure 5-11 *a* presents the cross-section of a 5 μm wide pore in the cC-12 coating revealing a conical channel of 10 μm wide at its base as a product of a powerful discharge. The adjacent coating had a thickness of about 12 μm which became thicker closer to the discharge channel. The height of the channel over the surface was about 10 μm . This indicated that more powerful discharges involved more melted coating

material and enhanced the formation of TiO_2 and the deposition of electrolyte species. It is suggested that this process was responsible for the thickening and, indirectly, the smoothening of the surface.

Figure 5-10. SEM images of the cross-sections of the nC-6 coating obtained by FIB: a) top-view before FIB sectioning (top) and cross-section (bottom), the dashed line marks the actual location of the cross-section; b) higher magnification image of the area marked in image a.

The trough-thickness morphology of the coatings consisted on a thin inner nanoporous layer ($< 1 \mu\text{m}$) in contact with the substrate and a thicker outer layer of compact coating and microporosity. The coating-substrate interface was relatively flat in comparison with the surface. The inner layer had an average thickness of 400 and 600 nm in the cC-6 and nC-6 coatings, respectively. The size of the pores found within the inner layer laid in the range between 200 to 400 nm. The inner layer in the cC-12 and nC-12 coatings had a thickness of $1 \mu\text{m}$ containing 200-400 nm pores.

The microporosity within the outer compact layer consisted on the discharge channels, rounded pores ($0.5\text{-}1 \mu\text{m}$) and a network of horizontal cavities at 20-50% of the coating thickness. Rounded pores with sizes between 0.5 to $1 \mu\text{m}$ were found within the outer coating which could be related to gas bubbles and sectioned channels (marked by black arrows and a black circle, respectively, in Figure 5-10 a). The cross-section of the nC-12 coating (Figure 5-11 b) showed bubbles within the outer compact layer underneath superficial blisters (type A discharge) and porosity (type C discharge).

Figure 5-11. SEM images of the cross-sections of coatings cC-12 (a) and nC-12 (b) coatings obtained by FIB.

The porous network was observed along the total length of the cross-sections ($\sim 40\text{-}50\ \mu\text{m}$) forming a sort of discontinuous plane that separated the inner nanoporous layer from the outer compact layer (white arrows in Figure 5-10 a, Figure 5-11). The discharge channels grew inwards in a conical shape. At their base ($\sim 50\%$ of the coating thickness) the channels were found to join the horizontal cavities network by several ramifications. The porous network could interconnect nearby discharge channels (white arrows in Figure 5-10 b). The formation of this porous network could be assigned to the action of type D and type E discharges inside the electrolyte-filled internal porosity of the coatings as described by Cheng et. al. [177].

The nC-12 coating volume having dimensions of $25\ \mu\text{m} \times \sim 20\ \mu\text{m} \times 20\ \mu\text{m}$ deep was cut out by combining FIB slicing and subsequent SEM imaging of the cross-section for further tomography measurements to study the horizontal cavities observed in Figure 5-10 and Figure 5-11. The top-view of the coating to be analysed is shown in Figure 5-12 a. The slicing-imaging was carried out from front-to-back as marked by the arrow. After

each slicing, an image of the new cross-section was acquired. 385 images were taken in the present case at an advance rate of ~ 48 nm per slice. The voxel dimensions in x , y and z were 22, 24, and 48 nm, respectively, having a voxel volume of $2.5 \cdot 10^{-5} \mu\text{m}^3$. A high-pass filter of $0.0025 \mu\text{m}^3$ was applied to remove noise contributions.

Figure 5-12. *a)* Top-view of the nC-12 coating area analysed in the tomography. The slicing-imaging direction is marked by the arrow from front to back. *b)* Population of pores according to their volume. Volume bin size $0.025 \mu\text{m}^3$. *c-d)* 3D reconstruction of the free volume (porosity) obtained using the Avizo software. *c)* Total free volume, *d)* Porosity group one, *e)* porosity group two, and *f)* porosity group three.

The 3D reconstruction of the free volume (i.e. porosity) is shown in Figure 5-12 *c*. The colours of the pores in the images were used to identify individual pores (images *d* and *e*) and interconnected porosity (image *f*). The average thickness of the coating was $\sim 13 \mu\text{m}$. The total volume analysed was $5.3 \cdot 10^3 \mu\text{m}^3$. The internal free volume was an 11 % of the total volume analysed. The total volume of coating, without porosity, was $4.8 \cdot 10^3 \mu\text{m}^3$. The detected pores could be divided into three groups according to their volume and population (Figure 5-12 *d*, *e*, and *f*). Groups one and two corresponded to rounded and elongated pores whose volumes laid in the range between 0.0025 and $1.33 \mu\text{m}^3$. The division between the first and the second group was done based on the significant reduction of the pore population (from ~ 3000 to ~ 300) with volumes above $0.025 \mu\text{m}^3$ (Figure 5-12 *b*).

The first group corresponded to pores with an equivalent sphere diameter (φ_{Eq}^{11}) between 170 and 360 nm having volumes in the range between 0.0025 and $0.025 \mu\text{m}^3$ (Figure 5-12 *d*). This group represented an 85 % of the total pores detected and a 13 % of the total free volume of the coating. These pores were mainly observed at the coating-substrate interface, although the finer pores ($V < 0.005 \mu\text{m}^3$) were also distributed within the outer compact layer. The second group consisted on rounded and elongated pores whose volumes laid in the range between 0.025 to $1.25 \mu\text{m}^3$ ($0.360 \mu\text{m} < \varphi_{\text{Eq}} < 1.33 \mu\text{m}$) accounting for a 9 % of the total free volume and a 15 % of the total population (Figure 5-12 *e*). Although a slightly higher density of these pores were found at the interface, they were homogeneously distributed across the thickness. The first group could be assigned to oxygen evolution during Ti oxidation at the coating-substrate interface, while the second group would correspond to gas generation at the outer compact layer.

The third group corresponded to a vast network of cavities, narrow ramifications and discharge channels as described previously (Figure 5-12 *f*). The 3D reconstruction confirmed that the horizontal cavities formed a sort of horizontal free-volume plane throughout the coating connecting different discharge channels. Despite this group corresponded to only a 0.11 % of the total pore population, it had a contribution to the total free volume of about 78 % ($\sim 373 \mu\text{m}^3$). The tomography also showed that a minimal part of this group was associated with vertical cracks and bubbles which were interconnected between them as a path within the outer compact layer. These features are related to the secondary micro-discharges originated by oxygen evolution within the outer compact layer, defects or cracking of the coating during solidification. Whereas the first analysis was performed slice by slice from front-to-back, a second top-to-bottom plane-by-plane reconstruction was performed in order to understand the evolution of the area covered by the internal porosity across the thickness. In this case, no discrimination according to volume or size was done. Figure 5-13 presents the progression of the ratio of the area covered by the porosity to the total area on each plane across the thickness. The ratio was found above 10 % in the thickness range between 3 to 7 μm reaching a maximum of ~ 25 %. This great increment was assigned to the contribution of the horizontal cavities, as it is indicated in

¹¹Equivalent spherical diameter: The diameter of a sphere enclosing the same volumen.

the inset in Figure 5-13. The free volume in the thickness range between 3 to 7 μm was about $368 \mu\text{m}^3$, which was 98 % the volume of the third porosity group shown in Figure 5-12 c. Another interesting feature observed thanks to the plane-by-plane top-to-bottom reconstruction was the porous surface of the inner nanoporous layer (Figure 5-12 g). In the image, it can be observed the outer compact coating (A in Figure 5-12 g) and the surface of the of the inner nanoporous layer. The pores of the inner layer (marked by black arrows in Figure 5-12 g) could be assigned to type D discharges taking place near the inner layer.

Figure 5-13. Evolution of the ratio between the covered area by the free volume and the total area analysed across through the thickness.

In summary, the coating had a porosity of approximately 10 % constituted mainly by horizontal cavities, ramifications and channels (78 % of the free volume), followed by a large density of nanopores at the coating-substrate interface (13 %), and micro pores (9 %). Most of the free volume was concentrated in the thickness range between 3 to 7 μm in which the porosity could cover a 25 % of the area.

5.2.2 Coatings fabricated in the S1 electrolyte

The S1 treatment produced highly porous coatings (Figure 5-14) due to the formation of: *i*) a great number of sub- and micro pores; *ii*) fine rounded pores (1 - 2.5 μm); and, *iii*) wide (2.5 - 11 μm) rounded and elongated openings housing multiple channels (Figure 5-15 a). The surfaces disclosed soft undulations and only disrupted by the pores and their edges (Figure 5-3 c). The size and number of the larger pores (*iii*) grew with the treatment time on both substrates (Figure 5-5), contributing significantly to the porosity which was found to be about 5 % at 120 s (Figure 5-7). As a result of the continuous formation of larger pores, the average roughness of the coatings increased from about 0.3 μm to $\sim 0.4 \mu\text{m}$ (Figure 5-9). Coatings on the CG substrate were found to have bigger pores than on the UFG substrate, hence leading to the larger average areas (Figure 5-5). However, the overall population was greater on the UFG coatings resulting in a porosity similar to the CG coatings (Figure 5-6). Dehnavi and co-workers [204] found that the number of pores increased for a duty cycle of 20 %

with respect to a duty cycle of 80 %. At the same time, the size of the pores at 20 % was smaller due to the lower intensity of the discharges. The authors hypothesized that if many more discharges occur per cycle at a constant current density, the current passing per discharge should be lower, thus resulting in less intense discharges. On the other hand, they also reported that the coating thickness increased with the decreased of the duty cycle suggesting a more homogeneous thickening. In the case of the S1 coatings, according to the average area and population charts (Figure 5-5 and Figure 5-6), weaker discharges took place on the UFG than in the CG substrate.

Figure 5-14. SEM images of the S1 coatings on both CG (*a* and *c*) and UFG (*b* and *d*) after 60 (*a* and *b*) and 120 s (*c* and *d*) treatment time.

The cross-section SEM images of the S1 coatings revealed that the through-thickness morphology (i.e. discharge-channels structures) of the coatings did not differ significantly neither with the substrate nor with the treatment time. Images in Figure 5-14 are representative images illustrating the characteristic through-thickness features of the coatings. The growth was mainly due to the deepening of the interface into the substrate due to consumption of Ti at the discharge sites, in contrast to the relatively flatter surface (Figure 5-15). The interface depth at the discharges sites was of the order of the thickness at the adjacent coating. Neither the thickness of the inner layer (~ 550 nm) nor the pore size (~ 200 nm) within this layer were seen to increase with the treatment time.

Figure 5-15. SEM images of the cross-sections of coatings cS1-6 (a) and nS1-12 (b) coatings obtained by FIB.

In correlation with the observations of the coatings surface, different pore morphologies were observed across the thickness. Sub- and micro surface pores (*i*) were identified with the fine pores within the outer layer of the coatings as pointed out by black arrows in Figure 5-15 *a*, which might correspond to type A and/or C discharges. Fine rounded pores (*ii*) such as the one marked by a black circle in Figure 5-15 *b*, consisted of single conical channels, whose base could split in few ramifications that reached the inner layer producing independent Ti consumption points. Rounded and elongated openings (*iii*) such as the ones showed in images *a* and *b* in Figure 5-15 were found to consist on complex porous structures formed by multiple channels and cavities. The oxidation of the Ti substrate forming the medium and large size pores (*ii* and *iii*) could be related to the action of type D and E discharges after a strong type B discharge. In fact, it could be hypothesized that medium pores evolved into large size pores by the action of type D and E discharges.

5.2.3 Coatings fabricated in the S2 electrolyte

The coatings produced by the S2 treatment were characterized by a pore morphology of fine spherical bumps (Figure 5-3 *d* and Figure 5-16). A bimodal surface morphology was found consisting of porous and smooth pore-less areas that may be related to the unstable sparking process observed during the PEO. The average

roughness of the S2 coatings was found between 0.3 and 0.4 μm (Figure 5-9), being larger for the coatings fabricated at 90 s. The instabilities in the current density and voltage observed were reflected in the pore generation. On the one hand, the maximum pore size grew slightly from 4.5 μm at 60 s to 6 μm at 90 s on the CG substrate while the total population decreased, in agreement with the thickening of the coating with more powerful discharges (Figure 5-5 and Figure 5-6). Between 90 to 120 s both the pore size and population decreased significantly to $\sim 3 \mu\text{m}$ and less than 10000 pores/ mm^2 , respectively. Hence the porosity decreased from $\sim 1.5 \%$ down to 0.5 % (Figure 5-7). On the other hand, both the pore size and population increased with treatment time on the UFG substrate having a maximum pore size at 120 s of 5 μm and a porosity of 1.5 %. This could explain the destruction of coating.

Figure 5-16. SEM images of the S2 coatings on both CG (*a* and *c*) and UFG (*b* and *d*) after 60 (*a* and *b*) and 120 s (*c* and *d*) treatment time.

Similar to the S1 coatings, the through-thickness morphology of the S2 coatings did not show significant changes with time or substrate. Representative SEM images are shown in Figure 5-17. The coatings had an irregular cross-section due to an almost symmetric thickening at both surface and interface at the discharge sites that doubled the thickness of the adjacent coating (Figure 5-17). The inner layer at the discharge sites thickened slightly with the treatment time from $\sim 400 \text{ nm}$ after 60 s to $\sim 550 \text{ nm}$ at 120 s. Similarly, the maximum pore size at the interface grew from 300 nm to 500 nm. Apart from this, there were no evident changes in the through-thickness morphology of the pores with time. The outer layer of the coatings had a compact appearance with no presence of bubble-like pores or type A and C discharges. The internal structure

of the pores consisted of conical channels whose base split into few ramifications at the inner layer (marked with black circles in Figure 5-17). The large discharge channels pointed by the black arrows seemed to have a columnar morphology, however, it is believed that this was an artefact due to the direction of the cross-section with respect to the pore vertical axis. The cavities observed beside the large channel in image *a* in Figure 5-17 are likely to be sectioned ramifications of the central channel suggesting the same morphology of the remarked channels.

Figure 5-17. SEM images of the cross-sections of coatings nS2-6 (*a*) and cS2-12 (*b*) coatings obtained by FIB.

5.3 Compositional analysis of the coatings via EDX

The average values obtained from local EDX measurements performed on the surface of the coatings are listed in Table 5-2. Oxygen and titanium were indeed the dominant elements in the coatings. The Ti to O atomic ratio was between 0.4 and 0.5 in all the coatings indicating an excess of oxygen for the formation of stoichiometric TiO_2 , which according to the Ti-O phase diagram precipitates on its own for contents of O above 64 at. % corresponding to an atomic Ti/O of about 0.56. Hence, the excess of O could point out to the association of this element with P and Ca. The deposition of P from the electrolyte in the coatings was confirmed. The P at. % descended for the C and S1 coatings with treatment time while it remained between 8 and 9 at % in the S2 coatings. In the case of the C coatings, this reduction could be assigned to the inclusion of Ca which grew with the treatment time on both substrates. The Ca/P atomic ratio grew accordingly from about 1.05 on both substrates to 1.4 on the CG substrate and to 1.5 on the UFG substrate. In the case of the S1, the stronger

discharges taking place at longer treatment times might have produced more oxidation of the substrate than deposition of P.

Table 5-2. Content of chemical elements measured by EDX. The presented data showed corresponds to the average of over 20 measurements on the surface of the samples.

Sample\Element	O at. %	P at. %	Ti at. %	Ti/O	Ca at. %	Ca/P
cC-6	58.4	7.2	25.9	0.5	7.8	1.1
cC-9	59.6	7.0	23.7	0.4	9.0	1.3
cC-12	61.1	6.4	22.8	0.4	9.0	1.4
nC-6	62.7	7.2	21.8	0.4	7.6	1.1
nC-9	60.7	6.6	23.7	0.4	8.3	1.3
nC-12	60.4	6.6	21.2	0.4	10.3	1.5
cS1-6	62.7	6.4	30.2	0.5	-	-
cS1-9	61.5	6.3	31.5	0.5	-	-
cS1-12	65.2	5.3	28.9	0.5	-	-
nS1-6	62.6	6.5	30.2	0.5	-	-
nS1-9	66.8	5.6	27.4	0.4	-	-
nS1-12	65.4	5.6	28.9	0.5	-	-
cS2-6	64.2	8.6	27.2	0.4	-	-
cS2-9	64.9	8.6	26.5	0.4	-	-
cS2-12	62.6	9.1	28.3	0.5	-	-
nS2-6	65.2	8.4	26.4	0.4	-	-
nS2-9	63.3	8.1	28.5	0.5	-	-
nS2-12	63.8	8.6	27.5	0.5	-	-

Figure 5-18 shows an area of the nC-12 coating analysed by EDX. The elementary composition at areas 1-3 are given in Table 5-3. An elementary mapping of a discharge affected region was also performed (Figure 5-18, images *b-d*. The mapped area is marked by a black rectangular on the upper image) showing the heterogeneous distribution of the elements around the discharge channels.

Indeed the ionization and plasma reactions taking place during the discharge process modified the chemical composition of the coatings, especially in the outer layer, promoting the deposition of compounds from the electrolyte. The deposition of the elements around the discharges was not homogeneous, as shown by the EDX maps performed in Figure 5-18. At the left side of the discharge channels (area 1) the Ca and Ti contents reached 23.0 and 32.1 at. %, respectively, while the O content dropped down to 34.2 at. %. On the other side of the channel (area 2), the O content increased to approximately 70 at. % whereas Ca and Ti contents dropped below average values.

Figure 5-18. EDX analysis of the nC-12 coating. Top image: SEM image of the area analysed EDX local (areas 1 to 3, Table 5-3). Bottom images: element maps in atomic percent corresponding to the area marked in image *a*.

A modest increase of P content was also observed at the high Ca and Ti content side but remained close to the average values. Brighter particles and debris present on the surface of the nC-12 sample (Figure 5-8) were analysed at area 3 in Figure 5-18. At this area, the Ca and P content were found to be about 11 at. % and 8.5 at. %, respectively (Table 5-3). This can be attributed to an enhanced deposition of electrolyte elements due to a current rising at the micro-cracks.

Table 5-3. Content of chemical elements measured by EDX on the nC-12 sample. The average elemental content of the sample is also given for comparison.

Sample\Element	O (at.%)	P at. %	Ti at. %	Ti/O	Ca at. %	Ca/P
Av. nC-12 ¹²	60.4	6.6	21.2	0.4	10.3	1.5
Area 1 ¹³	34.2	7.8	32.1	0.9	23.0	3.0
Area 2 ¹³	76.0	5.0	11.0	0.1	7.9	1.6
Area 3 ¹³	69.8	8.5	9.4	0.1	10.8	1.3
Area 4 ¹⁴	70.3	7.0	28.1	0.4	1.4	0.2
Area 5 ¹⁴	62.8	6.2	25.1	0.4	3.7	0.6
Area 6 ¹⁴	65.8	4.5	26.3	0.4	4.3	1.0
Area 7 ¹⁴	67.0	6.3	20.1	0.3	10.5	1.7

The heterogeneous deposition of the electrolyte elements was also evident across the thickness. Local measurements were performed on the cross-section of the nC-12 coating as shown in Figure 5-19. The data collected at areas from 4 through 7 in Figure 5-19 is shown in Table 5-3. Phosphorous was found to be preferentially deposited at the inner coating (area 4) while Ca was generally found at the outer compact coating (areas 5-7). The deposition of these two elements in the outer coating was heterogeneous, as it is put in evidence by comparison between areas 5, 6 and 7. The minimal Ca amount was found at area 5 (3.7 at. %) while P remained at average values (~ 6 at. %). Both EDX measurements 6 and 7 near the surface showed dissimilar P and Ca contents.

Figure 5-20 and Figure 5-21 present the elementary maps recorded on the surfaces of the nS1-12 and nS2-12 coatings, respectively. The distribution of the elements deviated from the average content values at the lobes of the discharge channels where the O at. % increased while the Ti and P contents decreased. According to EDX line scans performed on the nS1-12 and nS2-12 cross-sections (not shown), the P content decreased from the interface towards the surface in both cases. The measurements showed a decreased from 8 at. % to 4 at. % of P in the nS1-12 coating. However, the content increased slightly around cavities. In the case of the nS2-12 coating the P content decreased from 9 at. % to 6 at. %. These measurements were in agreement with the ones presented in Table 5-2.

¹² Average of the EDX local measurements on sample nC-12 (Table 5-2) for comparison.

¹³ Points in Figure 5-18.

¹⁴ Points in Figure 5-19.

Figure 5-19. SEM image of the cross-section of the coating nC-12. The elemental composition measured at areas from 4 through 7 is presented in Table 5-3.

Figure 5-20. Element maps in atomic percent corresponding to the area marked in image *a*.

Figure 5-21. element maps in atomic percent corresponding to the area marked in image *a*.

5.4 Phase composition of the coatings via XRD and Raman analyses

Figures 5-22 through 5-24 present the XRD spectra of the C, S1, and S2 coatings, respectively, on both CG and UFG substrates (left and right panels in the figures, respectively). Strong diffractions from the substrates were also detected due to the low thickness of the coatings.

Figure 5-22. XRD spectra of the coatings fabricated in the C electrolyte on the CG (left) and the UFG (right) substrates. Ti: CP Titanium. An: TiO_2 -anatase. Ru: TiO_2 -rutile.

The dominant phase in all the specimens was the TiO_2 anatase (ICDD: 98-015-6838). TiO_2 rutile (ICDD: 98-016-1909) was found in all the C coatings. The contribution of this phase grew with the treatment time (Figure 5-22). According to the Spurr function that describes the fraction of anatase in a mixture of TiO_2 anatase and

rutile [238],

$$X_A = 1 / \left(1 + 1.265 \cdot I_R / I_A \right)$$

the fraction of anatase decreased from ~0.93 on both substrates at 60 s to 0.77 on the CG substrate and to 0.71 on the UFG substrate for the 120 s treatment.

Figure 5-23. XRD spectra of the coatings fabricated in the S1 electrolyte on the CG (left) and the UFG (right) substrates. Ti: CP Titanium. An: TiO₂-anatase. Ru: TiO₂-rutile.

Figure 5-24. XRD spectra of the coatings fabricated in the S2 electrolyte on the CG (left) and the UFG (right) substrates. Ti: CP Titanium. An: TiO₂-anatase. Ru: TiO₂-rutile.

A minimal presence of rutile was also detected in the spectra of samples cS1-12 and nS1-12 (Figure 5-23) where the anatase fractions were 0.95 and 0.94, respectively. No rutile was found in the S2 coatings (Figure 5-24). The increase of the rutile content with the treatment time suggested that the local temperatures achieved in the coatings increased with the treatment time promoting the anatase to rutile transformation ($> 400\text{ }^{\circ}\text{C}$) [30]. No diffractions from P- and/or Ca-containing compounds were found in any of the coatings.

A large background was observed under the region corresponding to the two main diffractions of anatase and rutile ($2\theta\ 20^{\circ}$ - 35°) suggesting the presence of amorphous or highly disordered crystalline domains. In order to provide with a quantitative parameter on the crystallinity of the coatings, a procedure similar to the one used by Parfenov et. al. [150], based on the method developed by Keller and Dollase [239], was followed (Figure 5-25).

Figure 5-25. XRD background subtraction: *a* and *b*) Ti substrate diffractogram, *c* and *d*) PEO-coated sample. Images *a* and *c* represent the fitting of the spectrum with a 5 point background. Images *b* and *d* show the resultant spectra after subtraction.

Both the CG and UFG CP Ti also presented a background signal between 20° to 35° which was fit with a 5-point background in the Fityk software obtaining a flat signal (Figure 5-25 *a-b*). The same procedure was followed with the coatings' spectra to remove the background (Figure 5-25 *c*). Removing the background resulted in a XRD spectrum with a wide band (i.e. bump) in the range from 20° to $\sim 34^{\circ}$ (Figure 5-25 *d*). Diffraction peaks in the 20° - 34° range were fit with Voigt profiles, while the bump was fit with a Gaussian profile. The following relation was used to determine the crystalline fraction (X_c) of the coatings:

$$\chi_c = \frac{A_c}{(A_c + A_{bump})}$$

Where A_c stands for the total area of the diffraction peaks in the 2θ range between 20° and 35° that corresponded to the anatase and rutile main diffraction peaks. A_{bump} corresponds to the area of the wide band

in the same range. The X_C calculated for all the samples is shown in Figure 5-26. The lowest fractions of crystalline TiO_2 within the coatings were observed for the S2 coatings (~ 0.2) without significant differences between the substrates. The coatings fabricated on the UFG CP Ti in the C and S1 treatments presented lower crystallinity than their CG counterparts.

Figure 5-26. Calculated crystalline fraction X_C of all the PEO coatings.

Raman spectroscopy was used as a morphological tool to identify the TiO_2 phases and to assess the crystallinity [212], [240]. The intensity and width of the Raman peaks are associated to crystalline phases whereas wide bands represent defective crystalline domains and amorphous material. Figure 5-27 shows representative Raman spectra in the range between 300 to 700 cm^{-1} Raman shift of samples nC-12, nS1-12 and nS2-12. Two spectra per sample were plotted corresponding to channel-free areas and coating adjacent to discharge sites (solid and dashed lines, respectively). The inset in Figure 5-27 shows the spectra in the range between 0 and 300 cm^{-1} , where the main anatase band (144 cm^{-1} Raman mode E_g) is found.

The Raman spectra obtained at channel-free areas of the coatings (solid lines in Figure 5-27) showed the predominant presence of the TiO_2 -anatase, in agreement with the XRD measurements. The anatase contribution in the 300-700 cm^{-1} range consisted of three bands at 400, 515 and 640 cm^{-1} . The TiO_2 -rutile phase could also be observed at 447 and 610 cm^{-1} in the nC-12 and nS1-12 coatings. The anatase and rutile bands were superimposed over a wide background which indicated disorder in the crystalline domains and/or amorphous material. This could be related with the background observed in the XRD spectra. The spectra corresponding to the nC-12 coating presented well-defined peaks over a large background which indicated that the coating was formed by crystalline TiO_2 domains and amorphous material. In contrast, the nS1-12 coating had broader well-defined peaks and a minimal presence of background suggesting a large fraction of crystalline TiO_2 within the coating. Finally, the anatase peaks in the nS2-12 were wider and smaller than in the other two coatings and the spectra presented a wide background corresponding to a low level of crystallinity.

Figure 5-27. Raman spectra of the samples nC-12, nS1-12 and nS2-12. Solid lines correspond to the Raman spectra taken at discharge-free areas while dashed lines correspond to the Raman spectra taken in the vicinities of discharges. An: TiO₂-anatase. Ru: TiO₂-rutile. Inset shows the Raman spectra in the 0-300 cm⁻¹ Raman shift range.

The local nature of the Raman technique allowed to study the microstructure in the vicinities of large discharges (dashed lines in Figure 5-27). It was found that large fractions of rutile were formed at the discharge sites in the nC-12 and nS1-12 coatings. This indicated that rutile was mainly formed at the large discharge channels where the highest temperatures were achieved allowing the anatase to rutile transformation. In contrast, the nS2-12 coating was mainly amorphous at the discharge sites suggesting destruction of coating.

5.5 Transmission electron microscopy of the PEO coatings

Transmission electron microscopy was aimed to study: the effect of the CP Ti refinement on the coating-substrate interface; and to gain a better insight about the heterogeneous distribution of elements and the coexistence of crystalline TiO₂ and amorphous material across the thickness of the coatings.

5.5.1 Inner layer

The inner layers of the PEO coatings were found to maintain an essential layered-structure irrespective of the electrolyte and substrate (Figure 5-28 and Figure 5-29): 1st layer, a thin amorphous TiO₂ (am-TiO₂) layer in intimate contact with the substrate; 2nd layer, a thicker nanoporous-nanocrystalline TiO₂ (nc-TiO₂) layer; and 3rd layer, amorphous material region extending to the cavities of the outer layer within which pores of 0.3 to 1 μm in diameter were observed. This structure was only disrupted by porosity under the action of strong discharges (Figure 5-28 b).

Figure 5-28. Bright field TEM images at the inner layers of the cC-12 (*a* and *b*) and nC-12 (*c* and *d*) specimens.

Figure 5-29. Bright field TEM images at the inner layers of the nS1-12 (*a-c*) and nC-12 (*d*) specimens.

The first amorphous layer was found continuous in the cC-12 sample with a thickness of 50 nm (Figure 5-28 *a*). This layer was also found in the coatings fabricated on the UFG substrate nC-12, nS1-12 and nS2-12 (Figure 5-28 *c* and Figure 5-29 *a* and *c*, respectively) however it was thinner and discontinuous (Figure 5-28 *d* and Figure 5-29 images *b* and *d*, respectively) in comparison with the one found in the cC-12 sample. Whenever present, the amorphous layer had a thickness below 50 nm in the nC-12 and nS1-12 coatings and below 20 nm in the nS2-12. The discontinuity of the am-TiO₂ layer suggested that the UFG CP Ti induced its crystallization. The nc-TiO₂ layer had a thickness between 100 and 500 nm in the C coatings (Figure 5-28). This layer thickened in the nS1-12 coating from 200 nm at a channel-free section of the coating (Figure 5-29 *b*) to 400 nm under the characteristic large openings found in this coating (Figure 5-29 *a*). In the nS2-12 coating the nc-TiO₂ layer was 50-100 nm thick (Figure 5-29 *c-d*).

5.5.2 Outer compact layer (OCL)

The outer compact layer of the C coatings consisted of nc-TiO₂ domains with a grain size below 200 nm surrounded by amorphous TiO₂ (am-TiO₂) (Figure 5-30 *a* and Figure 5-31 *a* and *b*). No substantial differences were found in the microstructure of this layer in the cC-12 and nC-12 coatings. The action of discharges produced the formation of nc-TiO₂ isles or mixtures of nc-TiO₂ and am-TiO₂ after solidification (Figure 5-31 *b*). The outer compact layer of the nS1-12 coating was mainly composed by a layer of nc-TiO₂ bordered by am-TiO₂ (Figure 5-32 *a* and *b*). Islands of nc-TiO₂ were found embedded in am-TiO₂ close to large openings (black box in Figure 5-32 *a*). The nS2-12 coating was mainly formed by am-TiO₂ where islands of nc-TiO₂ were embedded (Figure 5-33). The walls of the internal cavities were amorphous in both C coatings and in the nS1-12 sample. This could be related to the deposition of electrolyte species and to higher cooling rates as they would be the contact point with the electrolyte once the sparks shut down.

Compositional analyses by EDX mappings and local measurements in the STEM mode were performed on the lamellas. As in section 6-3, no significant differences were found between coatings cC-12 (Table 5-4) and nC-12 (Table 5-5). The EDX analysis showed that O was distributed all over the coatings with contents between 60 and 70 at. %, while Ti was the dominant element at the crystalline domains (20 to 30 at. %) and in a lesser amount in the amorphous regions (~ 15 at. %).

Table 5-4. Elemental composition at the areas depicted in Figure 5-30 corresponding to the cC-12 specimen.

Area\Elem.	O (at. %)	Ti (at. %)	Ca (at. %)	P (at. %)	Ca/P
1 - interface	62.2	32.8	0.7	3.6	0.2
2 - interface	70.6	23.3	0.7	5.1	0.1
3 - am-TiO ₂	66.7	15.0	12.8	4.6	2.8
4 - nc-TiO ₂	70.5	21.4	4.6	3.1	1.5

Figure 5-30. TEM of the cC-12 specimen: *a)* Bright field overview of the lamella, *b)* HAADF mode for EDX analysis, *c-e)* Ti, Ca and P maps (at. %), respectively.

Figure 5-31. TEM of the nC-12 specimen: *a*) Bright field overview of the lamella, *b*) Bright field image of a nc-TiO₂/am-TiO₂ within the OCL, *c-e*) Ti, Ca and P maps (at. %), respectively, taken at the area marked in image *a*.

Figure 5-32. TEM of the cC-12 specimen: *a*) Bright field overview of the lamella, *b*) Bright field image of the OCL, *c*) HAADF mode for EDX analysis, *d* and *e*) Ti, and P maps (at. %), respectively, taken at the area presented in image *c*.

Figure 5-33. TEM of the nS2-12 specimen: *a*) Bright field overview of the lamella, *b* and *c*) Bright field images of the OCL.

Phosphorous was found to be preferentially deposited at the coating-substrate interface in the cC-12 and nC-12 coatings with contents up to ~ 9 at. % (areas 1 and 2 in Table 5-4 and 6-5, depicted in Figure 5-30 *b* and 6-31 *a*). Ca on the other hand was preferentially found within the OCL. However, it was also found to reach contents of up to ~ 8 at. % at the interface (area 2, Table 5-5). Both electrolyte elements, Ca and P, were preferentially found at the am-TiO₂ domains in the OCL with contents ranging from 6 to ~ 20 at. % and 4 to 8 at. %, respectively (area 3 in Table 5-4 and areas 3 and 4 Table 5-5).

Table 5-5. Elemental composition at the areas depicted in Figure 5-31 corresponding to the nC-12 specimen.

Area\Elem.	O (at. %)	Ti (at. %)	Ca (at. %)	P (at. %)	Ca/P
1 - interface	63.6	23.7	3.8	8.5	0.4
2 - interface	63.7	18.1	8.2	9.2	0.9
3 - am-TiO ₂	59.1	11.3	21.1	8.2	2.6
4 - am-TiO ₂	61.7	27.6	6.0	4.4	1.4
5 - nc-TiO ₂	63.3	27.5	5.2	3.6	1.4
6 - nc-TiO ₂	63.3	31.9	2.4	1.8	1.3
7 - nc-TiO ₂	61.4	28.9	5.0	4.1	1.2
8 - nc-TiO ₂	61.4	25.2	8.0	4.8	1.7

The Ca and P contents at the nc-TiO₂ domains laid in the ranges 2-8 at. % and 3-4 at. %, respectively, as shown by the local analyses at area 4 in the cC-12 specimen (Table 5-4) and areas 5 through 8 in the nC-12 (Table 5-5). The atomic Ca to P ratio was found above 1 within the OCL and above 1.5 at the am-TiO₂. The area analysed in Figure 5-31 *c-e* (corresponding to the box in image *a*) showed the sign of a type E discharge throughout the outer compact coating. At this area the deposition of P and Ca at the inner layer was enhanced (see Table 5-5 and Figure 5-31 *c-e*, areas 1 and 2). The OCL microstructure consisted on nc-TiO₂ bulk (areas 6 and 7) which was pushed towards the surface and an am- and nc-TiO₂ mixture region (areas 8 and 4) according to the fast solidification of the area. This indicated that the type E discharges modified the local phase composition by depositing am-TiO₂ from fresh Ca- and P-rich oxide and/or melting-and-quenching of previous material.

Table 5-6. Elemental composition at the areas depicted in Figure 5-32 corresponding to the nS1-12 specimen.

Area\Elem.	O (at. %)	Ti (at. %)	P (at. %)
1 - interface	65.2	29.5	4.0
2 - interface	66.2	30.7	3.1
3 - am-TiO ₂	60.1	33.1	5.6
4 - am-TiO ₂	66.1	24.6	9.2
5 - nc-TiO ₂	60.1	37.5	1.6
6 - nc-TiO ₂	63.6	34.4	2.0
7 - nc-TiO ₂	53.1	44.1	2.8
8 - am-TiO ₂	66.0	27.8	5.0
9 - am-TiO ₂	66.0	29.0	5.0
10 - am-TiO ₂	67.2	23.3	8.4
11 - am-TiO ₂	65.8	26.8	7.4

Similarly to the C coatings, the O and Ti contents in the nS1-12 coating were found between 60 and 70 at. % and between 20 and 40 at. %, respectively, irrespectively to whether the analysed areas were nc-TiO₂ or am-TiO₂ (Table 5-6). The P content at the nc-TiO₂ inner layer was 3-4 at. % (area 1, Table 5-6, Figure 5-32 *a*) and it did not significantly changed close to the large openings of the nS1-12 coating (~ 3 at. % area 2, Figure 5-32 *c*). However, at the am-TiO₂ the amount of P rose to 5.5 at. % at the opening-free section of the coating (area 3, Figure 5-32 *a*) and to ~ 9 at. % under the section of the coating affected by intense sparking (area 4, Figure 5-32 *c*). At the nc-TiO₂ in the outer layer the P content dropped to ~ 2 at. % (areas 5-7, Figure 5-32 images *b* and *c*) whereas at the am-TiO₂ it reached values around 5 at. % (points 8 and 9). Local measurements 10 and 11 were performed at the am-TiO₂ surrounding large pores within the OCL (Figure 5-32 images *b* and *c*). At these areas the P content was found between 7 and 9 at. %. The enhanced deposition of electrolyte derived species in the am-TiO₂ on the cavities walls was in agreement with the generation of type D and E discharges [177].

The content of O in the nS2-12 specimen was found to be about 68 at. % whereas the content of Ti varied between 21 and 29 at. % according to the content of P (Table 5-7). At the interface under the discharge channels, the content of P was found between 6 and 10.5 at. %. Within the am-TiO₂ in the outer layer it ranged between 5 and 7 at. % while it dropped down to 4 at. % at the nc-TiO₂ isles.

Table 5-7. Elemental composition at the areas depicted in Figure 5-33 corresponding to the nS2-12 specimen.

Area\Elem.	O (at. %)	Ti (at. %)	P (at. %)
1 - nc-TiO ₂	67.3	28.7	4.0
2 - am-TiO ₂	67.5	27.3	5.2
3 - am-TiO ₂	67.8	25.6	6.7
4 - am-TiO ₂	67.9	24.8	7.4
5 - am-TiO ₂	67.9	25.0	7.2
6 - am-TiO ₂	68.4	21.4	10.21

Figure 5-34. Segregation of Ca and P at the nc-TiO₂ grain boundaries. Ti, Ca and P maps (at. %).

Table 5-8. Elemental composition at the areas depicted in Figure 5-34 corresponding to the nC-12 specimen.

Area\Elem.	O (at. %)	Ti (at. %)	Ca (at. %)	P (at. %)	Ca/P
1 - am-TiO ₂	67.8	15.3	10.5	5.7	1.8
2 - nc-TiO ₂	68.9	21.9	5.3	3.5	1.5
3 - nc-TiO ₂	67.3	23.7	5.1	3.4	1.5

High magnification EDX maps were taken at the nc-TiO₂ regions in coating nC-12 (Figure 5-34) showing that Ca and P were segregated at the nc-TiO₂ grain boundaries (Table 5-8).

Diffraction patterns confirmed the formation of TiO₂ anatase, Ti₃O₅ and Ti₂O₃ in all the coatings (Figure 5-35). Rutile was found in the cC-12, nC-12 and nS1-12 specimens. According to the Ti-O phase diagram, precipitation of TiO₂ on its own takes place when local O contents exceed 64 at. %. Ti₂O₃ and Ti₃O₅ form at the narrow ranges of 61-62.5 and 62.5-64 O at. %, respectively. The diffraction patterns showed a high level of disorder probably due to the small crystallite size and to the non-homogeneous solidification. Blurred and

weak diffractions were a common feature. Most of the diffraction patterns presented a halo between the corresponding distances ~ 3.75 and ~ 2.5 Å overlapping of high intensity lattice families such as the most likely anatase (3.52 Å) and rutile (3.25 Å) phases which were the only ones observed in the XRD spectra. This phenomenon took place whenever the patterns were taken at areas with a great number of crystals. Few diffractions corresponding to α -tricalcium phosphate (α -TCP) were also found in the cC-12 and nC-12 specimens.

Figure 5-35. Diffraction pattern on sample nC-12.

5.6 Discussion

5.6.1 Coatings formation

The formation of the coatings was mainly driven by the generation of powerful type B through-thickness discharges originated at the coating-substrate interface [173]. Signs of weaker type A and C discharges were also observed in the C coatings. However these type of discharges did not play an important role in the growth of the coatings apart from the formation of blisters and sub-micron pores and the enhanced deposition of electrolyte elements at the surface.

According to Eq. (3.1), the intensity of the type B discharges would increase with the cell voltage, which, at the same time, increased with the treatment time (Figure 5-1), forming larger discharge channels in the coatings. The latter is in agreement with the average pore area trends of coatings fabricated in the C and S1 electrolytes (Figure 5-5). On the other hand, this might point out that the thinning of the S2 coatings with the treatment time was due to blasting of coating material towards the electrolyte without depositing at the surface. The formation of the internal porous structures, as pointed out in Section 5.2, and the deposition of electrolyte elements at the inner parts of the C and S1 coatings, see section 5.5.2, suggested the action of type D and E discharges [177].

Regarding the C coatings, the type B discharges grew in intensity with the treatment time (Figure 5-5). The change in morphology (Figure 5-4) and the stagnation of the coatings growth (Figure 5-2) observed from 60

to 90 s could be explained by the generation of fewer type B discharges and a more frequent action of weaker type D and E discharges. Type E discharges thickened the OCL adjacent to discharge channels (i.e. type B) smoothening the surface. The oxide mass production however, was not as significant as the one produced by the type B discharges but just enough to produce a homogeneous thickening. Other morphological modifications were observed through the action of these discharges. The porous surface of the inner nanoporous-nanocrystalline layer (Figure 5-12 g) suggested the action of type D discharges which could explain the relative flat coating-surface interface through homogeneous oxidation of the substrate (Figure 5-11). Both type D and E discharges were responsible for the expansion of the internal cavities of the coatings due to the heat and pressures generated (Figure 5-12 f). Finally, type E discharges modified the phase composition of the OCL layer enhancing the deposition of am-TiO₂ by fast solidification of new oxide and/or melting-and-quenching of previous oxide (Figure 5-31 c-e). This may explain the decrease of the crystallinity level from 90 to 120 s (Figure 5-26).

Diffraction patterns from the cC-12 and nC-12 specimens showed the presence of α -TCP. This phase could appear by crystallization of (ACP) through thermal transformation at 600-800 °C [241], [242]:

ACP could be present at the amorphous domains where the Ca/P atomic ratio reached values around 1.52. The temperatures required for the ACP to α -TCP transformation were easily attained during the present PEO. The XRD spectra did not show any peaks corresponding to α -TCP indicating that only a small amount of ACP transformed into α -TCP. Henceforth, it could be suggested that part of the Ca and P-rich am-TiO₂ found in the C coatings was ACP. α -TCP is a precursor of HA (Ca₉(HPO₄)(PO₄)₅(OH)) by the dissolution of Ca and P in physiological conditions (pH 7.2-7.4, 37 °C) [241]. HA has been shown to form in PEO coatings fabricated in the present C electrolyte for treatment times of 300-600 s [30]. Collagen secretion and early osteogenic ability of osteoblastic cells was assessed in the latter work for the 180 and 600 s coatings. The authors reported similar results for both coatings although no Ca/P-crystalline phases were observed in the 180 s coating. *In vivo* studies have shown that ACP results in a better osteoconductivity and bioactivity in comparison with HA [243].

The coatings fabricated in the S1 electrolyte were characterized by large openings (group *iii*, see section 5.2.2 Figure 5-14) which grew in size and number with the treatment time (Figure 5-5 and Figure 5-6). Comparing the coating surfaces with the coating-substrate interface it could be deduced that the growth of the S1 coatings was mainly driven by the oxidation of Ti rather than by deposition of oxide at the surface. This could explain the low contents of P (Table 5-2) and the high levels of crystallinity of these coatings (Figure 5-26). The TEM observation of the nS1-12 specimen confirmed that the complex porous structures were formed by type D or E discharges (Figure 5-32). Assuming that type D and E discharges are indirectly a consequence of type B discharges it might be hypothesized that pores of the *ii* group (single discharge channels) evolved into pores of the *iii* group by the action of type D and E discharges undermining the titanium substrate via oxidation.

The S2 coatings were mainly formed by type B discharges producing the thickening of the coating both at the

surface and at the coating-substrate interface (Figure 5-16 and Figure 5-17). The outer coating had a compact appearance with no presence of bubble-like pores. Rather than the large cavities described for the C and S1 coatings, the S2 coatings presented narrow ramifications at the base of the vertical discharge channels which could be assigned to type D discharges.

5.6.2 *Effect of grain refinement of the substrate in the fabrication of PEO coatings*

The grain refinement of CP Ti via equal channel angular pressing in the conform scheme plus drawing has been seen to affect the formation of the plasma electrolytic coatings in three ways: *i*) increasing the growth rate of the coatings on the UFG CP Ti with respect to the coatings on the CG CP Ti (i.e. thickness Figure 5-2, Section 5.1); *ii*) reducing the level of crystallinity of the coatings on the UFG CP Ti (Figure 5-26, Section 5.4); and, *iii*) inducing the crystallization of the am-TiO₂ layer at the coating-substrate interface (i.e. inner layer of the coatings Figure 5-28, Section 5.5.1).

The effect of the grain refinement of CP Ti on the growth rate of PEO coatings has been reported previously [37], [38], [149], [150] (see section 2.2.2). In the present work, the major differences were observed for the coatings fabricated for 60 s, suggesting that the UFG CP Ti surface played a more important role at the onset of sparking ($\sim V_B$). No substantial differences were observed neither in the chemical composition of the coatings (Section 5.3) nor the phase composition (Section 5.4) which could be attributed to the short treatment times applied. The results reported in the literature indicated that these differences might be observed for treatment times above 2-5 mins when high temperature phases such as rutile or α -TCP appear earlier on the UFG CP Ti. The results from the present work and from the literature survey suggest that the refinement of CP Ti promotes the generation of discharges, achieving larger thicknesses and higher local temperatures with respect to coatings fabricated on CG CP Ti for the same treatment time. For this, the dielectric breakdown of both the growing anodic TiO₂ film ($\sim V_B$) must be favoured.

The aforementioned works [37], [38], [149], [150] concluded that these differences could be attributed to the higher chemical activity of the surface of UFG CP Ti due to a larger fraction of high-energy grain boundaries and dislocations [202]. Indeed, defects on the surface of UFG CP Ti act as passive film nucleation sites increasing the corrosion resistance of the material [99], [101], [244]. Due to the greater number of nucleation sites, a homogeneous distribution of microdefects might be formed within the anodic film which could serve as more TiO₂ nucleation points and oxygen evolution sites. In agreement, Reshadi et. al. [149] showed that the onset of dielectric breakdown of the oxide film on UFG CP Ti took place at longer times and at higher voltages than on CG CP Ti, which could be attributed to a thicker passive film and to oxygen evolution. Similarly, bubbles from oxygen evolution appeared earlier on the surface of the UFG CP Ti samples in the present work, although no differences in the cell voltage or current density between the CG and UFG CP Ti were found. Microdefects act also as dielectric breakdown points. Accordingly, high density of microdefects would lead to a higher density of discharges [203], which would subsequently result in earlier homogeneous sparking conditions [149] and, subsequently, thicker coatings [33], [175], [176], [204]. Unfortunately, the present work

lacked data at the onset of sparking. Nevertheless, the PEO coatings on the UFG CP Ti at 60 s of treatment, slightly after the onset of sparking, were thicker than the ones on the CG CP Ti in each and every treatment (Figure 5-2, Section 5.1). It could be said that the anodic TiO_2 film formed on the surface of UFG CP Ti laid the grounds for earlier homogeneous sparking.

During full sparking conditions ($> V_B$) the formation of the coatings was dependent on the electrolyte characteristics. At 60 s, the average pore area was slightly larger in the C coatings on the UFG CP Ti than on the CG CP Ti suggesting that the earlier thickening on the UFG CP Ti led to stronger discharges. The differences in growth rate of the S1 coatings agreed with the theories proposed in [33], [175], [176], [204] where a higher density of weaker type B discharges in the nS1 coatings led to higher growth rates. Regarding the S2 coatings, the treatment seemed to lead to the destruction of coating. However the destruction was manifested earlier on the UFG CP Ti (from 60 s to 90 s) than on the CG CP Ti (from 90 s to 120 s).

The lower levels of crystallinity presented in the coatings fabricated on the UFG CP Ti (*ii*, Figure 5-26) could be attributed to a higher deposition of electrolyte-derived species which have been seen to deposit preferentially as amorphous material and are known to inhibit the crystallization of TiO_2 (P and EDTA) [201]. However, the elemental analysis of the coatings did not present significant differences when comparing the coatings fabricated on both substrates (Table 5-2). On the other hand, higher local temperatures on UFG CP Ti could be expected from stronger discharges (C treatment), higher discharge densities (S1 treatment) and thicker oxide layers (i.e. less heat dissipated to the substrate), which would promote crystallization. In fact, as already commented, most of the works comparing PEO of CG and UFG CP Ti have shown that high temperature phases such as rutile appear earlier on UFG CP Ti than on CG CP Ti. Henceforth, the dissimilarities in the crystallinity levels should be a consequence of the ability of the treatments to form or to destroy nc- TiO_2 according to the discharge characteristics of each treatment and the coatings morphology history.

For instance, it was pointed out that, among other aspects, type E discharges replaced nc- TiO_2 for am- TiO_2 within the OCL of the C coatings. It might be possible that a greater number of E discharges took place in the coatings fabricated through the C treatment on the UFG CP Ti due to larger cavities produced by the stronger type B discharges. The cS1 coatings presented larger openings (group *iii*) than the ones of the nS1 coatings. According to the earlier reasoning that these openings were the consequence of type D and E discharges, it could be hypothesized that a greater number of these discharges took place in the cS1 than on the nS1 coatings. In turn, the cS1 coatings comprised more oxidation of the substrate explaining the higher crystallinity levels.

As it was commented in point *iii*), UFG CP Ti induced the am- TiO_2 crystallization of the inner layer of the coatings. This could be assigned to the growth of a more crystalline anodic TiO_2 film on the UFG CP Ti in comparison to the CG CP Ti at voltages below the V_B . In fact, as commented earlier, bubbles were observed earlier on the surface of the UFG CP Ti samples which could be related to a more crystalline anodic film. Yao et. al. [37] showed a dense 100 nm thick nc- TiO_2 separating the substrate from the nanoporous-nc TiO_2 inner layer in the 20 mins PEO coating on UFG CP Ti. The nc- TiO_2 crystallization from am- TiO_2 under sparking

conditions is known to take place with the treatment time due to the local high temperatures [192]. On the other hand, crystallization could also be enhanced by increased ionic transport [192], [231], [245]. A higher fraction of nc-TiO₂ could facilitate the dielectric breakdown of the inner layer and the subsequent discharge generation due to the higher conductivity and defects (dielectric constant of nc-TiO₂ and am-TiO₂, 18.2 and 39.7 F/m, respectively [246]). In turn, the generation of discharges at the inner layer of the coatings on UFG CP Ti would be eased increasing the growth rate during the PEO regime.

5.7 Conclusions

- Plasma electrolytic oxidation coatings were successfully fabricated on both CG and UFG CP Ti in the three electrolytes for treatment times of 60, 90 and 120 s. Each treatment produced specific morphologies and growth dynamics according to the discharge characteristics allowed by the electrolytes.
- Type B discharges were observed to be the main responsible for the growth of the coatings. Type D and E discharges played also an important role in the modification of the coatings morphology and contributed to the deposition of electrolyte-derived species especially in the case of the C and S1 coatings. Signs of the action of weak type A and C discharges were observed in the C coatings.
- The grain refinement of CP Ti via equal channel angular pressing in the conform scheme plus drawing has been seen to affect the formation of the plasma electrolytic coatings in three ways: *i*) increasing the growth rate of the coatings on the UFG CP Ti with respect to the coatings on the CG CP Ti; *ii*) reducing the level of crystallinity of the coatings on the UFG CP Ti; and, *iii*) inducing the crystallization of the am-TiO₂ layer at the coating-substrate interface. No substantial differences were observed neither in the chemical composition of the coatings nor the phase composition, which could be attributed to the short treatment times applied.
- *i*) The increased growth rate of the coatings fabricated on the UFG CP Ti was presumably related to a thicker and more defective anodic TiO₂ at the onset of sparking with respect to the one formed on the CG CP Ti. It was assumed that the nature of this film laid the grounds for an earlier homogeneous thickening. *ii*) The lower levels of crystallinity presented in the coatings fabricated on the UFG CP Ti were attributed to the ability of the treatments to form or to destroy nc-TiO₂ according to the discharge characteristics of each treatment and the coatings morphology history. In the case of the C coatings it was assigned to a greater number of type E discharges as a consequence of the larger cavities within the nC coatings with respect to the cC ones. Regarding the S1 coatings it was hypothesized that the higher level of crystallinity of the cS1 coatings could be due to increased oxidation of Ti as a consequence of greater number of type D discharges. *iii*) The enhanced crystallization of the am-TiO₂ layer in intimate contact with the substrate in the coatings fabricated on the UFG CP Ti could be assigned to: more crystalline anodic TiO₂ films before sparking, higher local temperatures, and increased ionic transport from the UFG substrate.

6 Mechanical characterization of PEO coatings

This section presents a thorough and comprehensive study of the mechanical properties and tribological performance of the fabricated PEO coatings. The results presented in the sections 7.1 and 7.2 of this chapter demonstrate that the mechanical properties of highly porous PEO coatings cannot be accurately measured and comprehended by means of nanoindentation directly on the surface of the coatings due to the presence of pores, which affect the accuracy of the H and E measurements, using the Oliver and Pharr method [216]. The results in the second section show that H and E measured depend on the fractions of nanocrystalline and amorphous TiO₂ present in the volume tested by nanoindentation. These results correlate well with the work of Herre et. al. [247] on the deformation behavior of nanocrystalline and amorphous TiO₂. The findings could be extrapolated to explain the differences in fracture toughness between treatments presented in the first section and the nanoscratching performance.

Despite their lowest thickness and hardness, the coatings fabricated in the S2 electrolyte appeared to have a highest fracture toughness due to the higher fraction of ductile am-TiO₂ delaying the failure during the tribological tests. The brittle behaviour of the S1 coatings led to the lowest critical loads but to an acceptable wear behaviour due to the hardness provided by the large fraction of nc-TiO₂ in the microstructure. The C coatings presented a poor mechanical performance considering their thickness. The PEO-related microstructural defects and porosity within the outer compact layer promoted the brittle behaviour of this layer. The horizontal cavities induced the early failure of the coatings during the tribological tests.

Considering the results found in this chapter, an alternative approach to the common PEO hardening approaches is presented in section 7.4.3 to improve the mechanical performance of the coatings.

6.1 Nanoindentation on the surface

30 load control cyclic indentations up to a maximum load of 1 N were performed on the surface of each coated sample. Each cyclic indentation provided with hardness (H) and Young's modulus (E) vs. contact depth (h_c) curves. All the curves per sample were averaged using Origin software and plotted against the h_c/t_0 ratio (t_0 , sample nominal thickness according to Figure 5-2) in Figures 6-1 and 6-2. According to the well-established 10 % rule of thumb, reliable film-alone mechanical properties can be obtained for maximum indentation depths below 10 % of the coating thickness [215]. It should be taken into account that tip rounding effects could be significant for maximum indentation depths below 30 % of the tip radius ($R_{tip} \sim 200$ nm in this study).

At low indentation depths (< 10 % of the thickness) the coatings were observed to have similar mechanical properties irrespective of the PEO treatment with $H \sim 5$ GPa (Figure 6-1) and E between 60 and 100 GPa (Figure 6-2). The hardness of the coated surfaces laid in the range between 1.5 and 4 GPa at larger depths below the hardness of both substrates.

Figure 6-1. Hardness (H) vs. contact depth to thickness ratio (h_c/t_0) of the PEO coatings fabricated on both substrates in the three electrolytes for 120 s.

The E values were observed to increase with the indentation depth as the contribution of the substrate also increased with the exception of the S1 coatings which remained at a plateau at about 65 GPa. This substrate effect was not observed for the hardness measurements due to the fact that elastic fields induced under the indenter are about 3 times larger than plastic fields [248].

Figure 6-2. Young's modulus (E) vs. contact depth to thickness ratio (h_c/t_0) of the PEO coatings fabricated on both substrates in the three electrolytes for 120 s.

The SEM and AFM images of the indentations on the coatings showed irregular prints (due to surface roughness and internal porosity) and brittle circumferential and radial cracks on the surface (Figure 6-3, Figure 6-4 and Figure 6-5).

Figure 6-3. Representative print of the indentations on the surface of the nC-12 coating: *a*) SEM image; *b*) AFM image of the print in *a*; and *c*, FIB milled cross-section of the print in *a*. Dashed line in image *a* shows the location of the cross-section.

Several cracks were found in the C coatings while fewer were observed in the S1 coatings. The S2 coatings barely presented cracking. The FIB milled cross-section of the indentations on the C coatings (Figure 6-3 image *c*) revealed multiple cracks through the OCL in most cases associated to internal porosity. The coating densified as the porous microstructure was deformed. The cross-section after indentation of the S1 and S2 coatings presented a defectless appearance due to the closure of the internal porosity and cracks (Figure 6-4 and Figure 6-5, respectively). These results indicated the brittle behaviour of the coatings that is associated with the ceramic nature of TiO_2 . According to presence of cracks on the surface, the S2 coatings had the highest fracture toughness while the C coatings had the lowest and the S1 coatings an intermediate toughness.

The results presented correlate well with the experimental data from mechanical characterization of thin PEO coatings on CP Ti and its alloys ($H \sim 3\text{--}6$ GPa and $E \sim 50\text{--}120$ GPa, Table 6-1) reported in the literature [186], [205]–[207], [249]–[254]. It should be pointed out, however, that there are reasons to believe that the H and E measurements could have been altered by the porosity and the brittle fracture of the coatings. Sub- and micropores within the OCL of the coatings might have acted as stress concentrators and crack initiators, resulting in a lower net fracture toughness, especially in the case of the C coatings. Fracture of the material might also decrease the stiffness of the material as elastic stored energy is dissipated during fracture [255]. Following the fracture of the outer layer of the coatings, the closure of the porosity might affect the calculations

of the H and E by the Oliver and Pharr method [205]. The densification of the porosity might lead to higher indentation depths (h_c , Eq. (3.3)), increasing the contact area (A_c , Eq. (3.4)), and subsequently decreasing the H and E values (Eq. (3.5) and Eq. (3.7), respectively). This could explain the lower H values of the coatings at large indentation depths where the effect of the substrate should be significant.

Figure 6-4. Representative print of the indentations on the surface of the nS1-12 coating: *a*, SEM image; *b*, AFM image of the print in *a*; and *c*, FIB milled cross-section of the print in *a*. Dashed line in image *a* shows the location of the cross-section.

Figure 6-5. Representative print of the indentations on the surface of the nS2-12 coating: *a*, SEM image; *b*, AFM image of the print in *a*; and *c*, FIB milled cross-section of the print in *a*. Dashed line in image *a* shows the location of the cross-section.

Table 6-1. Comparison of data from earlier reports on the mechanical properties of PEO coatings on CP Ti and its alloys. Hardness and Young's modulus values correspond to the data for contact depths below the 10 % of the coating thickness. Notice that the data presented correspond to nanoindentation and nanoscratching testing on the surface of the coatings with Berkovich tip unless specified otherwise: HV, Vickers hardness; ‡, micro testing Rtip ~ 200 µm; †, Ball-on-disk; and, ‡, Pin-on-disk.

Electrolyte	Thickness (µm)	H and E (GPa)	Wear performance	Ref.
0.5-1 M H ₂ SO ₄	-	H ~ 3-4 E ~120 - 140	-	[207]
1.4 M H ₃ PO ₄	0.6-1.3	H ~ 4-6 E ~ 125	-	[186]
0.14 M (CH ₃ COO) ₂ Ca·H ₂ O 0.06 M NaH ₂ PO ₄ ·2H ₂ O	3.6- 4.1	H > 5 E ~ 65	Severe plastic deformation and fracture at 220 mN	[205]
Anodic Oxidation (AO): 0.14 M (CH ₃ COO) ₂ Ca·H ₂ O 0.06 M NaH ₂ PO ₄ ·2H ₂ O. Thermal treatment at 600 °C (TT600)	AO: 3.6 AO+TT600: 3.5	H: (AO) ~ 6 GPa, (TT600) ~ 8 E: (AO) 40 (TT600) ~ 120.	Severe plastic deformation and fracture at 220 mN	[250]
1 M H ₂ SO ₄ . Treatment time 60 s.	0.9	H ~ 4 E ~ 100	Lc > 400 mN. Plastic deformation of the fine porous morphology.	[252]
0.14 calcium acetate monohydrate 0.06 M sodium biphosphate dihydrate	3.6	H ~ 3	Lc: 135 mN.	[256]
1.5 g/l NaAlO ₂ 4.5 g/l Na ₃ PO ₄	-	H ~ 6.5 E ~ 70 (50 % of the coating thickness)	-	[251]
0.2 M (CH ₃ COO) ₂ Ca·H ₂ O 0.1 M NaH ₂ PO ₄ ·2H ₂ O	HA layer: 7.71. TiO ₂ layer: 6.44	-	‡ Lc: 26.5 N apatite. 48.5 N TiO ₂	[257]
5 g/l Na ₃ PO ₄ ·12H ₂ O. 2 g/L KOH	4 - 9	-	‡ Lc: 26 N	[33]
TiO ₂ -based Ca and P-containing coating	20	-	‡ Lc: 36 N	[258]
(CH ₃ COO) ₂ Ca·H ₂ O + Na ₂ SiO ₃ ·5H ₂ O + Na ₃ PO ₄ ·12 H ₂ O	5-40	-	‡ Lc: 27.8 to 33.2 N	[259]

Solution A: 0.2 M Na ₂ SiO ₃ ·5H ₂ O 0.04 M Ca-beta- glycerophosphate. Solution B: 0.1 M Si(CH ₃ COO) ₄ + 0.04 M Ca-beta-glycer + 0.2 M NaOH	Up to 200	-	‡ Lc > 25.4 N. Adhesion decreases with SBF immersion time.	[260]
10.5 g/l Na ₂ SiO ₃ , 2 ml/l H ₃ PO ₄ , 2.8 g/l pKOH	30-50	-	† Failure at 600 s. Flake delamination at the track.	[261]
0.02 M C ₃ H ₇ CaO ₆ P 0.15 M C ₄ H ₆ O ₄ Ca.	2-8	H 2-10 E 50-140	† Failure delayed with the forming voltage.	[206]
0.05 M Na ₃ PO ₄ ·12H ₂ O	10-20	-	† 10 µm wear track. Compact appearance and good adhesion.	[262]
20 g/L Na ₂ SiO ₃	2.1	H ~ 200 Hv (~2 GPa)	‡ Failure within the first 50 s.	[253]
20 g/l NaH ₂ PO ₂ ·H ₂ O 1.48 g/L Ca(CH ₃ COO) ₂ ·H ₂ O 7.44 g/L EDTANa ₂	10	H ~ 7.92	‡ Wear rate = 28.3·10 ⁻¹⁰ kg/Nm	[254]
0.2 M Ca(C ₂ H ₃ O ₂) ₂ H ₂ O 0.02 M C ₃ H ₇ CaO ₆ P. Hydrothermal treatment 10 h at 190 °C.	7-9	-	‡ Wear rates: MAO 1.5 ·10 ⁻⁴ mm ³ /Nm. MAO+HT 0.75. Extensive deformation and smearing.	[38]

It should be noted that these measurements represent the mechanical properties of the coated system accounting for the porosity of the coatings and substrate, so they cannot be taken as the mechanical properties of the phases in the present coating. In order to eliminate the effect of porosity and substrate, nanoindentations were performed on the ion-polished cross-section of the coatings.

6.2 Nanoindentation on the cross-section of the coatings

Figure 6-6 presents the calculated E values versus the H values measured by displacement controlled (DC) indentations up to 150 nm on the cross-section of the nC-12, nS1-12 and nS2-12 specimens. The data was fitted in Origin. The data in Figure 6-6 correspond to the indentations performed on compact defect-less areas resulting in the load vs. depth curves free of any artefacts such as pop-ins. The indentation prints did not present any pile-up, sink-in or radial cracks.

Figure 6-6. Young's modulus (E) versus Hardness (H) of the nC-12, nS1-12 and nS2-12 coatings. Linear fit equations are presented in Table 1.

The data presented an evident scatter showing similar E and H values for the three coatings. Comparing the average E and H values (presented in Table 6-2, together with their standard deviation and the maximum and minimum values) it could be said that the nS1-12 coating presented the stiffest response ($H \sim 8.7 \text{ GPa} \pm 1.2$ and $E \sim 96.3 \pm 9.5 \text{ GPa}$) while the nS2-12 appeared to be the softest coating ($H \sim 6.2 \pm 0.7 \text{ GPa}$ and $E \sim 86.9 \pm 7.5 \text{ GPa}$). According to the linear fit of the data (Table 6-2), the E/H ratios demonstrated that the microstructures developed after each PEO treatment led indeed to different mechanical behaviours.

Table 6-2 . Average (Mean), standard deviation (Std. Dev.), maximum (Max.) and minimum (Min.) of the E , H and H/E_r ratio for the three samples (first four rows). The 'Fit' row presents the equations of the linear fits in Figure 6-6. Radius of the plastic (R_p) and elastic (R_e) zones, respectively.

	nC-12			nS1-12			nS2-12		
	$E \text{ (GPa)}$	$H \text{ (GPa)}$	H/E_r	$E \text{ (GPa)}$	$H \text{ (GPa)}$	H/E_r	$E \text{ (GPa)}$	$H \text{ (GPa)}$	H/E_r
Mean	92.17	6.55	0.07	96.27	8.56	0.09	86.85	6.18	0.07
Std. Dev.	13.37	1.12	0.01	9.45	1.23	0.01	7.50	0.71	0.01
Max.	120.94	8.89	0.08	111.90	10.79	0.11	101.35	7.83	0.09
Min.	57.63	3.77	0.05	75.39	6.44	0.07	68.26	4.67	0.06
Fit	$E = 9.86 H + 27.59$			$E = 3.63 H + 65.19$			$E = 7.07 H + 43.16$		
$R_p \text{ (}\mu\text{m)}$	0.54			0.51			0.54		
$R_e \text{ (}\mu\text{m)}$	1.63			1.53			1.63		

The mechanical response depended ultimately on the plastic and elastic deformation produced under the tip (H and E , respectively). Both plastic and elastically deformed volumes can be assumed as a hemisphere as it is represented in Figure 6-7. The radius of the plastic zone (R_P) can be estimated through the Chen and Bull relationship between the R_P and the maximum penetration depth as a function of the H to reduced modulus (E_R) ratio [217]:

$$R_P/h_{max} = -12.91 H/E_R + 4.55 \quad \text{Eq. (7.1)}$$

The radius of the elastic zone (R_E) can be considered as 3 times the R_P [248]. The H/E_R ratios for the three coatings and the subsequent estimations for the R_P and R_E are presented in Table 6-2. The TEM observations revealed that the outer compact layer of the coatings consisted of nc- and am-TiO₂ domains (see section 5.5.2). It is reasonable to think that if the size of both crystalline and amorphous volumes within the coatings was of the order or larger than the size of the deformed volumes, the mechanical responses might differ. This is represented in the schematic in Figure 6-7 where image *a* and *b* represent the indentation of a volume with the predominant presence of nc- and am-TiO₂, respectively. This was the case of the C coatings, where the amorphous regions could occupy areas having a size of over 1 μm .

Figure 6-7. Schematic of the plastic and elastic deformation volumes under the indenter. a) Predominant presence of nc-TiO₂. b) Predominant presence of am-TiO₂.

The elemental composition at the indents was measured by EDX at circular areas of $\sim 3 \mu\text{m}$ in diameter in the nC-12 sample (estimated elastic region). A second batch of indentations (included in Figure 6-6) was performed on the nC-12 coating at sites spotted with large Ca and P contents by EDX mapping of the cross-section. The H and E data from the nC-12 specimen were plotted against the Ca at. % measured at the indentations in Figure 6-8. It could be observed that most of the scatter took place for the Ca contents below 7 at. % having an average hardness of $6.9 \pm 1 \text{ GPa}$ and an average E of $95 \pm 12 \text{ GPa}$ (region A in the graph). The scatter was drastically reduced for a narrow range of the Ca content between 7 and 9 at. % (region B, average H and E of $7 \pm 0.17 \text{ GPa}$ and $100 \pm 6 \text{ GPa}$, respectively). For Ca contents above 9 at. % (region C) the data showed a softer response and certain dispersion ($H = 6 \pm 0.7 \text{ GPa}$ and $E = 94 \pm 5 \text{ GPa}$). EDX of the TEM lamellas from sample nC-12 (see section 6.5.2) showed that nc-TiO₂ domains had Ca and P contents in the

ranges between 3.5 and 8 at. % and between 3 and 4 at. %, respectively, while the amorphous material presented Ca contents from 6 up to 20 at. % and P contents from 4 to 8 at. %. Hence, zones A and C in Figure 6-8 could be assigned to either predominantly nc-TiO₂ or am-TiO₂ volumes, respectively (Figure 6-7), while zone B was considered as a mixture region.

Figure 6-8. H and E modulus of the nC-12 coating against the Ca at. % measured at each indentation.

Table 6-3. The E and H and Ca and P contents measured at the indents of the lamellas a, b, c and d.

Lam.	E (GPa)	H (GPa)	Ca at. %	P at. %
a	93	6.2	11	7
b	94	7	7.4	6.7
c	96	5.2	4.5	5.2
d	113	8	3.7	5.8

In order to confirm this, TEM lamellas were extracted under few indentations in three regions. Four lamellas are presented herein as example: lamella *a* in region C, lamella *b* in region B and lamellas *c* and *d* in region A. Table 6-3 shows the mechanical properties and the elementary composition measured at the indents corresponding to the lamellas *a* through *d*.

Figure 6-9 presents the TEM BF images of the lamellas under the indentations, which are marked by dark arrows. These images proved that the microstructures under the indentations were: *a*) predominantly am-TiO₂ with the presence of nc-TiO₂ isles; *b*) a mixture of nc- and am-TiO₂; and, *c*) and *d*) nc-TiO₂. Therefore, it was confirmed that regions with the higher local fractions of am-TiO₂ had a softer response, and that the scatter of the mechanical properties was mitigated with the mixture of am- and nc-TiO₂. It was also confirmed that fully nc-TiO₂ presented the highest scatter.

Figure 6-9. TEM BF images of the lamellas *a*, *b*, *c* and *d*, under the indentations indicated by the arrows.

It is worth noticing the differences in *H* and *E* values and in microstructure between the indentations of lamellas *c* and *d*. On the one hand, the lamella *c* showed the presence of wide grain boundaries and elongated crystallites having a size that ranged between 20 to 100 nm resulting in the *H* of 5.2 GPa and the *E* of 96 GPa. The inset in Figure 6-9 *c* is a TEM BF image of the same region at lower magnification showing the presence of a bubble and a crack running through the bulk of nc-TiO₂ under the indentation. Henceforth, the microstructure under indentation *c* could be the result of either a type A, C, D or E discharge. On the other hand, the microstructure under indentation *d* consisted of fine globular grains with diameters between 20 to 30 nm and Ca and P-rich grain boundaries providing enhanced hardness (8 GPa) and elastic modulus (113 GPa).

Ceramic materials are characterized by a great dispersion in their mechanical properties and a brittle behaviour

due to the inherent presence of numerous intra- and intergranular flaws. Nanocrystalline ceramics (having crystallite sizes within tens of nanometres) have been shown to possess certain strain rate sensitivity and plasticity levels unseen in classic ceramics at the expense of H and E [263]. Recent studies claimed that plasticity by grain mobility (i.e. rotations, shifts and grain boundary sliding [264]–[266]), densification of mesoporosity and intergranular fracture are preferred to intragranular crack propagation as the maximum flaw size is limited by the grain size (Griffith criterion) [247], [267]. The former mechanisms being promoted by the reduced grain size [268]. The presence of PEO-related defects, such as in the case of indentation c , might assist the plastic deformation and reduce the elastic recovery resulting in the lower H and E values. These defects might also compromise the fracture toughness of the OCL. On the contrary, the nc-TiO₂ with Ca and P-rich grain boundaries (see Figure 5-34 in section 5.5.2) under indentation d might limit the slip of crystallites and prevent intergranular cracking assisting the elastic recovery resulting in a slightly stiffer overall response. Unfortunately, in the case of the nS1-12 and nS2-12 coatings the elastic regions hardly fit in the thickness of the coatings and no distinction between crystalline and amorphous mechanical response could be done, as in the case of the nC-12 coating. However, a similar correlation could be established considering the microstructures developed in the nS1-12 and nS2-12 coatings. The XRD, Raman and TEM analyses in chapter 5 showed that the nS1-12 coating was mainly formed by nc-TiO₂, while the nS2-12 coating had a larger fraction of am-TiO₂.

The H data presented in this section (6–8 GPa, Figure 6-6), which could be definitely assigned to the mechanical properties of the coatings' material, was slightly higher than the H values at low indentation depths in section 7.1 (5.5 GPa, < 10 % the coatings thickness, Figure 6-1). On the other hand, the E values were similar but tended to decrease with the indentation depth (Figure 6-2). This confirms the effect of porosity and brittle fracture on the nanoindentation measurements on the surface. It is reasonable to think that given the large scatter observed in the cross-section, the measurements of the first section could also present statistically lower H , E and toughness.

6.3 Nanoscratching

This section presents the outcomes of the adhesion and wear tests carried out by nanoscratching on the coatings fabricated at 120 s in all the electrolytes on both CG and UFG substrates. The section is further divided into loading tests and constant force reciprocating tests.

6.3.1 *Progressive loading scratch tests*

The C coatings presented a complex behaviour strongly dependent on their internal porosity. Four damage modes were observed along the scratch tracks: Lc1, Lc1', Lc2 and Lc3. Figure 6-10 *a* presents an overview of a representative scratch. Images from *b* through *d* present representative images of the four damage types. White arrows indicate the direction of the scratch. The critical normal loads at these points are presented in Table 6-4. The first two modes, Lc1 and Lc1', were related to the damage of the OCL: Lc1, tensile cracks at the scratch track (Figure 6-10 *b*); and Lc1', fracture of the OCL (Figure 6-10 *c*). Beyond Lc1, the tensile cracks

grew inwards and towards the adjacent coating assisted by the presence of sub- and micro-pores within the OCL. The growth of the cracks allowed the stylus to deepen into the coating causing the forward shearing of OCL sections behind the tip (Lc1'). This would subsequently lead to the densification of the coating under and ahead of the tip inducing the Lc2 failure.

Figure 6-10. SEM images of ramping scratches at 1 mN/µm up to a normal load of 500 mN on the nC-12 coating: a) overview of a full scratch, b) Lc1, tensile cracks at low load, c) Lc1', cohesive damage of the OCL, d) Lc2, detachment of the OCL, and e) Lc3, final cut-through

Table 6-4. Critical loads in N of the ramping scratches on the cC-12 and nC-12 samples.

Sample	Lc1	Lc1'	Lc2	Lc3
cC-12	103 ± 41	140 ± 41	181 ± 47	253 ± 47
nC-12	132 ± 38	158 ± 49	196 ± 60	273 ± 83

The Lc2 failure consisted on the dramatic detachment of large OCL portions ahead of the indenter, revealing the inner layers of the coating. (Figure 6-10 *e*). The loosening could be observed on the left side of the track at the initial point and at higher loads (Figure 6-10 *e*, A). The failure was, however, prevented at certain points (Figure 6-10 *e*, B). It is believed that this failure was strongly related to the horizontal cavities observed within the C coatings which, as confirmed by tomography (see Figures 5-12 and 5-13), would represent a weak horizontal plane. The OCL ahead of the indenter without underlying support would bent under the tip pressure and fracture ahead of the contact point (as in a deflected cantilever), whereas wherever physical support existed the OCL would remain attached. Plastically deformed coating was observed at the scratch track along and after the Lc2 critical point until the tip eventually cut-through into the substrate at higher loads (Lc3, Figure 6-10 *d*). The fact that the inner porous layer of the coating was observed in the SEM micrographs indicated that no adhesion failure took place. Raman measurements at the detached areas showed TiO₂ anatase peaks (Figure 6-11 *a*).

The critical loads for the cC-12 and the nC-12 coatings are presented in Table 6-4. The large scatter in the critical loads should be assigned to the heterogeneous nature and distribution of the porosity within the coatings. No significant difference was observed between the coatings, although the averages were lower in the case of the cC-12 coating presumably due to the lower thickness. The Lc1' and Lc2 failures took place at about 140-150 mN and 180-200 mN, respectively, for both coatings. The friction coefficients (COF) grew during the initial stages up to ~ 0.9 at the Lc1' the Lc2 points (Figure 6-12). A fairly constant regime was attained after the Lc3, when the tip reached the substrate. The COF were characterised by large fluctuations that could be related to the roughness and the fracture of the OCL before Lc1' and due to the abrasive wear of the substrate and crashing of the coating ahead of the tip beyond Lc3.

The loading scratches caused significant wedge spallation of the S1 and S2 coatings as in a hard-on-soft coated system (representative scratches are shown in Figure 6-11 *b* and *c-d*, respectively) [219]. Tensile cracks at the scratch track were rarely observed before the spallation in the S1 coatings (Figure 6-11 *b*) and nS2-12 coating (Figure 6-11 *d*). However, several buckling cracks appeared at the scratch track in coating cS2-12 before spallation (Figure 6-11 *c*). The mechanism by which the spallation was caused is believed to be as follows: cracks were formed somewhere ahead of the indenter (compressive shear cracks or bending cracks) and at the scratch track (due to buckling ahead of the tip or tensile stresses behind the tip). As the indenter moved forward cracks ahead of the indenter grew in a concave shape (A in the images), while cracks at the scratch track grew outwards in a divergent fashion to the adjacent coating (B in the images), forming a wedge with a shell-like shape. At the same time the cracks propagated through the coating reaching the coating-substrate interface. The bending of the substrate promoted the growth of the cracks along the interface. At some point the wedge

was finally detached allowing the tip to reach the substrate. The surface at the detached areas was analysed by Raman spectroscopy revealing weak anatase peaks (Figure 6-11 *e, f*). This indicated that the spallation was due to a cohesive failure through the inner layers of the coatings rather than to an adhesion failure between the coating and substrate. Another interesting feature found at the fracture surfaces in the S1 coatings were darker areas that were related to sectioned coating at the large openings described in the microstructural section (black arrows in Figure 6-11 *b*). These large openings would represent weak spots due to their large and complex porosity. In addition, the depression caused by the oxidation of Ti would lead to further deepening of the tip.

Figure 6-11. *b-d*) Representative secondary electron images of loading scratches on samples nS1-12, cS2-12 and nS2-12, respectively. *a, e* and *f*) Raman spectra at adjacent coating and failure regions of the loading scratches in samples cC-12, cS1-12, and cS2-12, respectively.

The chart in Figure 6-13 presents a comparison of the averaged critical loads for the OCL loosening of the coatings cC-12 and nC-12 (Lc2) and the wedge spallation of the coatings fabricated in the electrolytes S1 and S2. Given the scatter of the results, all the coatings presented similar critical loads being the S2 coatings slightly more resistant to failure. It must be taken into account the strong effect of the characteristic porosity in the C

coatings. The largest critical loads registered for the cS2-12 coating with respect to the nS2-12 one must be related with the slightly larger thickness. This thickness effect was not observed for the S1 coatings that could be explained by the larger population of pores and openings found in the nS1-12 specimen, increasing the probability of these weak spots to be in the path of the scratch.

Figure 6-12. Evolution of the friction coefficient during the loading scratches. Upper panel: nC-12. Lower panel: nS1-12 and nS2-12 coatings. Approximated positions of the critical loads are indicated.

Figure 6-13. Critical loads at the moment of the coatings' detachment.

6.3.2 Constant load reciprocating scratching

The objective of the tests was to evaluate the wear rate of the coatings before failure. In order to do this, it was necessary to find a load that not only caused the ultimate failure of the coatings but also induced gradual damage with the number of cycles. Constant normal load scratches were performed at 50, 100 and 150 mN for 10, 20 and 30 cycles over a length of 70 μm in all the coatings. The tests performed at 50 mN only caused the crushing of the coatings at the scratch track and cracks at the adjacent coating. At 100 and 150 mN, the OCL loosening in the C coatings (Lc3) and the spallation of the S1 and S2 coatings were observed at the coating adjacent to the scratches before any wear or delamination at the scratch track took place. Henceforth, the detachment at the adjacent coating was taken as the critical failure for these tests. At 150 mN, the failure of the coatings took place within the first 10 cycles in most of the specimens due to the fact that this load was quite close to the critical loads registered during the loading scratches (Figure 6-13). On the contrary, the damage was induced progressively at 100 mN. The critical failure was observed beyond 20 cycles in all the samples which allowed to measure the wear rate with the number of cycles before failure. It was required to increase the number of cycles up to 50 for the samples nS1-12 and cS2-12 samples in order to cause the failure.

Figure 6-14. SEM images of the wear tracks after the reciprocating constant normal force tests before (*a*, *c* and *e*) and after failure (*b*, *d* and *f*) at 100 mN. Images *a* and *b* correspond to the nC-12 coating after 20 and 30 cycles, respectively. Images *c* and *d* to the nS1-12 coating after 40 and 50 cycles, respectively. Images *e* and *f* to the cS2-12 coating after 40 and 50 cycles, respectively.

Representative scratches at 100 mN are shown in Figure 6-14. These scratches correspond to the nC-12 sample for 20 and 30 cycles (images *a* and *b*, respectively), and to nS1-12 (*c* and *d*) and cS2-12 (*e* and *f*) samples for 40 and 50 cycles, respectively. The images on the left and right columns illustrate the appearance of the scratches before (images *a*, *c* and *e*) and after the failure (images *b*, *d* and *f*), respectively. At the scratch track the coatings were smooth with no signs of abrasive wear. The friction coefficients were constant between 0.15 and 0.2 in all the samples. The wear rates, calculated following Eq. (3.8), are plotted against the number of cycles in Figure 6-15. Open symbols indicate the number of cycles for which failure took place. At those points, the volume generated by the coatings' detachment was also accounted for.

Figure 6-15. Wear rates vs. number of cycles. Dashed lines correspond to the wear rates obtained on both the CG and UFG substrates at 100 mN for 10 cycles. Open symbols indicate the failure of the coatings.

The C coatings presented the loosening of the OCL between 20 to 30 cycles (Figure 6-14 *b*). Before the failure, brittle fracture of the OCL was observed (Figure 6-14 *a*). The evolution of the wear rates suggested that the brittle spallation was more severe during the first 10 cycles. Interestingly, the wear rates for the nC-12 coating measured after 10 and 20 cycles ($5 \cdot 10^{-2}$ and $3.5 \cdot 10^{-2}$ mm³/N·m, respectively) were lower than the ones measured for the cC-12 coating ($7 \cdot 10^{-2}$ and $5 \cdot 10^{-2}$ mm³/N·m, respectively). However, after the failure the wear rate of the nC-12 coating was almost twice the wear rate of the cC-12 coating ($1.1 \cdot 10^{-1}$ and $5.5 \cdot 10^{-2}$ mm³/N·m, respectively). This could be explained by the fact that the nC-12 coating was thicker than the cC-12 coating, i.e. the former withstood better during the first 20 cycles but the volume detached was larger. The shape of the fractures indicated the fracture of several portions suggesting a progressive detachment of the OCL. The S1 and S2 coatings did not present significant damage of the adjacent coating before spallation (Figure 6-14 *c* and *e*). The spallation of the coatings took place between 20 to 30 cycles in the case of the cS1-12 and nS2-12 samples and beyond 40 cycles for the nS1-12 and cS2-12 coatings (Figure 6-14 *d* and *f*). These differences

could be related to the fact that the later coatings were thicker than their counterparts. The wear rates of the coatings decreased with the number of cycles beyond 10 cycles ($\sim 10^{-2} \text{ mm}^3/\text{N}\cdot\text{m}$) and in all the cases they were found below the ones calculated for both the CG and UFG before the spallation and of the same order after the spallation (between $1.5 \cdot 10^{-2}$ and $\sim 2.5 \cdot 10^{-2} \text{ mm}^3/\text{N}\cdot\text{m}$).

6.4 Discussion

6.4.1 Mechanical properties of nc-TiO₂ and am-TiO₂

The results in the section 6.2 showed that H and E measured depended on the fractions of nc- and am-TiO₂ present in the volume tested by nanoindentation. Herre et. al. [247] showed that am-TiO₂ particles had a hardness of $2.8 \pm 0.2 \text{ GPa}$ and a Young's modulus of $19.3 \pm 4.1 \text{ GPa}$ while nc-TiO₂ particles (crystallite size between 5 and 10 nm) had a hardness of $10.7 \pm 2 \text{ GPa}$ and an elastic modulus of $99.7 \pm 18.3 \text{ GPa}$. They also demonstrated that amorphous particles admitted larger elastic deformations than anatase particles and presented a ductile behaviour beyond the yielding point. The mechanical properties measured in the present work correlate well with the findings of Herre and co-workers [247]. Indentations over nc-TiO₂ domains in the nC-12 coating and on the nc-TiO₂ nS1-12 coating showed values of hardness of ~ 7 and 8.6 GPa , respectively, and E around 96 GPa for both coatings. The indentations on the surface (section 6.1) caused brittle fracture of the C and S1 coatings. On the other hand, lower H and E values were determined for the amorphous domains of the nC-12 coating (H and E of 6 and 94 GPa , respectively) and the less crystalline nS2-12 coating (H and E of 6 and 87 GPa , respectively). Few cracks were found after the indentation of the S2 coatings suggesting a higher fracture toughness which, according to Herre et. al. [247], could be related to the larger fraction of am-TiO₂ providing ductility to the coatings. It is also worth to point out that the S2 coatings showed the most compact OCL among all the coatings.

For a narrow range of Ca content (6 – 9 at. %) a nc- and am-TiO₂ mixture microstructure (Figure 6-9 b) led to reduced scatter of the data while maintaining acceptable levels of H and E (7 GPa and 100 GPa , respectively). This type of microstructure is assumed to provide the H of nc-TiO₂ and the ductility of am-TiO₂, which might represent a great opportunity to improve the mechanical performance of the C coatings. The conditions for the homogeneous distribution of this microstructure during short treatment times should be further investigated though. Nevertheless, the TEM observations suggested that they could be formed during the solidification of turbulent flows of plasma and melted material that follows a discharge (Figure 5-31 b). This points out that a high discharge density might produce a homogeneous distribution of nc- and am-TiO₂ mixtures.

It can be concluded that the presence of am-TiO₂ reduced slightly the H and E of the coatings but counterbalanced the brittle behaviour of nc-TiO₂ preventing the PEO coatings from fracture.

6.4.2 Mechanical performance during scratch testing

In principle, the thicker the coating, the higher the critical load and/or number of cycles to failure [269]. From the results in section 6.3, and given the thickness of the coatings, it could be concluded that the performance of the S1 and S2 coatings during both loading and constant load scratching tests were superior to the one of

the C coatings (almost twice as thick as the S1 and S2 coatings) and improved the wear resistance with respect to the uncoated substrates. The poor mechanical behaviour of the C coatings was assigned to: *i*) the low fracture toughness of the OCL due to PEO-related defects and sub- and microporosity within the OCL, as discussed in previous sections (sections 7.1 and 7.2); and, *ii*) the weak plane formed by the horizontal cavities that led to the early failure of the coatings (Lc3 failure mode. Section 7.3). Despite their lower thicknesses, the ductility of the S2 coatings led to higher spallation loads (~ 230 and 180 mN) in comparison with the S1 coatings (~ 160 mN). However, both treatments presented similar wear rates and number of cycles to failure. The wear resistance of the S1 coatings could be assigned to the higher hardness of the coating material while the ductility of the S2 coatings delayed the wear failure.

Souza et. al. [205] and Lima and co-workers [250] performed loading nanoscratches on Ca/P-containing PEO coatings (~ 4 μm thickness) using a Berkovich tip (Table 6-1). The authors found severe plastic deformation and detachment at critical loads above 220 mN. The critical loads were higher than the ones found for the C coatings (Lc2, 180-200 mN). However, the comparison has to be made carefully as different tip geometries could induce different stress fields. The wear rate found in [250] was of $1.1 \cdot 10^{-4} \text{ mm}^3/\text{N} \cdot \text{m}$ without considering detachment of the adjacent coating.

Most of the tribological studies in the literature are not comparable with the present work as they use micro indenters (i.e. Rockwell C with a tip radius of 200 μm) or are based on macro pin-on-disk or ball-on-disk tests (Table 6-1). Due to the tip size used in these studies the applied pressures are lower than in the case of the present study where the tip radius was of 10 μm . For instance, Alsaran and co-workers [38] performed pin-on-disk tests (tip diameter 5 mm) on a Ca and P containing PEO coating (7-9 μm thickness) on UFG CP Ti for a sliding distance of 84 m at 2 N. According to the track width (~ 200 μm), the applied pressure was ~ 63 MPa, whereas in instrumented nanoscratching the applied pressures are of the order of GPa. The reported wear rate in [38] was of $1.5 \cdot 10^{-4} \text{ mm}^3/\text{N} \cdot \text{m}$, one order of magnitude lower than the wear rates before failure (40 cycles, equivalent sliding distance of 2.8 mm) of the nS1-12 and cS2-12 coatings (Figure 6-15). On the other hand, detachment of the adjacent coating, as in the present work, is not considered as a failure mode since the size of the detached area is several orders of magnitude smaller than the scratch track width. Instead, detachment at the wear track is defined as failure which takes place at significantly larger critical loads and wear distances than adjacent detachment. Aliasghare et. al. [261] identified the failure by delamination at the wear track at 600 s, equivalent to 200 cycles. Gowtham et. al. [33] measured critical loads of up to 26 N for 4 to 9 μm thick coatings by progressive loading micro-scratch testing (Rockwell C with a tip radius of 200 μm). Similarly, Chen and co-workers [258] performed progressive loading micro-scratches on a 20 μm thick PEO TiO₂-based Ca and P-containing coating. The critical load found was 36 N.

6.4.3 Approaches to improve the mechanical performance of the PEO coatings

In order to ensure the long term success of the coated implants it is important to preserve the integrity of the coatings as they provide the necessary corrosion resistance and composition to improve the cell-implant

interactions. Common approaches to improve the mechanical performance of PEO coatings consist on increasing the hardness by the precipitation of hard ceramic phases such as alumina (Al_2O_3) or rutile (TiO_2) (Table 6-1). These approaches include the addition of aluminates and silicates to phosphate-based electrolytes [251], [262], [270] and the enhanced crystallization of TiO_2 rutile through the application of higher voltages during the PEO treatment [206], [270] or via post-thermal treatments [250], [271]. Thermal treatments such as the one in [250] could be a convenient approach to increase the H and the tribological performance of the coatings in the present study, especially in the case of the C coatings.

However, debris generation of ceramic PEO coatings seems unavoidable and unless it is minimize these approaches might aggravate abrasive wear or fretting fatigue situations due to the hardness of the debris liberated. In line with this, according to the H measurements in section 6.2, the debris liberated by the S1 coatings would be the hardest amongst all the coatings (~ 8 GPa) while the debris from the S2 coatings would be the softest (~ 6 GPa). In the event of friction between the coated-implant and any other surface, the soft debris might deform plastically and reduce the friction coefficient between surfaces. On the other hand, the hardening approaches just discussed are not aimed to reduce the porosity of the coatings which has been seen to decrease dramatically the fracture toughness of the coatings.

We propose an alternative approach based on the tuning of the frequency and duty cycle of the applied signal during the PEO treatment which has been proven as successful approach to produce denser and thicker coatings [33] and to enhance the deposition of amorphous material [176]. The aim of this approach is twofold: *i*) produce denser and thicker coatings for short treatment times, minimizing the porosity within the coatings; and *ii*), enhance the deposition of am- TiO_2 . The enhancement of amorphous phases is further aimed to: provide ductility to the coatings in order to counterbalance the brittle character of TiO_2 ; and to produce softer debris. Following equations 3.1 and 3.2, the discharge characteristics can be controlled in pulsed DC and AC modes when the t_{on} (positive pulse time) approximates the total time of the discharges cascade during a type B discharge (section 6.6.1). The cascade of discharges is shut down when the t_{on} is shorter than the total discharge cascade time, reducing the pore size and coating material generated. As in the present study, the cycle time is of ~ 3.3 ms ($f = 300$ Hz), while the t_{on} is of ~ 1.7 ms (50 % duty cycle). Gowtham et. al. [33] fabricated coatings at 50 and 1000 Hz using for each frequency duty cycles of 0.1 and 0.95 on CP Ti in a Na_3PO_4 based electrolyte. The coatings fabricated at 1000 Hz were thicker and denser than their 50 Hz counterparts. At the same time, comparing the coatings fabricated at 1000 Hz, the one with a 0.95 duty cycle (t_{on} 0.95 ms) was thicker and denser than the coating fabricated with a 0.1 duty cycle (t_{on} 0.1 ms). These results indicated that the coating mass production per cycle was larger for longer t_{on} times [175] (high duty cycle), however, the accumulated mass production for a given time span ($>$ signal period) is larger for higher frequencies, when the density of discharges per time is higher. In addition, using high frequencies led to finer porous microstructures resulting in denser coatings. The sample fabricated at high frequency-high duty cycle in [33] also presented the higher scratch and corrosion resistance which can be assigned to the thickness and compactness of the coating. Wang and co-workers [176] fabricated PEO coatings on CP Ti varying the frequency in the range between 100 to

5000 Hz in a Ca- and P-containing electrolyte using EDTA chelating agent. XRD measurements showed that as the frequency was increased the coatings were more amorphous. As it was found in the present work, electrolyte derived species deposit preferentially as amorphous phases and inhibit the crystallization of TiO_2 [201]. The results in [176] indicated that the greater number of discharges involved greater deposition of amorphous phases. However, the cooling process after a discharge is independent of the pulse time and secondary discharges might take place as heated and melted TiO_2 are more conductive than the cooled TiO_2 [176]. This side-effect led to a high porosity in the coating fabricated at 5000 Hz.

In conclusion, this approach appears promising to fabricate dense and thick coatings with an enhanced deposition of amorphous phases that could improve the mechanical performance of PEO coatings. Besides, they showed to be beneficial for other aspects such as corrosion resistance and cell-to-coating interactions. Nevertheless, the application of this approach should be further investigated to find the appropriate combination of frequency and duty cycle for each electrolyte in order to obtain the desired coating microstructure in terms of compactness, composition and phases.

Regarding the application of this approach to the C treatment it might provide with a high density of discharges obtaining the nc- and am- TiO_2 mixture-like microstructures observed in Figure 5-31 *b* and Figure 6-9 *b* which were considered to provide acceptable H and E (7 GPa and 100 GPa, respectively) while reducing the scatter of the mechanical properties.

6.5 Conclusions

- The mechanical properties of highly porous PEO coatings cannot be accurately measured and comprehended by means of nanoindentation directly on the surface of the coatings due to the presence of pores, which affect the accuracy of the H and E measurements.
- The results in the second section showed that H and E measured depend on the fractions of nanocrystalline and amorphous TiO_2 present in the volume tested by nanoindentation. In agreement with the work of Herre et. al. [247], am- TiO_2 provided with ductility to the coatings explaining the higher fracture toughness of the S2 coatings.
- Henceforth, the mechanical properties of the coatings depended strongly on the characteristic microstructure and porosity within the OCL of each treatment. The differences observed in the mechanical properties when comparing each treatment on both substrates were more likely related to the thickness differences observed in Chapter 5.
- Despite their lowest thickness and hardness, the coatings fabricated in the S2 electrolyte appeared to have a highest fracture toughness due to the higher fraction of ductile am- TiO_2 delaying the failure during the tribological tests. The brittle behaviour of the S1 coatings led to the lowest critical loads but to an acceptable wear behaviour due to the hardness provided by the large fraction of nanocrystalline TiO_2 in the microstructure. The C coatings presented a poor mechanical performance considering their thickness. The PEO-related microstructural defects and porosity within the outer compact layer promoted the brittle

behaviour of this layer. The horizontal cavities induced the early failure of the coatings during the tribological tests.

- An alternative approach to the common methods for hardening of PEO coatings was proposed. The proposed approach consisted on the variation of the frequency and duty cycle of the PEO applied signal.

7 Corrosion characterization of PEO coatings

7.1 Open circuit potential evolution

The evolution of the open circuit potential (OCP) of the substrates and the PEO-coated samples was recorded for 1 h in the Hank's + D-glucose solution at 37°C (Figure 7-1). The OCP values of the samples after 1 h immersion can be found in Table 7-1.

Figure 7-1. Evolution of the Open Circuit Potential (OCP) of the CG and UFG CP Ti substrates and PEO coatings during 1 hour of immersion in Hank's + D-glucose solution.

Table 7-1. Open Circuit Potential (OCP), corrosion potential (E_{corr}) and passivation current density (j_{pass}).

Sample	OCP (mV)	E_{corr} (mV)	j_{pass} (A/cm ²)
CG substrate	-348	-357	$1.95 \cdot 10^{-6}$
cC-12	64	-79.62	$2.18 \cdot 10^{-8}$
cS1-12	68.94	-46.82	$2.75 \cdot 10^{-8}$
cS2-12	43.85	-36.95	$3.38 \cdot 10^{-8}$
UFG substrate	-288	-290	$1.79 \cdot 10^{-6}$
nC-12	-71.75	-164.5	$1.16 \cdot 10^{-7}$
nS1-12	14.31	-78.31	$1.31 \cdot 10^{-7}$
nS2-12	-0.3	-70.7	$1.24 \cdot 10^{-7}$

The bare substrates presented an increasing evolution of the OCP indicating the formation of a passive oxide film. The UFG CP Ti had a nobler response (-288 mV_{Ag/AgCl}) than the CG CP Ti (-348 mV_{Ag/AgCl}). The PEO-coated samples reached positive steady OCP values 300-400 mV higher than the substrates except for the case of the nC-12 coating. The coatings fabricated on the CG substrate reached steady OCPs above 44 mV_{Ag/AgCl}

(Table 7-1), whereas the OCP of the nS1-12 and nS2-12 coatings were ~ 14 mV_{Ag/AgCl} and nearly zero, respectively, and negative in the case of the nC-12 coating (-72 mV_{Ag/AgCl}). The decrease of the OCP values of the PEO-coated samples could be assigned to the permeation of the electrolyte within the porosity of the coatings.

7.2 DC polarization response

DC polarization tests were performed in all the samples after 1 h of immersion in the Hank's + D-glucose solution at 37 °C (Figure 7-2). The corrosion potentials (E_{corr}) and passivation current densities (j_{pass}) can be found in Table 7-1. The CG and UFG substrates showed E_{corr} of -357 mV_{Ag/AgCl} and -288 mV_{Ag/AgCl}, respectively. An active-passive transition segment was observed just above the corrosion potential during which Ti^{3+} and Ti^{4+} ions are formed and incorporated into the passive film [272]. A well-defined passive region at a j_{pass} of 1.8 - 1.95 $\mu\text{A}/\text{cm}^2$ that extended for about 1.2 V was observed after the transition segment.

Figure 7-2. Direct current polarization curves of the CG and UFG CP Ti substrates and PEO coatings during 1 hour of immersion in Hank's + D-glucose solution.

The E_{corr} of the PEO-coated samples were nobler than the ones of the bare substrates as indicated in Table 7-1, being higher in the case of the coatings fabricated on the CG substrate. In comparison with the bare substrates, the PEO-coated samples showed a shorter transition region indicating the presence of a pre-existing TiO_2 barrier layer (i.e. nanoporous-nanocrystalline inner layer of the coatings) that supported the passivation process. The j_{pass} of the PEO-coated UFG samples were of one order of magnitude lower (0.12 - 0.13 $\mu\text{A}/\text{cm}^2$) than the ones of the substrates and of two orders of magnitude with respect to the coatings on the CG substrate ($2 \cdot 10^{-2}$ - $3.4 \cdot 10^{-2}$ $\mu\text{A}/\text{cm}^2$). However, the passive region was more stable in the coatings on the UFG substrate

than the one of the coatings on the CG substrate. The nC-12 had a passive region in the range between 0.24 and 1.38 V_{Ag/AgCl}. The nS1-12 and nS2-12 coatings reached the passive state slower than the nC-12. Among the coatings on the CG substrate, the cC-12 coating had the lowest j_{pass} followed by the cS1-12 and the cS2-12. After the passive segment all the samples experimented an increase of the current density related to oxygen evolution.

The UFG CP Ti showed a nobler response and a faster passivation than the CG CP Ti. The surface structure of UFG CP Ti has been proven to be more corrosion resistant than CG CP Ti in most media [99]–[101]. It is believe that the high fraction of high-angle grain boundaries and dislocations on the surface of UFG CP Ti provide more oxide nucleation points than on the CG CP Ti, hence leading to a more rapid passivation of the surface. The OCP evolution and DC polarization results showed that there was a clear difference in the electrochemical response between the coatings fabricated on the CG and the coatings fabricated on the UFG substrate. It was shown in chapter 5 that the substrate refinement influenced the microstructure of the inner barrier layer (see section 5.5.1) while the electrolyte influenced the characteristics of the OCL (i.e. composition, crystallinity, morphology, etc.). It can be inferred from the results that the microstructure of the inner barrier layer played a major role while the thickness of the coatings and the nature of the OCL induced subtle differences. According to the TEM findings comparing the inner layers of the cC-12 and nC-12 coatings (Figure 5-28), the cC-12 had a continuous ~50 nm-thick amorphous coating-substrate interface that was partially crystallized in the nC-12 as well as in the nS1-12 and nS2-12 coatings (Figure 5-29). It has been shown that nc-TiO₂ has a lower dielectric constant (18.2 F/m) in comparison with the one of am-TiO₂ (39.7 F/m) i.e. nc-TiO₂ is more conductive than am-TiO₂ [246]. Hence, the nobler response of the PEO-coated CG CP Ti with respect to the PEO-coated UFG CP Ti could be assigned to this am-TiO₂ layer that acted as a capacitor blocking the charge transfer and delaying the corrosion. The same reason would explain the less stable passivation on the CG CP Ti as the am-TiO₂ would impede the Ti³⁺ and Ti⁴⁺ migration to be incorporated into the more stable nc-TiO₂ passive film.

7.3 EIS response

The total impedances ($|Z|_{0.01\text{Hz}}$) of the bare and coated samples for the immersion times from 1 hour to 14 days are shown in Figure 7-3. After 1 hour of immersion the cS2-12 and nS2-12 coatings presented the highest total impedance (1540 and 1190 kΩcm², respectively) in comparison with the rest of coated samples and the bare substrates (200-400 kΩcm²), which could be assigned to the compactness and amorphous character of these coatings. Although demonstrating better corrosion resistance in DC polarization test, the PEO-coated samples in the C and S1 electrolytes had $|Z|$ values similar to the bare substrates for 1 hour in SBF. This behaviour might be explained by the fact that PEO coatings on CP Ti have a capacitive response in neutral electrolytes that extends to frequencies lower than the capacitive response of non-coated CP Ti and well below the lower limit studied here (0.01 Hz) [36]. Hence the coated samples could present a higher total impedance if the frequency swept had been extended to frequencies lower than 0.01 Hz. After 14 days of immersion the total impedance of the cS2-12 and nS2-12 coatings descended to 800 kΩcm². The total impedance of the C and S1

coatings decreased after the 3 day of immersion to 200-300 kΩcm² where they remained constant. The bare substrates presented a dissimilar response with the immersion time which is described in the following subsection.

Figure 7-3. Total impedance $|Z|$ of the CG and UFG CP Ti substrates and PEO coatings during 1 hour of immersion in Hank's + D-glucose solution.

The EIS experimental data was fitted using Randles equivalent circuits whose elements were selected in accordance with the response obtained. The circuits are composed by pairs of in-parallel resistors and constant phase elements (CPE) to account for the effects of the films morphology; i.e. a R_b/CPE_b pair would represent the resistance and the capacitance of the barrier oxide film. Unlike pure capacitors, CPE elements adjust better to the response of non-homogeneous films (i.e. rough, discontinuous, porous, etc. films). The impedance of this type of element is calculated as follows:

$$Z = 1/[CPE(j\omega)^n]$$

Where j is the imaginary number ($-1^{1/2}$), ω the radial frequency and the exponential n , that lays in the range 0-1, represents the approximation to a pure capacitor ($n = 1$). A resistor element representing the resistance of the electrolyte (R_{el}) was included in all the circuits. Additional elements to account for diffusion or space charge regions effects were used whenever necessary. The fitting of the data was performed using the ZView software ensuring a fitting goodness below 0.01.

7.3.1 Substrates response

The Nyquist, imaginary vs. real part of the impedance (Z'' vs. Z'), and Bode plots, impedance modulus ($|Z|$)

and phase (theta) versus frequency, for both the CG and UFG substrates are presented in Figure 7-4 and Figure 7-5, respectively. Both substrates presented essentially a capacitive behaviour ($\theta > 50^\circ$, plot *c* in Figure 7-4 and Figure 7-5) for almost the whole range of swept frequencies except for the CG substrate after 14 days of immersion in SBF where the phase angle decreased greatly for frequencies < 1 Hz. The capacitive response of the substrates is generally assigned to the dielectric nature of the barrier TiO_2 thin film formed and a space charge region that develops at the film-substrate interface [36], [273].

Figure 7-4. Complex plot (a) and Bode plots (b and c) of the CG CP Ti in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

Figure 7-5. Complex plot (a) and Bode plots (b and c) of the UFG CP Ti in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

Accordingly, the EIS response of the substrates was fitted with the equivalent circuit shown in Figure 7-6 which consisted on two in-parallel CPE/R elements connected in-series accounting for the effects of the barrier film (CPE_b/R_b) and the space charged region ($\text{CPE}_{sc}/\text{R}_{sc}$). The fitted data using the aforementioned circuit is presented in Table 7-2.

Figure 7-6. Randles equivalent circuit for the fitting of the EIS response of the CG and UFG CP Ti.

Table 7-2. Equivalent circuit parameters for the CG and UFG CP Ti for 1h, 3d, 7d and 14d of immersion in Hank's + D-glucose solution.

Sample	Immersion time	$ Z $ ($k\Omega cm^2$)	CPE_{sc} ($\mu S s^n cm^{-2}$)	n	R_{sc} ($k\Omega cm^2$)	CPE_b ($\mu S s^n cm^{-2}$)	n	R_b ($k\Omega cm^2$)
CG Substrate	1 h	245.53	163.30	0.75	4.81	45.72	0.89	347
	3 d	121.16	51.70	0.82	13.44	56.60	0.83	119.20
	7 d	8.37	46.90	0.83	17.09	90.54	0.84	77.77
	14 d	16.18	38.50	0.86	11.10	520.00	0.79	9.67
UFG Substrate	1 h	293.99	159.00	0.74	7.74	45.90	0.97	401
	3 d	415.36	50.04	0.93	21.52	17.90	0.84	2470
	7 d	601.02	-	-	-	-	-	-
	14 d	640.00	51.20	0.87	14.11	17.50	0.86	2700

The UFG CP Ti substrate demonstrated a higher total impedance ($294 k\Omega cm^2$) with respect to the CG CP Ti ($245.53 k\Omega cm^2$), in agreement with the OCP and DC polarization results. The CG substrate lost its passivity with the immersion time as it could be seen from the $|Z|$ values decreasing from $245.53 k\Omega cm^2$ after 1 hour of

immersion down to $16.18 \text{ k}\Omega\text{cm}^2$ after 14 days. The thickness of the barrier film can be estimated following the equation:

$$d \text{ (nm)} = \frac{\varepsilon_0 \cdot \varepsilon}{C}$$

Where ε_0 the vacuum permittivity (8.85 F/m) and ε is the relative permittivity of TiO_2 (39.7 F/m for amorphous titania [246]), and C the capacitance (F/cm^2) which can be inferred from:

$$C = \frac{1}{2\pi f_{\theta\max} Z_{\theta\max}}$$

Being $f_{\theta\max}$ and $Z_{\theta\max}$ the frequency and impedance at the maximum phase angle of the corresponding time constant. According to these equations the thickness of the barrier film on both substrates for all the immersion times was between 1 and 5 nm.

The CPE_b of the barrier film provides information on its nature in such a way that lower CPE_b indicates a more compact film [33] and vice-versa, higher CPE_b might indicate a more porous and flawed film [274]. At 1 hour both substrates had similar R_b and CPE_b values suggesting that the barrier films were structurally similar. However, after 3 days of immersion, the CPE_b of the CG CP Ti increased indicating a more porous/defective film while, on the contrary, the CPE_b of the UFG CP Ti decreased indicating a more compact oxide layer. Grain boundaries and dislocations are more chemically active than bulk defectless material, meaning that a higher release of Ti ions is expected at those defects. Given the results just mentioned, it might be suggested that the net Ti^{3+} and Ti^{4+} release was higher in the case of the UFG CP Ti due to the high density of high angle grain boundaries and dislocations resulting in a more compact passive layer. In addition, it was commented in section 8.1, that the high density of surface defects also provides with several more points for TiO_2 nucleation.

The EIS response of the substrates obtained in the present study contrast with other values found in the literature where the CPE_b values were one order of magnitude higher and the $|Z|$ of the CG CP Ti did not decreased [105], [273]. In both aforementioned articles the surfaces of the Ti substrates were treated with HF and HF/ HNO_3 before the electrochemical tests, producing a passive TiO_2 that ensured the corrosion protection at immersion times up to 28 days. In the present work, due to technical difficulties, the surfaces of the CG and UFG substrates were only grounded (SiC-600) and no chemical treatment was applied. Henceforth, the bare substrates had a peak-valley surface. It is believed that this surface morphology led to the decrease of the CG CP Ti $|Z|$ as the barrier oxide might not grow homogeneously at peak and valleys.

7.3.2 PEO coatings response

The coatings fabricated in the C and S1 electrolytes presented two time constants in the high frequency range between 10^2 and 10^4 Hz and below 1 Hz , respectively, for the first hour of immersion (plots *c* in Figure 7-7 through 7-10). Both time constants were related with the outer and inner parts of the coatings, respectively [275]. The high frequency component appeared as an undeveloped semicircle in the complex plots (inset in

plots *a*). A diffusion-related slope could be found in the range between 1 to 10 Hz in the phase angle plot. This diffusion effect was less evident for the cS1-12. The EIS experimental data of the C and S1 coatings was fitted with the equivalent circuit shown in Figure 7-11 where the contribution from the inner layer of the coatings was represented by an in-parallel CPE_b/R_b element nested into the CPE_{ocl} and R_{ocl} elements that corresponded to the response of the OCL and the micro-porosity within it (i.e. discharge channels and cavities). A finite length Warburg diffusion element with open circuit terminus was included in the equivalent circuit to account for mass-transfer processes. The EIS fitting parameters for the PEO coatings fabricated in the C and S1 electrolytes are presented in Table 7-3.

Figure 7-7. Complex plot (*a*) and Bode plots (*b* and *c*) of the cC-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

For immersion times of 3 to 14 days, the high frequency component disappeared for the C coatings and the nS1-12 sample whereas it was mitigated in the cS1-12. In addition, the diffusion influence extended towards higher frequencies. This effect has been already reported in SBF, artificial saliva and NaCl [150], [249], [276] and has been assigned to the permeability of the PEO coatings [36], i.e. the internal cavities of the coatings are filled by the electrolyte with the immersion time [277]. In fact, the R_{ocl} values for 3 to 14 days approximated the electrical resistance of the electrolyte ($< 100 \Omega\text{cm}^2$) in the cC-12, nC-12 and nS1-12 coatings. At the same time the W_o -T and W_o -R increased (Table 7-3). Warburg diffusion may represent both the mass-transport through the cavities of the coatings and the diffusion of species through the oxide [277]. This suggests that between 1 hour to 3 days of immersion the cavities of the coatings were filled with electrolyte and that the number of vacancies within the OCL was reduced by the oxidation of the TiO_2 [273].

The influence of the OCL in the cS1-12 was still evident for long immersion times in comparison with its counterpart (R_{ocl} -cS1-12: 1h, $\sim 3.6 \text{ k}\Omega\text{cm}^2$; 3d, $\sim 0.76 \text{ k}\Omega\text{cm}^2$. R_{ocl} -nS1-12: 1h, $\sim 13.4 \text{ k}\Omega\text{cm}^2$; 3d, $\sim 0.08 \text{ k}\Omega\text{cm}^2$). The cS1-12 coating had fewer and wider openings in comparison with the nS1-12 coating. As observed in the cross-sections, the bigger the openings, the greater the number of channels forming an

entangled porous structure which could impede the mass-transport and could get clogged easier by corrosion products [36].

Figure 7-8. Complex plot (a) and Bode plots (b and c) of the nC-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

Figure 7-9. Complex plot (a) and Bode plots (b and c) of the cS1-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

Table 7-3. Equivalent circuit parameters for the cC-12, nC-12, cS1-12 and nS1-12 coatings 1h, 3d, 7d and 14d of immersion in Hank's + D-glucose solution.

Sample	Immersion time	$ Z $ ($M\Omega cm^2$)	CPE_{oc1} ($\mu S s^n cm^{-2}$)	n	R_{oc1} ($k\Omega cm^2$)	WO-T (s)	n	WO-R ($k\Omega cm^2$)	CPE_b ($\mu S s^n cm^{-2}$)	n	R_b ($M\Omega cm^2$)
cC-12	1 h	0.29	0.91	0.80	0.44	123.70	0.39	36.46	40.00	0.92	1.89
	3 d	0.25	0.75	0.75	0.04	127.00	0.53	41.13	46.70	0.89	1.72
	7 d	0.22	5.62	0.56	0.04	102.90	0.51	26.87	43.90	0.92	22.50
	14 d	0.24	7.54	0.59	0.04	59.40	0.57	42.05	41.50	0.95	7.56
nC-12	1 h	0.23	1.45	0.75	0.40	0.20	0.44	2.20	63.40	0.87	0.42
	3 d	0.22	7.44	0.57	0.06	50.89	0.55	46.55	41.20	0.95	3.27
	7 d	0.22	9.69	0.55	0.06	178.00	0.58	122	42.80	0.99	50.20
	14 d	0.19	10.60	0.57	0.06	440	0.54	92.53	42.22	0.96	218
cS1-12	1 h	0.37	0.28	0.89	3.59	3.19	0.19	20.45	34.80	0.90	1.17
	3 d	0.23	0.63	0.82	0.76	0.51	0.51	4.06	68.20	0.91	1.39
	7 d	0.20	0.72	0.81	0.53	0.27	0.40	1.99	82.60	0.93	1.14
	14 d	0.21	0.68	0.82	0.55	0.24	0.40	2.26	84.00	0.92	1.34
nS1-12	1 h	0.32	0.22	0.83	0.59	0.1	0.13	31.36	56.6	0.93	0.79
	3 d	0.28	5.87	0.60	0.09	52.23	0.55	46.14	41.70	0.98	10.24
	7 d	0.27	5.61	0.60	0.09	65.3	0.53	35.46	40.3	0.97	2.04
	14 d	0.27	4.35	0.63	0.08	69.04	0.51	28.27	40.30	0.95	8.03

Figure 7-10. Complex plot (a) and Bode plots (b and c) of the nS1-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

Figure 7-11. Randles equivalent circuit for the fitting of the EIS response of the cC-12, nC-12, cS1-12 and nS1-12 coatings.

Figure 7-12. Complex plot (a) and Bode plots (b and c) of the cS2-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

The S2 coatings presented a capacitive behaviour ($\theta > 50^\circ$, plot c in Figure 7-12 and Figure 7-13) with two time constants in the high and low frequency ranges which became more evident with the immersion time. The W_o element was substituted by an in-parallel CPE_{sc}/R_{sc} in series with the CPE_b/R_b component representing the formation of a space charge region (Figure 7-14) [275].

The fitting parameters can be found in Table 7-4. The high frequency component shifted towards higher frequencies for the time span of 3-14 days in both samples indicating a higher influence of the outer part of the coating. In fact, in contrast to samples cC-12, nC-12 and nS1-12, the R_{ocl} of both S2 coatings increased with the immersion time (Table 7-3). It is worth to remind here that the S2 coatings were much compact than the C and S1 coatings and that the internal porosity was mostly limited to columnar discharge channels. Henceforth, the R_{ocl} increase might be related to the formation of TiO_2 at the bottom of the discharge channels effectively making the coating more compact.

Figure 7-13. Complex plot (a) and Bode plots (b and c) of the nS2-12 coating in Hank's + D-glucose solution for the immersion times from 1 h to 14 days.

Figure 7-14. Randles equivalent circuit for the fitting of the EIS response of the cS2-12 and nS2-12 coatings.

Regarding the response of the inner barrier layer: *i*) the R_b of all the coatings were shown to be higher than the ones of the substrate demonstrating the improvement in the corrosion resistance; and, *ii*) the R_b after 1 hour of immersion was higher in the coatings on the CG substrates in comparison with their UFG counterparts (Table 7-3 and Table 7-4) explaining the nobler response showed in the polarization tests. The CPE_b of the barrier film provides information on its nature in such a way that lower CPE_b indicates a more compact film (i.e. am- TiO_2) [33] and vice-versa, higher CPE_b might indicate a more porous and flawed film (i.e. nc- TiO_2 , more conductivity) [274].

Table 7-4. Equivalent circuit parameters for the cS2-12 and nS2-12 coatings 1h, 3d, 7d and 14d of immersion in Hank's + D-glucose solution.

Sample	Immersion time	$ Z $ ($M\Omega cm^2$)	CPE_{oc1} ($\mu S s^n cm^{-2}$)	n	R_{oc1} ($k\Omega cm^2$)	WO-T (s)	n	WO-R ($k\Omega cm^2$)	CPE_b ($\mu S s^n cm^{-2}$)	n	R_b ($M\Omega cm^2$)
cS2-12	1 h	1.54	3	0.65	0.43	1.24	0.99	30	1.33	0.78	72.7
	3 d	0.90	3.46	0.73	16.94	1.41	0.89	9.44	4.26	0.69	21
	7 d	-	-	-	-	-	-	-	-	-	-
	14 d	0.77	3.75	0.72	15.93	0.99	0.99	4.54	4.22	0.61	57.6
nS2-12	1 h	1.19	4.30	0.65	0.22	2.76	0.99	0.08	1.31	0.93	13.8
	3 d	1.13	4.34	0.60	0.19	6.61	0.77	0.34	0.6	0.99	21.9
	7 d	1.04	2.34	0.79	0.56	0.37	0.99	48	3.46	0.65	5.11
	14 d	0.80	2.79	0.79	0.53	0.37	0.99	51.	4.8	0.64	5.39

The effect of the crystalline/amorphous nature of the inner barrier layer on the electrochemical performance of the coatings was discussed in section 7.2. This discussion was supported by the CPE_b values obtained for 1 hour in SBF. The CPE_b values of the cC-12 and cS1-12 coatings (40 and $34.8 \mu S s^n cm^{-2}$, respectively) were lower than the CPE_b of the nC-12 and nS1-12 (63.4 and $56.7 \mu S s^n cm^{-2}$, respectively) demonstrating that the later were more conductive due to the larger fraction of nc-TiO₂. The CPE_b of the S2 coatings were identical and one order of magnitude lower than the other coatings ($\sim 1.3 \mu S s^n cm^{-2}$). Given that the thickness of the am-TiO₂/nc-TiO₂ barrier layer of these coatings was lower than the ones of the C and S1 coatings (according to TEM BF images ~ 200 nm and over 400 nm, respectively, see section 5.5.1), these low CPE_b values may as well represent to some extent the amorphous material on top of the am-TiO₂/nc-TiO₂ layer.

Comparing the results between 1 hour and 3 days of immersion the lower stability of the passive film in the CG-coated samples was also corroborated. The CPE_b of the CG-coated samples increased at the third day of immersion while the R_b was constant for the cC-12 and cS1-12 coatings suggesting the growth of a defective barrier film. In the case of the cS1-12 the R_b also increased what could be assigned to a thicker-porous layer [274]. In all the coatings fabricated on the UFG CP Ti the CPE_b decreased and the R_b increased suggesting that the film formed after 3 days had less defects due to the stable passivation of these coatings, as discussed previously in section 7.2. Beyond 3 days of immersion the CPE_b of the C coatings remained constant while the R_b increased. Both the CPE_b and R_b of the S1 coatings remained constant. In the case of the S2 coatings the R_b decreased which might be related to the clogging of the discharge channels and a subsequent increased contribution from the OCL.

7.4 Discussion

In agreement with previous reports, UFG CP Ti showed a better electrochemical behaviour than CG CP Ti. This improvement is assigned to the high density of high-angle grain boundaries and dislocations which provided with more TiO₂ nucleation points (section 7.1) and, as hypothesized in section 8.3.1, a higher net Ti ions release.

The PEO treatment of CP Ti has been proven to improve the corrosion resistance and the passivation. The results suggested that the nature of the inner layer formed during PEO, affected directly by the substrate state (see section 5.5.1), played a major role in the electrochemical performance of the coatings. The coatings fabricated on the CG substrate showed a nobler response but the passivation of the coatings on the UFG CP Ti was more stable. It was assumed in section 7.2 that the more dielectric behaviour of am-TiO₂ insulated the CG substrate but impede the transfer of Ti^{3+} and Ti^{4+} slowing down their incorporation into the nc-TiO₂ and decreasing the stability of the barrier layer. This was supported by the CPE_b values presented in section 7.3.2. It was shown that after 1 hour of immersion, the barrier film of the PEO-coated CG samples was more compact and resistive than the one of the UFG samples, in agreement with the presence of the am-TiO₂ layer. However, after 3 days of immersion the CPE_b of the CG-coated samples increased while the CPE_b of the UFG-coated

samples decreased. This indicated that Ti ions had been effectively incorporated into the barrier film of the UFG coatings due to the conductivity of the nc-TiO₂, while the supply of ions was blocked by the am-TiO₂ in the CG coatings.

7.5 Conclusions

- In comparison with the CG CP Ti, the refined surface of UFG CP Ti led to more TiO₂ nucleation points and higher Ti³⁺ and Ti⁴⁺ release, improving the corrosion resistance.
- The PEO treatment of CP Ti in both CG and UFG states improved the corrosion resistance.
- The differences in the electrochemical behaviour of CG- and UFG-coated samples were assigned to the nature of the inner layer of the coatings (i.e. barrier film).
- The presence of a continuous am-TiO₂ layer in intimate contact with the CG CP Ti substrate was assumed to impede the migration of ions which couldn't be incorporated in to the barrier film. Although this led to a nobler response of the coatings fabricated on the CG CP Ti, it compromised the stability of the inner layer for immersion times beyond 1 hour.
- On the contrary, the more crystalline barrier film of the coatings fabricated on the UFG CP Ti allowed the migration of Ti ions. In turn, the corrosion potentials of the coatings were lower than the ones of the coatings on the CG CP Ti. However, this favoured the formation of a more compact and stable barrier layer at longer immersion times.

8 Cell assessment on PEO coatings

8.1 Qualitative examination of hMSCs morphology

The morphologies of adipose derived hMSCs co-cultured with the investigated samples was examined qualitatively by using inverted microscopy at seed, 24 and 72 hours in culture. Cells showed appropriate adhesion and growth at 24 hours after seeding. The morphology of cells displayed smooth cytoskeleton in the control wells after 24 hours compared to a more rounded shape morphology, while cells showed a more rounded shape morphology in co-cultures with bare and PEO-coated samples at the same time point. At 72 hours, cells started to spread on the surfaces and displayed cytoplasmatic extensions and better focal adhesion similarly to those observed in control groups after 24 hours in culture. Cell size and morphology was similar in all PEO-coated samples with the exception of the nC-12 group, where larger cell size and appearance of filopodial extensions was observed. This results suggested that hMSCs displayed appropriate polygonal cell shape characteristic of this cell type when co-cultured with titanium samples and demonstrated the capacity of nC-12 coating to promote stronger and more accentuated cytoskeleton attachments.

8.2 Proliferation of hMSCs

The analysis of cell proliferation revealed a difference between the behavior of hMSCs on titanium samples vs control groups (Figure 8-1). The mean proliferation of cells on titanium samples was lower than in the control cultures. These results might be explained by a lower growth surface area encountered by hMSCs when seeded with titanium samples due to the areas occupied by the pieces. In comparison to control cultures, after 2 days in culture, the cell densities started to exhibit statistical differences of values between 16 and 32 % reductions with titanium samples. The same tendency was observed at 4 and 6 days in culture. Both CG and UFG CP Ti showed the lowest proliferation rates, being the CG CP Ti group the one with the largest difference with respect to the control group. In contrast, cell densities of hMSCs co-cultured with the nC-12 group maintained the highest cell densities of all the PEO-treated conditions, implying the beneficial role of the nC-12 coating. This was maintained among the different time points.

8.3 Metabolic activity of hMSCs

The metabolic activity of the hMSCs cells after 48 hours of culture for each surface condition is showed in Figure 8-2. The PEO-coated groups displayed similar results among them showing a metabolic activity reduction around 23 % compared to the control groups. However, cells demonstrated appropriate adhesion and proliferation on the titanium samples. This indicated no toxicity of the titanium samples on hMSCs. The cellular viability increased on the PEO-coated samples with respect to the bare substrates by 10 %. Finally, the metabolic activity of adherent cells on the nC-12 group was slightly higher than the rest of the PEO-coated samples, reaffirming that the nC-12 condition had better biocompatibility than the rest of the groups.

Figure 8-1. Cell proliferation of hMSCs cultured with CG and UFG CP Ti and PEO-coated samples at 48, 96 and 144 hours. Data are presented as mean $\pm$ standard deviation of at least three independent experiments. Differences were analyzed with $p < 0.05$ being considered statistically significant.

Figure 8-2. Metabolic activity of the hMSCs cells on the control, bare CG and UFG CP Ti, and PEO-coated samples after 48 hours of culture. Data is expressed as mean $\pm$ standard deviation with $p < 0.05$ with the blank and test controls.

8.4 hMSCs morphology and cytoskeleton distributions by SEM

The morphology of the hMSCs after 72 hours of culture time on the surface of the titanium samples was studied by SEM. The images were taken in backscattered electrons mode. All the samples presented a good density of cells which had elongated shapes that indicated the formation of lamellipodia protrusions and focal adhesion to the extra-cellular matrix (ECM). These results demonstrated that the hMSCs were able to adhere to the surfaces and reached the spreading and migration stages. Images *a* and *b* in Figure 8-3 correspond to the surfaces of CG and UFG CP Ti samples, respectively. It can be seen that the cells adapted to the ploughed surface. hMSCs on the surfaces of samples cC-12, nC-12, nS1-12 and nS2-12 adopted elongated and polygonal shapes. Whereas the size of the hMSCs on sample cC-12 was about 200 μm , the cell size on samples nC-12 and nS1-12 was found over 300 μm , in accordance with proliferation results. Also in agreement with the proliferation measurements, the nS2-12 sample showed a larger density of rounded-like hMSCs suggesting that the proliferating stage was reached later for this group with respect to the other PEO-coated groups.

Figure 8-3. SEM images taken in backscattered electrons mode of the surfaces after 72 hours in co-culture with hMSCs. a, CG CP Ti; b, UFG CP Ti; c, cC-12; d, nC-12; e, nS1-12; and f, nS2-12.

8.5 Conclusions

Human Mesenchymal Stem cells were seeded with the bare CG and UFG substrate and the PEO-coated samples. The proliferation results showed that all PEO treatments studied improved the proliferation of the hMSCs by 16-32 % with respect to the non-treated samples for 2, 4 and 6 culture days. It was also shown that the metabolic activity of hMSCs on the non-treated samples was 10 % lower in comparison to the PEO-treated samples at 48 hours of culture. This improved results might be related to a more favourable surface topography towards protein adsorption promoting the formation of focal contact. Comparing the CG and UFG CP Ti, the UFG substrate group showed slightly higher proliferation rates than the CG one. Among the coatings, the nC-12 coating presented the highest proliferation rates at all the time points and the highest metabolic activity.

9 General conclusions on PEO coatings and future work

9.1 Conclusions on the PEO treatment of CG and UFG CP Ti

The following conclusions can be drawn from the comparison of the PEO treatment of both CG and UFG CP Ti:

- Plasma electrolytic oxidation coatings were successfully fabricated on both CG and UFG CP Ti in the three electrolytes for treatment times of 60, 90 and 120 s. The electrolytes used were: an alkali and an acidic phosphate-based electrolytes generally used in the anodization and PEO treatment of CP Ti for the enhancement of the corrosion and wear resistance, and a third Ca and P-containing complex electrolyte with the aim to produce an osteoconductive coating. Each treatment produced specific morphologies and growth dynamics according to the discharge characteristics allowed by the electrolytes.
- The grain refinement of CP Ti via equal channel angular pressing in the conform scheme plus drawing has been seen to affect the formation of the plasma electrolytic coatings in three ways: *i*) increasing the growth rate of the coatings on the UFG CP Ti with respect to the coatings on the CG CP Ti; *ii*) reducing the level of crystallinity of the coatings on the UFG CP Ti; and, *iii*) inducing the crystallization of the am-TiO₂ layer at the coating-substrate interface. No substantial differences were observed neither in the chemical composition of the coatings nor the phase composition, which could be attributed to the short treatment times applied.
- The mechanical properties of the coatings depended strongly on the characteristic microstructure and porosity within the OCL of each treatment. The differences observed in the nanoscratching tests when comparing each treatment on both substrates were more likely related to the thickness differences observed in Chapter 5, i.e. growth rate: the coatings fabricated for 120 s using the alkali and complex electrolytes on UFG CP Ti and the acidic on CG CP Ti were thicker than their counterparts.
- The UFG microstructure provided with a better corrosion protection in comparison to the CG one. On the other hand, the corrosion protection of both CG and UFG CP Ti was improved with the PEO treatments.
- The differences in the electrochemical behaviour of CG- and UFG-coated samples were assigned to the nature of the inner layer of the coatings (i.e. barrier film). To be more specific, they were related to the partial crystallization of the am-TiO₂ in intimate contact with the UFG CP Ti.
- The PEO-coated groups presented higher proliferation rates and metabolic activity than the non-coated CG and UFG CP Ti groups. Among the coatings, the one fabricated for 120 s in the complex electrolyte on the UFG CP Ti presented the highest proliferation rates at all the time points and the highest metabolic activity. These results suggested that the PEO treatments studied in the present Thesis might provide with enhanced osteoinductive capabilities to the surface of UFG CP Ti.

9.2 Future work

The following future work areas are proposed:

- In order to provide with a better understanding of the effect of the UFG microstructure of CP Ti on the PEO process and the coatings formation, the study should be focused at the onset of the V_B and sparking initiation.
- The human body is frequently under cyclic loading conditions such as in the case of walking and chewing. Hence, the implanted materials must have a high fatigue limit in the high cycle regime. Surface modification approaches might have a detrimental effect on the fatigue performance of metallic materials. Although one of the goals of this Thesis was to produce thin coatings with the aim of minimizing any possible detrimental effect on the mechanical properties of CG and UFG CP Ti, the fatigue performance of PEO-coated UFG CP Ti should be investigated.
- *In vitro* proliferation and metabolic activity assessment of osteoblast-like cells differentiated from hMSCs is envisioned as future work in order to assess the osteoinductive capabilities of the present coatings.
- The nC-12 coating appeared in the *in vitro* tests of Chapter 8 as the most promising candidate for its application as osteoconductive coating. However, this coatings showed a poor mechanical performance due to the excessive porosity and PEO-related defects which could jeopardize in the short-term the ability of this coating to enhance the adhesion and proliferation of hMSCs and might cause excessive debris liberation. An alternative approach to the common methods for hardening of PEO coatings was proposed in section 6.4.3. based on the tuning of the frequency and duty cycle of the AC voltage signal during the PEO processing. The aim of this procedure is to produce thicker and more compact coatings with a finer microstructure and a homogenization of nc- and am-TiO₂. In addition, a thicker and more compact coating might also provide with a better corrosion protection. However, the application of this approach should be further investigated.

10 References

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