Coating of Polyethylene Medical Devices

Marta Alves da Silva
Licenciada em Física e Química Ensino pela Faculdade de Ciências da
Universidade do Porto

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Professora Maria Ascensão Ferreira da Silva Lopes
Faculdade de Engenharia, Universidade do Porto

Professora Maria Helena Fernandes
Faculdade de Medicina Dentária, Universidade do Porto

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... To my parents

“Man's mind, once stretched by a new idea, never regains its original dimensions.”

O.W. Holmes, Sr. 1858

“If I have seen further it is by standing on the shoulders of giants.”

Isaac Newton, 1675
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ABSTRACT

The aim of this work consisted in developing an approach to coat ultra high molecular weight polyethylene (UHMWPE) with commercial pure Titanium (cpTi) or with commercial pure Titanium and Hydroxyapatite (cpTi/HA), and characterize physicochemical and biologically the coated materials. This approach may be useful on the development of a novel acetabular component in which the metallic shell is substituted by a coating, and therefore made of just one piece, thus having the great advantage of the reduction of implant costs. The association of Hydroxyapatite (HA), known to be a bioactive material, with Titanium was attempted in order to improve osteointegration of the medical device envisaged.

The selection of the coating technique most suitable to accomplish this work had to take into account that UHMWPE material has a low melting point. After attempting a wide variety of coating techniques, DC Magnetron Sputtering technique was selected to deposit commercial pure Titanium (cpTi-UHMWPE) and DC/RF Magnetron co-Sputtering was selected to co-deposit commercial pure Titanium and Hydroxyapatite (cpTi/HA-UHMWPE).

Analyses to the coatings surface revealed that both presented a homogeneous coverage of the substrate with few cracks and defects. In the case of the co-deposited coatings of commercial pure Titanium and Hydroxyapatite, analyses revealed a uniform distribution of calcium, phosphorus and titanium elements. cpTi/HA coatings presented a higher adhesion to UHMWPE than cpTi coatings.

cpTi/HA coatings were found to be less hydrophobic and less negatively charged than cpTi ones. According to the Owens and Wendt approach, both presented a total surface tension of the same order of magnitude. cpTi/HA coatings polar component was found to predominate over the dispersive one, in opposition to cpTi coatings.

The biological assessment of the coated materials in vitro was performed evaluating the behavior of human osteoblastic bone marrow cells cultured onto the samples. In order to infer the need of a pre-incubation period, cpTi-UHMWPE and cpTi/HA-UHMWPE samples were divided into two groups, A and B. Samples of group A were seeded in the “as-prepared” condition (i.e., not submitted to a pre-incubation treatment) and samples of group B were incubated with fully supplemented culture medium over 24 hours.

cpTi-UHMWPE A and B samples presented a similar behaviour and cultured cells exhibited the complete proliferation/differentiation sequence, including the expression of
alkaline phosphatase activity (ALP) and the formation of a mineralized matrix. Pre-incubation treatment did not reveal significant influence in these samples, once both cpTi-UHMWPE A and B samples presented no considerable differences in their biological performance.

Conversely to cpTi-UHMWPE, and especially for group A, fewer cells were able to attach onto cpTi/HA-UHMWPE samples. This is related to the presence of poor crystalline HA and the deleterious effects of excessive material leaching during the culture period. Samples subjected to the pre-incubation treatment revealed a superior performance than the samples which were not subjected to the pre-incubation treatment. The relative low number of cells present in cpTi/HA-UHMWPE B surface exhibited a normal morphology and formed small cell clusters over the surface. Furthermore, high levels of ALP were observed and exuberant cell-mediated matrix mineralization occurred at earlier incubation time, suggesting that cpTi/HA-UHMWPE enhanced osteoblastic differentiation compared to that observed on cpTi-UHMWPE.

The developed approach to deposit on UHMWPE a coating made of commercial pure Titanium or a multi-component coating, made of commercial pure Titanium and Hydroxyapatite, may have potential to be used in medical devices such as hip prostheses. According to the attained characterization results, the coatings achieved in this work seem suitable to accomplish the latter application.
RESUMO

O presente trabalho teve como objectivo o desenvolvimento de uma abordagem para revestir polietileno de elevado peso molecular (UHMWPE), com Titânio comercialmente puro ou com Titânio comercialmente puro e Hidroxiapatite, e proceder à caracterização físico-química e biológica das amostras revestidas. Esta abordagem poderá ser útil ao desenvolvimento de um novo componente acetabular das próteses de anca em que a cápsula metálica é substituída por um revestimento passando a ser constituída por apenas uma peça, contribuindo para a redução dos custos dos implantes. Com o intuito de melhorar a osteointegração deste dispositivo médico, tentou-se associar a Hidroxiapatite, conhecida pela sua bioactividade, com o Titânio.

Quando da selecção da técnica de revestimento mais apropriada para alcançar os objectivos supracitados, teve que se ter em consideração o facto de o UHMWPE ter um ponto de fusão baixo. Após experimentar várias técnicas de revestimento, selecionou-se a técnica DC Magnetron Sputtering para revestir o Titânio comercialmente puro (cpTi-UHMWPE) e a técnica DC/RF Magnetron Co-Sputtering para co-depositar o Titânio comercialmente puro e a Hidroxiapatite (cpTi/HA-UHMWPE).

Análises feitas à superfície dos revestimentos revelaram que ambos apresentavam uma cobertura do substrato homogénea com poucas fissuras e defeitos. No caso dos revestimentos de Titânio comercialmente puro e Hidroxiapatite, as análises revelaram que os elementos cálcio, fósforo e titânio se encontravam uniformemente distribuídos. Os revestimentos cpTi/HA apresentaram uma maior adesão ao UHMWPE que os revestimentos de Titânio comercialmente puro.

Os revestimentos de cpTi/HA revelaram ser menos hidrofóbicos e apresentaram uma carga menos negativa que os de cpTi. De acordo com a aproximação de Owens e Wendt, ambos apresentaram uma tensão superficial total da mesma ordem de grandeza. Contrariamente às amostras cpTi-UHMWPE, a componente polar das amostras cpTi/HA-UHMWPE revelou predominar em relação à componente dispersiva.

A caracterização biológica dos materiais revestidos in vitro foi realizada através da avaliação do comportamento de células osteoblásticas de medula óssea humana cultivadas nas amostras. De modo a inferir acerca da necessidade de um período de pré-incubação, as amostras cpTi-UHMWPE e cpTi/HA-UHMWPE foram divididas em dois grupos, A e B. As amostras do grupo A foram semeadas sem serem submetidas a um tratamento de pré-
incubação e as amostras do grupo B foram incubadas durante 24 horas em meio de cultura inteiramente suplementado.

As amostras cpTi-UHMWPE A e B apresentaram um comportamento similar e as células cultivadas exibiram uma sequência completa de proliferação/diferenciação, incluindo a expressão da actividade da fosfatase alcalina (ALP) e a formação de uma matriz mineralizada. Uma vez que estas amostras não apresentaram quaisquer diferenças consideráveis no seu comportamento biológico, conclui-se que a pré-incubação não teve qualquer influência significativa nestas amostras.

Contrariamente às amostras cpTi-UHMWPE, poucas células foram capazes de aderir à superfície das amostras cpTi/HA-UHMWPE, principalmente nas amostras do grupo A. Este facto é explicado pela presença da HA pouco cristalina e pelos efeitos nocivos do lixiviamento excessivo do material durante o período de cultura. As amostras sujeitas ao tratamento de pré-incubação revelaram um melhor desempenho que as amostras que não foram pré-incubadas. O relativo baixo número de células existente na superfície das amostras cpTi/HA-UHMWPE_B exibiram uma morfologia normal e formaram pequenos grupos celulares ao longo da superfície da amostra. Além disso, foram observados níveis elevados da ALP e a formação de uma abundante matriz mineralizada num período de incubação mais precoce. Tal sugere que as amostras cpTi/HA-UHMWPE, quando comparadas com as amostras cpTi-UHMWPE, possam aumentar a diferenciação osteoblástica.

A aproximação desenvolvida para depositar no UHMWPE um revestimento feito de Titânio comercialmente puro ou um revestimento multi-componente feito de Titânio comercialmente puro e Hidroxiapatite, poderá ter potencial para ser usada em dispositivos médicos, como é o caso das próteses de anca. De acordo com os resultados obtidos da sua caracterização, os revestimentos desenvolvidos neste trabalho parecem adequados a esta aplicação.
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CHAPTER 1

GENERAL INTRODUCTION
1. OUTLINE

A biomaterial is defined, according to the National Institutes of Health Consensus Development Conference, as “any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body”. Biomaterials cover a wide range of applications where synthetic materials and modified natural materials are interfaced with biology.

The number of patients requiring and receiving biomedical implants, to correct skeletal defects and heal diseases, is continuously growing. The articular degeneration and osteoporosis are the main causes for the use of articular prostheses in the populations, and are associated with the continuous increase in life expectancy.

In the case of the development of artificial joints, they are designed to remove diseased areas of the joint and replace them with implants specifically designed to restore those joint’s function and stability. Thus, considerations about the physiological loads that the implants are going to be subjected and the materials of which they are going to be made of, must be taken into account. Material choices must consider the immune system biocompatibility, the environment, corrosion issues, friction and wear of the articulating surfaces, and the implant fixation (through osseointegration or bone cement).

Since the 60’ and as a result of several technological developments, different types of hip prostheses (design and materials) have been used clinically. More than a million of total hip prostheses are implanted all over the world each year. This procedure is successful in more than 90% of the patients, presenting good results after up 10 years follow-up. Unfortunately, some problems persist without clinical satisfactory solution, mainly those associated with peri-prosthetic osteolysis and prostheses loosening. These problems lead to second and third revision surgeries, with severe drawbacks in economic terms and extremely high medical risks for the patient. Therefore, new developments that offer improved durability and longer life, than current models in use today, are demanded.


2. **BONE BIOLOGY**

Bone is a highly specialized living tissue that supplies an internal support system to all higher vertebrates. Its mineralized extracellular matrix confers to the skeleton a distinct rigidity and strength whereas maintaining some degree of elasticity.\(^8\) Besides serving of structural support, bone also protects human vital organs and bone marrow, which contains a variety of precursor cells, and maintains calcium homeostasis.\(^8, 9\)

Bone is composed of an organic matrix, which is strengthened by deposits of calcium salts. The organic matrix is approximately constituted by 95% of type I collagen and 5% of proteoglycans plus noncollagenous proteins.\(^8\) The salts deposited in this matrix consist of crystalline and amorphous calcium phosphate phases. The exact structure of bone mineral has not been defined due to the sub-microscopic structure of its apatite crystals.\(^10, 11\) Besides, it has been reported that the morphology, size and chemistry of apatite crystals, vary according to their position within the skeleton, with age, species and health.\(^12, 13\)

Immature bone has been reported to be deficient in calcium and hydroxyl ions. However, with age, bone crystal approaches, despite never reaching, hydroxyapatite stoichiometric formula \((\text{Ca}_{10}\text{(PO}_4)_6\text{(OH)}_2)\).\(^14\) Bone mineral’s main chemical constituents are calcium, phosphate and carbonate, with a smaller amount of other ionic elements.\(^15\) Despite the role of ions such as sodium, magnesium, strontium, barium and fluoride in bone mineral is not entirely understood, it’s accepted that these ions play a major role in the biochemistry of bone.\(^14, 16\)

Attending the morphology of bone, it can be classified as woven and lamellar. Woven bone is found during embryonic and fetal development, in healthy adult skeleton at ligament and tendon insertions and under pathologic conditions such tumors or healing fracture. It is characterized by an irregular and disorganized pattern of collagen fiber orientation and osteocyte distribution.\(^17\) Woven bone can be rapidly produced when stimulated mechanically, remodeling into dense lamellar bone.\(^18\) Lamellar bone is found in cortical and cancellous bone (Figure 1).\(^17\)

Cortical bone presents a compact structure with its collagen fibrils densely packed forming concentric lamellae.\(^8\) Lamellae are, in cortical bone, arranged in osteons, which can be classified as primary osteons or secondary osteons (Haversian systems). Primary osteons are formed during woven bone consolidation and the secondary ones are formed
via resorption of preexisting bone. Haversian systems account for most of the adult bone tissue.¹⁹

![Figure 1](image1.png)

**Figure 1** – Bone section (left) and radiograph (right) of the proximal femur in the frontal plane illustrating cortical and cancellous bone.²⁰

Cancellous bone presents a spongy structure with its matrix loosely organized and porous⁸, and with lamellae running parallel to the trabeculae¹⁹. Structural differences, between cortical and cancellous bone, are due to the different functions which they are associated to. While cortical bone supplies mechanical and protective functions, cancellous bone supplies the metabolic ones.⁸

Bone is composed of four different cell types. Its surface is composed of osteoblasts, osteoclasts and bone lining cells, whereas its mineralized interior is permeated by osteocytes (Figure 2).⁸

![Figure 2](image2.png)

**Figure 2** – The origins and locations of bone cells.²¹
Chapter 1 - General introduction

Osteoblasts are the completely differentiated cells responsible for the production of the bone matrix.\(^8\) They produce bone matrix proteins including type I collagen and take charge of mineralization of the tissue.\(^22\) Matrix deposition is usually polarized toward the bone surface. However, from time to time this deposition becomes generalized, surrounding the osteoblasts, which develop into osteocytes.\(^8\)

Osteocytes are mature osteoblasts trapped within the bone matrix. They are responsible for its maintenance\(^{23,24}\), being capable of synthesize and also resorb matrix to a limited extent\(^8\). They are thought to act as transducers, sensing the mechanical and chemical environment around bone, and subsequently transmitting the information achieved to the osteoclasts and osteoblasts, producing an appropriate cellular response.\(^9\)

Bone lining cells are inactive cells present at surface’s bone, which do not undergo any of the processes of bone formation or resorption.\(^8,24\)

Osteoclasts are large and multinucleated cells responsible for bone’s resorption.\(^8,24\) These cells rest directly on the bone surface and have two plasma membrane specializations: a ruffled border and a clear zone. While the ruffled border is the area of the plasma membrane where the bone resorption takes place; the clear zone refers to a microfilament-rich and organelle-free area of the plasma membrane surrounding the ruffled border, which serves as point of attachment of the osteoclasts to the underlying bone matrix.\(^8\)

Bone is constantly being destroyed and reformed. This continuous remodeling process is made in order to maintain bone volume and calcium homeostasis.\(^25\) Bone modeling and remodeling work together during the skeleton growth, in order to define the appropriate skeletal shape, maintain proper serum levels of ions, and repair structurally compromised regions of bone.\(^26\)

During the normal bone remodeling process, osteoclasts remove bone that is subsequently replaced by the osteoblasts. However, changes in bone’s loading environment can also cause bone’s resorption by osteoclasts. According to Wolff’s Law, bone develops in response to the loading environment that it experiences. Therefore, bones that are not subjected to a sufficient loading will lose tissue mass whereas bones subjected to higher loadings than previously will add bone in order to reduce the stress experienced. These bone mass changes are related to the increase and decrease in bone’s structural arrangement and not to a change in the amount of mineral per unit volume of collagen at the material level.\(^9\)
3. **HIP PROSTHESES**

The advances made in the field of medical sciences contributed greatly for the increase of life expectancy. Because people live more, their joints will start wearing out, requiring their repair in order to maintain their life quality. 27

The hip (Figure 3) is one of the most commonly replaced joints. Like the other joints in the skeletal system, it’s of its responsibility to transfer load from one bone to another and allow the motion between the bones. It is basically a ball-and-socket joint limited by the bony and ligamentous structures of the joint.28

![Figure 3 – Anatomy of the hip joint (adapted from reference 29).](image)

Because it is subjected to high levels of cyclic mechanical stress, it is expected that after 50 or more years of activity, or because of degenerative or rheumatologic disease, it starts wearing out, having to be repaired.30 Total hip prostheses are formed by three components (Figure 4): the femoral stem and femoral head, which may be integrated or modular, and the acetabular cup. While the femoral head and acetabular cup combination provide the joint’s bearing surface, the femoral stem transfers the load to the femur and provides resistance to the bending moment caused by the anatomy of the joint.28

Each year are implanted worldwide more than a million of total hip prostheses,6 having a life expectancy of approximately 15 years.7 Hip prostheses wear particles incite component loosening, deleterious biological responses, osteolysis, mechanical instability, decreased joint mobility, increased pain, and, at last, implant failure. Consequently, younger and more active patients need, frequently, revision surgeries.31
Much research has been made in order to decrease hip prosthesis’s wear rates, thus improving their life expectancy. Ideally, hip prostheses should have a longer life expectancy than the patients who are subjected to total hip arthroplasty.  

Figure 4 – Components of hip prostheses (adapted from reference 32).

3.1. HISTORICAL OVERVIEW

The first attempts to restore mobility to deformed hip joints dates from 1820s, and consisted in removing the damaged femoral and acetabular bone involved. Later, in the 1830-1880s the use of interpositional membranes (made of wooden blocks and animal soft tissue) between the femoral head and the acetabulum became the subsequently attempts to restore patients mobility.33

The first prosthetic hip replacement was performed in 1891 by Theodore Gluck, who used a cemented ivory ball to replace the femoral head. Despite being a not successful procedure, many other attempts were made, in order to develop a hip replacement prosthesis.34

In 1938, Philip Wiles made the first total hip arthroplasty, using a stainless steel ball held to the femur with a bolt and a stainless steel acetabular liner held with screws.35 The poor corrosion resistance of early stainless steel in vivo and the high levels of stress of short-stemmed prostheses, conducted this attempt to failure.33

In 1951, G. K. McKee and J. Watson-Farrar adapted Philip Wiles hip prostheses design, using a stainless steel cup and a long-stemmed prosthesis. However, this design also failed, due to the poor corrosion resistance of the stainless steel that was later
successfully substituted by a cobalt-chromium alloy. Soon after, in order to improve their prostheses, they incorporated a spherical femoral head undercut at the neck to reduce the impingement of the head on the rim of the acetabular prosthesis and provide for a greater range of mobility.

The use of acrylic bone cement became the next step on the history of the evolution of total hip arthroplasty, since it reduced the rates of loosening associated with metal-metal hip prostheses.

The first successful hip joint prosthesis was developed by John Charnley. In 1960, he developed a “low friction arthroplasty” device, which’s femoral and acetabular sides were made of shells of polytetrafluoroethylene (PTFE). The excessive debonding and wear debris of PTFE led to the immediate failure of this prostheses design. In order to solve this problem he developed a model with an acetabular component having a thick wall of PTFE articulating on a small head. Though, this design also produced massive wear debris and consequently severe inflammation and failure of the prosthesis. In 1962, PTFE was replaced by high-density polyethylene, which was not as friction-free as PTFE but was 1000 times more wear resistant. This model became the basis for future designs of total hip arthroplasty, still being used nowadays (Figure 5).

Figure 5 – Example of a typical current total hip arthroplasty (adapted from reference 32).
3.2. CURRENT HIP ARTHROPLASTY

Nowadays, hip joint prosthesis remains very similar to those used in 1970s. Nevertheless, today’s market presents us a wide range of materials and geometries varieties. Hip joint prostheses are fabricated from titanium, stainless steel, special high-strength alloys, ceramics, composites and ultrahigh-molecular-weight polyethylene. At the moment, hip prosthesis are usually made of a titanium or cobalt-chromium alloy femoral stem (cemented with poly(methyl methacrylate), PMMA, or press fit into place), connected to a “modular” cobalt-chromium alloy or ceramic head that articulates on a ultrahigh-molecular-weight polyethylene (UHMWPE) or ceramic acetabular cup fitted into a titanium or cobalt-chromium cup liner cemented, screwed, or press-fit into place (Figure 6).

![Figure 6](image)

**Figure 6** – (a) Example of the three types of acetabular cups used in current total hip arthroplasty. From left to right: metallic cup liner, metallic acetabular cup, ceramic acetabular cup and polymeric (UHMWPE) acetabular cup. (b) Example of the three types of bearing couples used in current total hip arthroplasty. From left to right: metal-on-metal, ceramic-on-ceramic and metal-on-polymer.

In order to enhance the short- and long-term performance of hip prostheses, a variety of surface coatings are currently being used. These surfaces promote bone
ingrowth providing an enhanced fixation. They consist of roughened titanium, porous coatings made of cobalt chromium or titanium beads, titanium wire mesh, plasma-sprayed titanium and bioactive nonmetallic materials such as hydroxyapatite or other calcium phosphate compositions.\textsuperscript{33}

\textbf{Metal-on-metal bearing surfaces}

Metals constitute the first materials used to replace joints.\textsuperscript{33, 38} They present suitable properties required on total joint arthroplasty such as high strength, ductility, fracture toughness, hardness, corrosion resistance, formability and biocompatibility.\textsuperscript{33} Commercially pure titanium (98-99.6\% pure titanium) and Ti-6Al-4V are the most common grades of titanium metals used in implants. The titanium oxide (TiO\textsubscript{2}) film that forms on titanium metals gives them superior corrosion resistance compared with stainless steel and Co-Cr-Mo alloys.\textsuperscript{33, 38} Besides, it is also believed that this oxidized surface is responsible for Ti implants become osseointegrated \textit{in vivo}.\textsuperscript{38} Torsional and axial stiffness (moduli) of Ti metals are closer to those of bone, providing (theoretically) less stress shielding than Co alloys and stainless steel. However, care must be taken in the design geometry and in the fabrication of Ti components, because of their sensibility to geometrical factors which reduces their effective strength by increasing their susceptibility to crack propagation.\textsuperscript{33, 38} Ti-6Al-4V is plus than 15\% softer than Co-Cr-Mo alloys. Because of it, Ti-6Al-4V has relatively poor wear and frictional properties when compared with Co-Cr-Mo alloys. Disadvantages of titanium as a material for medical use include relatively low shear strength, poor wear resistance and difficulties in fabrication.\textsuperscript{38} Therefore, compromises have to be done and sometimes some material’s properties have to be sacrificed for the improvement of another’s. In the case of hip prostheses chemical inertness is sacrificed for wear resistance when Co-Cr-Mo substitutes Ti alloys in prostheses bearing surfaces.\textsuperscript{33}

\textbf{Ceramic-on-ceramic bearing surfaces}

Ceramic-on-ceramic bearings in current use are made of alumina.\textsuperscript{7, 33, 39} The use of ceramic-on-ceramic’s bearing couples is explained for its superior wear resistance when compared to metal-metal and metal-polymer bearing surfaces. Their ionic bonds and chemical stability are relatively biocompatible. Moreover, ceramics present resistance to further oxidation, high stiffness and low friction. These improved properties require the use of full-density, controlled, small and uniform grain size (usually less than 5μm)
ceramic materials. Ceramic’s small grain size, poor porosity and high purity together, with an improvement of manufacture techniques, contributed to their higher fracture toughness and wear resistance. Nowadays, the fracture of ceramic-on-ceramic surface bearings is estimated to occur 1/2000 over 10 years. In spite of improving ceramic’s bearing surfaces reputation, this still constitutes the great limitation of these articulations, obliging the surgeon to revise the hip prosthesis.

**Metal-on-polymer and ceramic-on-polymer bearing surfaces**

Polymers used in orthopedic applications present mechanical properties such as yield stress, creep resistance and wear rate, which are controlled by their molecular chain structure, molecular weight, degree of branching or of chain linearity. Hip prostheses with bearing surfaces made of metal-on-polymer, have an acetabular cup of UHMWPE that usually articulates with a femoral ball of cobalt-chromium alloy. The ones made of ceramic-on-polymer have an acetabular cup of UHMWPE that usually articulates with a femoral ball of alumina or zirconia. Comparatively to alumina, zirconia presents superior mechanical properties. Because of it, zirconia has been used in femoral heads of hip prostheses. The main problem associated to this type of bearing surfaces is related to the debris of UHMWPE resulting in the release of wear particles to the body, enhancing inflammatory reaction and eventually development of osteolysis. In order to improve UHMWPE wear resistance, production of a higher cross-linking of polyethylene is being made through the use of chemical and radiation techniques.

4. **Coating Techniques**

In orthopaedic technology development, is usually used to proceed to the coating of total joint replacement components. Coatings can improve corrosion and wear resistance as well as the load-bearing strength of artificial joints. Besides, their surfaces promote bone ingrowth, providing an enhanced fixation and enabling the mechanical interlocking of the implant with the bone.

A variety of surface coatings are currently being used, which consist of roughened and porous coatings made of metallic materials, such as titanium, or bioactive nonmetallic materials, such as hydroxyapatite. Surfaces with micrometrical roughness, of the size of bone cells, guarantee a good osteointegration, anchorage and stability.
Coating with calcium phosphates materials, such as hydroxyapatite, accelerates the process of bone growth along the implant’s surface, when placed in the vicinity of viable bone or differentiated bone-forming cells.43

Coatings may be applied by many different techniques, which allow coating thickness varying from several microns to several millimetres. Thin coatings are usually applied by Physical Vapour Deposition (PVD), Chemical Vapour Deposition (CVD) and Chemically Formed Processes (CFP). Thicker coatings can be deposited by High Velocity Oxy-Fuel (HVOF), plasma and flame spraying together with Plasma Transferred Arc (PTA), weld over-laying and laser cladding.44

4.1. PLASMA SPRAYING TECHNIQUE

Plasma sprayed coatings are often used in different orthopedic devices for surface hardening, in order to improve their wear, corrosion and fatigue performance.5 In plasma spraying technique, the powder of the coating material is heated to near or above its melting point and then accelerated in the direction of the substrate. At the moment of the impact, it forms a coating made of many layers of overlapping thin lamellar particles or splats. Almost any material, that is able to be melted without decomposing, can be used as a coating material. In relation to the substrate, for most applications, it is not heated above 150ºC. The coating thickness achieved in this coating technique ranges from 0.05 to 0.5 mm and in a few applications may exceed 5mm.45 Despite of supplying a wide range of possibilities, this technique presents some limitations. The thermal shocking, thermal expansion disparity and other induced stresses during plasma deposition, are sources of material cracking or degradation.46 The coatings are considerably stronger in compression than in tension and present poor resistance to locate loading.47

4.2. PHYSICAL VAPOR DEPOSITION (PVD)

Physical Vapor Deposition (PVD) technique consists in an atomistic deposition process in which the material being deposited is vaporized from a solid or liquid source, in the form of atoms or molecules. Then, it is transported in the form of vapor through a vacuum or low pressure gaseous (or plasma) environment to the substrate where it condenses.48
PVD techniques improve the materials wear resistance and usually, they are used to obtain films with thicknesses ranging from few nanometers to thousands of nanometers, with typical deposition rates of 1-10 nanometers per second. Nevertheless, they are also used to form multilayer coatings, graded composition deposits, very thick deposits and freestanding structures. They can deposit films of elements and alloys as well as compounds, using reactive deposition processes.

PVD coatings can be performed at temperatures below 500ºC, allowing substrates such as bearing steels and titanium or aluminum alloys to be coated. The coating temperature can even be reduced enough to allow polymers to be coated. In relation to the substrates, these can have a very small size or not, and a shape with a complex geometry or not.

The main sorts of PVD techniques are sputter deposition, vacuum evaporation and ion plating.

### 4.2.1. Sputtering Technique

Sputtering process may be described as the ejection of particles from a condensed-matter target, made of the coating material, due to the impingement of energetic projectile particles. This process is initiated by the first collision between the incident ions and the atoms of the target surface, which is followed by the second and third collisions between the atoms of the target surface. Because of the successive collisions, the displacement of these atoms will in time become more isotropic, allowing the atoms to escape from the surface. During the process, the target is mounted in an opposite position in relation to the substrates, in a vacuum chamber, which is then evacuated to a base pressure that usually ranges from $10^{-6}$ to $10^{-10}$ Torr, depending upon the process (Figure 7).

In order to provide the ion bombardment necessary for the sputtering process, the vacuum chamber is usually backfilled using a continuous flow of a gas such as argon and then the glow discharge is established. To initiate the positive-ion bombardment, is applied a negative potential, usually between 0.5 and 5 kV, to the target, while the substrate is grounded.

Target materials used in this technique can be made of metal, where a DC power supply can be used, or of semiconductors and isolators materials, where is used a RF power supply.
Recent trends of the sputtering process find the use of magnetrons in both DC and RF applications.\textsuperscript{50} Besides, the use of co-sputtering has been, also, widely used in the development of multi-element coatings.\textsuperscript{53}

4.2.2. DC / RF MAGNETRON SPUTTERING AND MAGNETRON CO-SPUTTERING

The application of a magnetic field allows the deflection of the electrons in order to stay near the target surface (Figure 8). Besides, with an appropriate arrangement of the magnets, the electrons can be made to circulate on a closed path on the target surface.\textsuperscript{48} While DC discharge methods are generally used for sputtering metals, RF potential must be applied to the target when sputtering non-conducting materials.\textsuperscript{49, 50}
Magnetron sputtering has the advantage of coating large areas with reduced substrate heating, allowing their range of materials application.\textsuperscript{50} Besides, with this technique is possible the achievement of thin films with great adhesion, uniform thickness, and the ability to coat implants with complex three-dimensional geometries.\textsuperscript{54}

Co-deposition of different materials is a common way to produce films and coatings of multi-elements by Physical Vapor Deposition.\textsuperscript{53} In magnetron co-sputtering, two or more separate magnetrons are set up with targets of different materials, being the film composition controlled by the relative power supplied to each sputtering target (Figure 9).\textsuperscript{54-56}

The application of this technique allows the establishment of dense and well-adhered thin coatings.\textsuperscript{55}

\section*{5. Physicochemical and Mechanical Characterization Techniques}

Materials characterization forms the basis for understanding the potential of a new medical device material in order to have success when the device is put into use in a specific application.

Innumerable pre-clinical studies are performed before a medical device is to be used in daily surgery, starting by its physicochemical and mechanical characterization.

The microstructure is one of the most important characterization features for a medical device material, since it usually controls mechanical properties and can influence surface behaviour due to the presence of phase boundaries, missing grains or inclusions.
The surface of a biomaterial becomes an interface with tissues upon implantation, and the interaction, which takes place between the surface atoms of the biomaterial and the biomolecules of the biological system, are important for the biological response to biomaterials. Therefore, characterization of surface features is of vital concern. A single surface analysis method rarely provide sufficient information to understand complex biological interactions, and therefore a multitechnique approach is usually used to provide a more complete picture of the surface. The most important problem is often the determination of which surface feature is relevant to the biological and biomechanical performance of a medical device.\textsuperscript{58} For example, features such as topography and surface charge are well known to influence the adsorption of proteins and other biological constituents, and affect the response of tissues in contact with it.

Standard mechanical testing, relating to the performance of new medical device materials for articular prostheses include fatigue, wear, static load and contact stress analysis.

A strategy of a characterization program should be defined for each specific medical device material, having in mind its final application and the analytical cost of various instrumental methods available, as well as the level of accuracy required for each characterization features.

5.1. X-RAY DIFFRACTION (XRD)

X-ray diffraction (XRD) is a technique used to identify the crystalline phases present in materials and to measure their structural properties such as strain state, grain size, epitaxy, phase composition, preferred orientation and defect structure. This technique is also used to determine the thickness of thin films and multilayer and atomic arrangements in amorphous materials and at interfaces.\textsuperscript{59}

XRD is a noncontact and nondestructive technique and all elements can be studied by it. Nevertheless XRD is more sensitive to elements with higher atomic numbers (Z), than from the elements with a low atomic number. This makes XRD technique sensitivity being dependent on the material studied.\textsuperscript{59}

Crystals consist of planes of atoms, which are spaced a distance $d$ apart and are uniquely distinguished by its Miller indices (hkl).\textsuperscript{59} X-rays wavelengths are approximately equal to the distance $d$ between the atomic planes. Thus, when an incident beam of x-rays meet the crystal, radiation reinforced peaks of several
intensities, may be formed. These diffraction peaks occur when there is a constructive interference from the X-rays scattered by the atomic planes. The condition for the occurrence of constructive interference is given by Bragg’s law:

\[ n\lambda = 2d_{hkl} \sin \theta_{hkl} , \]  

where \( n \) is the diffraction order, \( d_{hkl} \) is the distance between the atomic planes and \( \theta_{hkl} \) is the angle between the atomic planes and the incident (and diffracted) X-ray beam (Figure 10).

![Figure 10 – Diffraction of an X-ray beam (adapted from reference 61).](image)

### 5.2. **Fourier Transform Infrared Spectroscopy (FTIR)**

FTIR spectroscopy is a technique based on the interaction of infrared radiation with a material, analyzing the molecular vibrations induced by the radiation. It is a standard analytical method that allows the knowledge on specific chemistries and the orientation of structures. When material’s molecules are exposed to infrared light, the radiation which frequencies matches their fundamental modes of vibration, is absorbed. Besides, this absorption occurs only for those vibrations that induce oscillating dipoles perpendicular to the surface.

An IR spectrum shows peaks (or troughs) that correspond to the frequencies at which radiation is absorbed, and, by using the Fourier transform, the absorbance at each frequency can be rapidly determined. Since groups of atoms have distinctive fundamental modes of vibration, the peaks (or troughs) of a FTIR spectrum represent...
material’s specific chemical bonds and chemical functional groups. We can say that, an IR spectrum of a determined material constitutes its “fingerprint”.38

5.3. **SCANNING ELECTRON MICROSCOPY (SEM)**

Scanning electron microscopy allows the morphological, micro-structural and micro-analytical characterization of a material. With this technique it is possible to acquire images containing topographical, chemical composition (atomic number), crystalline structure and elemental composition information. These features can be directly correlated with the surface morphology of the region that is being scanned.63

In SEM, a relatively high-energy electron beam (typically 5 – 100 KeV) is scanned across the sample’s surface. The incident electrons transfer sufficient energy for the secondary electrons to be emitted from each spot of the sample where the focused electron beam impacted. The intensity of these secondary electrons depends of the atomic composition of the sample’s surface and of the geometry of the features that are being observed.38, 62 Since the secondary electrons have a low-energy, their penetration depth is very low. This way only secondary electrons generated near the surface can escape from the bulk and be detected. Thus, SEM constitutes a surface analysis method.62

The primary electrons which are elastically scattered back from the sample’s surface, called backscattered electrons, carry some not specific chemical information. The information achieved in backscattered electron mode, is reflected in the form of brighter (higher atomic numbers) and darker (lower atomic numbers) regions in the images. In order to obtain more specific chemical information, is necessary the use of complementary techniques such as energy- or wavelength-dispersive X-ray analysis. These methods are based on the emission of X-rays, which energy or wavelength is characteristic of the elements from which they originate, following the ejection of secondary electrons.38 Non conductive samples observed in the SEM are generally coated with a thin, electrically grounded layer of metal to minimize the accumulation of negative charges from the electron beam. 38, 62
5.4. ZETA POTENTIAL

The electric charge of a material surface is one of the main physical factors involved in the biological evolution of the tissue around an implant. It depends on numerous factors such as the nature of the material in contact with the tissues, the composition of the surrounding body fluid, the inflammatory situation, the environmental pH, etc. In order to improve the knowledge of the mechanisms involved in the biological integration of materials with tissues, is imperative to consider the relationship between electric charge on a surface and protein adsorption.64, 65

The determination of a biomaterial’s zeta potential, allows the knowledge of its electric surface properties.65 It can be measured by electrophoresis or alternatively by streaming potential/current methods.65, 66 The majority of zeta potential studies have been made on particulate dispersions. However, there are materials in the form of fibers, films and other macroscopic structures counting high density particles or granules (Ø>100μm), which zeta potential can’t be determined using the electrophoresis techniques. In these cases, streaming potential measurements are very useful. While the electrophoresis methods are based in the application of an electric field which causes relative movement between the two phases, in the streaming potential/current methods the movement results from the application of a force which gives rise to the potential. 67

Results obtained from a zeta potential measurement consist on the potential of a material in an ionic solution, at the boundary between the Stern layer and the diffuse layer (Figure 11).

Figure 11 – Schematic diagram of the electric double layer in zeta-potential (ζ) measurement.65
The Stern layer consists of the layer of anions that attach to the surface charged. Ions concentrations near the surface decrease further from the surface, forming the diffuse layer. Zeta potential is generated when a liquid is forced to flow directly through a small gap formed by two sample surfaces. During zeta potential measurement, charge carriers that are temporarily bound to this electric double layer, are removed from it by the external flow with pressure, allowing the measurement of the potential between two electrodes.\textsuperscript{65}

Zeta potential, $\zeta$, is calculated from:

$$\zeta = \frac{V_s}{\Delta p} \cdot \frac{\eta \varepsilon \varepsilon_0}{L/A} \cdot \frac{1}{R}$$ \hspace{1cm} (2)

where, $V_s$ is the streaming potential, $\Delta p$ the hydrodynamic pressure difference across the sample, $\eta$ is the viscosity, $\varepsilon$ the permittivity of the liquid, $\varepsilon_0$ the permittivity of free space, L and A are the length and cross sectional area of the sample, and R corresponds to the electrical resistance across it.\textsuperscript{67}

$V_s/\Delta p$ determination can be made by direct measurement of the potential generated by a given, constant, pressure. The values of $\eta$, $\varepsilon$, and $\varepsilon_0$ are constant for a given temperature and for the liquid being used. R value can be measured directly. However, if streaming current, $I_s$, and streaming potential, $V_s$, are measured simultaneously, R value can be determined from the ratio $(V_s/\Delta p)/(I_s/\Delta p)$.\textsuperscript{67} L/A term can be determined using Fairbrother and Mastin\textsuperscript{68} or Chang and Robertson\textsuperscript{69} approaches.

**5.5. Wettability**

Biomaterials wettability is a very important feature to take into account, since both \textit{in vitro} and \textit{in vivo} studies are based on the wetting capability of biomaterials by liquid-like substances, such as the media and body fluid.\textsuperscript{70} Wettability is influenced by three major factors: the surface free energy of both solid and liquid, solid’s surface topography and liquid’s viscosity.\textsuperscript{71}

The wettability of a surface is characterized by contact angle analysis, which measures the surface tension of a solvent droplet at its interface with a homogeneous surface.\textsuperscript{72} The foundation of contact angle technique is the three-phase equilibrium which occurs at the contact point at the solid/liquid/vapor or solid/liquid/liquid interface (Figure 12).\textsuperscript{73,74} The liquid drop put on the solid surface, will modify its shape under the pressure of the different surface/interfacial tensions, until getting equilibrium.\textsuperscript{74}
The surface tension of a liquid in equilibrium with its vapor, $\gamma_{LV}$, and the contact angle of a liquid drop resting on a solid surface, $\theta$, are related through Young’s equation:76, 77

$$\gamma_{LV} \cos \theta = \gamma_{SV} - \gamma_{SL} \quad (3)$$

where $\gamma_{SV}$ is the solid-vapor surface tension and $\gamma_{SL}$ is the solid-liquid interfacial tension. Thus, from the experimental values $\gamma_{L}$ and $\theta$, it is possible to obtain the difference $\gamma_{SV} - \gamma_{SL}$.78

The interaction energy at the solid-liquid surface is known as work of adhesion, $W_{SL}$, and may be calculated using Young-Dupré’s equation:

$$W_{SL} = \gamma_{LV} + \gamma_{SV} - \gamma_{SL} \quad (4)$$

Wettability can be quantified by this work of adhesion. While a high work of adhesion is indicative of a good wetting, a low work of adhesion is indicative of a poor wetting.79

In order to obtain the interfacial tensions $\gamma_{SV}$ and $\gamma_{SL}$ separately both equations (3) and (4) can be used if a third equation describing the work of adhesion is known. However, there is considerable controversy about this issue.78 One of the possibilities is to consider that the work of adhesion, $W$, can be taken as the geometric mean of the work of cohesion solid-solid, $W_{SS}$, and the work of cohesion liquid-liquid, $W_{LL}$:78

$$W_{SL} = (W_{SS} \cdot W_{LL})^{1/2} = 2(\gamma_{SV} \cdot \gamma_{LV})^{1/2} \quad (5)$$
This equation combined with equation (4) leads to the Rayleigh-Good equation:  

\[ \gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2(\gamma_{SV} \cdot \gamma_{LV})^{1/2} \]  

(6)

Combining this equation with equation (3) is possible to determine \( \gamma_{SV} \) from experimental values of \( \gamma_{LV} \) and \( \theta \). The determination of \( \gamma_{SL} \) is done directly using Young’s equation.  

Despite of being widely used, the geometric mean approach has some conditions that restrict its range of applicability. According to Good, works better for systems where the dominant cohesive and adhesive forces are of the same type. In order to correct the deviations of the geometric mean approach, is usually introduced an empirical correction factor, called the interaction parameter \( \Phi \), for which Neumann proposed an empirical expression leading to the Neumann’s equation of state for interfacial tensions:  

\[ \cos \theta = -1 + 2 \left( \frac{\gamma_{SV}}{\gamma_{LV}} \right)^{1/2} e^{-0.0001247(\gamma_{LV} - \gamma_{SV})^2} \]  

(7)

The rearrangement of this equation with Young’s equation, leads to a relation between \( \gamma_{SV} \), \( \gamma_{SL} \) and \( \gamma_{LV} \) in a two component, three phase system composed by a sessile drop on a solid flat surface.  

Another common approach used to obtain \( \gamma_{SV} \) and \( \gamma_{SL} \) from contact angle measurements is due to Fowkes. While Neumann had introduced a correction factor, Fowkes suggested an additive approach, according to which the surface tensions of the bulk phases could be described as a sum of independent contributions, each coming from the different types of intermolecular interactions existent:

\[ \gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2(\gamma_{SV}^d \cdot \gamma_{LV}^d)^{1/2} \]  

(8)

where \( \gamma_{SV}^d \) and \( \gamma_{LV}^d \) are the dispersion components of the solid and the liquid surface tensions, respectively. Combining this equation with Young’s equation, it’s possible to
determine $\gamma_{SV}^d$ from the experimental values of $\theta$ and $\gamma_{LV}$. This approach is only valid when both solid and liquid are nonpolar. However, it stills being a reasonable approach when only one of them is nonpolar.\(^7\)\(^8\)

Owen and Wendt’s approach\(^8\)\(^3\) lengthened Fowkes ideas to those cases where both dispersive and polar intermolecular forces operate, which happens when both liquid and solid are polar.\(^7\)\(^8\) Thus, they proposed that the total surface tension can be expressed as a sum of disperse ($\gamma_d$) and polar components ($\gamma_p$). According to Owen and Wendt, the solid-liquid interfacial tension, $\gamma_{SL}$, can be calculated from the following equation:\(^7\)\(^8\)

$$\gamma_{SL} = \gamma_{SV}^d + \gamma_{LV}^d - 2(\gamma_{SV}^d \cdot \gamma_{LV}^d)^{1/2} - 2(\gamma_{SV}^p \cdot \gamma_{LV}^p)^{1/2}$$ (9)

Using Young’s equation and equation (9) the components of the solid’s surface tension can be determined taking the values of contact angles measured with a pair of testing liquids whose dispersive and polar surface tension components are known.\(^7\)\(^8\)

Another additive approach is due to Wu\(^8\)\(^4\), and is designated as the harmonic-mean approach. According to Wu, the substitution of the geometrical mean by the harmonic mean in the work of adhesion calculation, conducts to betters results, in the case of the surfaces of low energy.\(^8\)\(^0\)

Van Oss’s approach\(^8\)\(^5\) is non-addictive being based on the solid surface tension decomposition into two components: Lifshitz-van der Walls, $\gamma_{LW}$, and acid-base, $\gamma_{AB}$, components.\(^8\)\(^0\) Lifshitz-van der Walls component takes into account the dispersion (London), induction (Debye) and orientation (Keesom) interactions ($\gamma_{LW} = \gamma^d + \gamma^i + \gamma^o$). The acid-base one results from the transfer of electrons between an electron donor and an electron acceptor ($\gamma_{AB} = 2\sqrt{\gamma^+ \gamma^-}$).\(^8\)\(^0\) For the total work of adhesion:\(^7\)\(^8\),\(^8\)\(^0\)

$$W_{SL} = 2(\gamma_{SV}^{LW} \cdot \gamma_{LV}^{LW})^{1/2} + 2(\gamma_{SV}^+ \cdot \gamma_{LV}^-)^{1/2} + 2(\gamma_{SV}^- \cdot \gamma_{LV}^+)^{1/2}$$ (10)

Using the definition of this work of adhesion in the equation (4) and combining with Young’s equation, it’s possible to determine LW and AB components of the solid’s surface tension from contact angle measurements of three testing liquids with known values of the surface tension components.\(^7\)\(^8\),\(^8\)\(^0\)
Contact angle’s experimental measurement can be done using the sessile drop technique, in which a droplet of a properly purified liquid is put in the solid by means of a syringe or a micropipette. However, there are a number of other ways to measure the contact angle such as the captive air bubble method, the capillary rise method and the Wilhelm plate method.

According to the contact angle, it’s possible to characterize a surface’s wettability. The condition $\theta < 90^\circ$ indicates that the surface is wet by the liquid (hydrophilic) and $\theta > 90^\circ$ indicates a nonwetting surface (hydrophobic). The limits $\theta = 0^\circ$ and $\theta = 180^\circ$ indicate complete wetting and nonwetting respectively (Figure 13).

![Figure 13 – Contact angle and wettability (adapted from reference 86).](image)

In order to make a straightforward interpretation of the contact angle, when studying a surface’s wettability, it’s necessary to assume that: The solid surface is rigid, immobile and nondeformable; the solid surface is highly smooth; the solid surface is uniform and homogeneous; the liquid surface tension is well-known and constant doesn’t changing during the time course of the measurement; the solid surface doesn’t interact in any other way with liquid other than the three-phase equilibrium; the liquid spreading pressure on the solid is zero; the solid surface groups cannot reorient or re-equilibrate in response to changes in environment.

### 5.6. Adhesion Testing: Pull-Off Test

A good adhesion is a fundamental requirement of any film-substrate system. According to the ASTM definition (D907-70), adhesion is “the state in which two surfaces are held together by interfacial forces which may consist of valence forces or interlocking forces, or both”. It consists on the strength that joins two different objects
or materials. On the other hand, cohesion is related to the strength in a single material due to interatomic or intermolecular forces.\textsuperscript{48}

A good adhesion requires strong chemical bonding between different atoms, close contact between different materials, a high fracture toughness of the materials in contact, low residual stress in the interfacial region and no degradation mechanism operating. Even if the chemical bonding between the different atoms involves a weak bond, the adhesion can still be good if these atoms are in good atomic contact. The interface, interfacial material and nearby material should have a high fracture toughness and no flaws which act as stress concentrators and initiate cracks under stress. The measurement of the apparent adhesion is usually done through the application of an external force to the thin film structure, to a level that causes failure between the film and substrate, or in the material near the interface. This external force supplies energy to the system, causing the strain and fracture of chemical bonds. The loss of adhesion can involve both adhesive and cohesive failure: it can occur at a sharp interface between materials, in an interfacial region containing both materials, in the near-interface region of the substrate or in the near-interface region of the deposited film or between films in a layered film structure. It can occur over a large area originating film delamination from the substrate, or over a small area causing pinholes in the film.\textsuperscript{48}

From a thermodynamic point of view the work $W_A$ necessary to split an unit area of two phases forming an interfaces is expressed by:\textsuperscript{48,88,89}

$$W_A = \gamma_f + \gamma_s - \gamma_{fs}$$

(11)

where $\gamma_f$ and $\gamma_s$ are the specific surface free energies of film and substrate, and $\gamma_{fs}$ is the specific interfacial free energy. A positive $W_A$ indicates adhesion and a negative one indicates deadhesion.\textsuperscript{89} If the rupture occurs at the interface $fs$ (film/substrate), the failure is adhesive. If it occurs within $f$ (film) or $s$ (substrate), it is a cohesive failure.\textsuperscript{88} Adhesion testing is done in order to evaluate the coating adhesion after film deposition and the coating under stresses similar to those encountered in subsequent processing, storage, and service. This testing should also evaluate the stability of the adhesion in the service environment, subjecting the coating to environmental stress.\textsuperscript{48} Adhesion tests are usually used to provide comparative measurements and not to obtain an absolute measurement. In several cases, different tests will give different values and even show a
different failure mode. Adhesion test methods include mechanical pull tests; shear tests; scratch, indentation, abrasion and wear tests; deformation tests; energy-deposition tests, fatigue tests, and others.

The simplest of the mechanical tensile tests include direct pull-off. The pull-off test (Figure 14) is performed through the fixation of a loading fixture, usually called dolly or stub, by an adhesive to a coating.

![Figure 14 – Illustration of the pull-off adhesion testing (adapted from reference 91).](image)

Using a pull-off adhesion tester, a load is increasingly applied to the surface until the dolly is pulled off. The force required to pull the dolly off, or the force the dolly withstood, yields the tensile strength. The adhesion failure, which is exposed by the fracture surface, occurs along the weakest plane within the system comprised of the dolly, adhesive, coating system and substrate.

The difficulties inherent of this test, and which affect the measured final adhesion strength, are related to the fact that:

- Simple tensile tests are difficult to perform, involving most of the times, a mixture of tensile and shear forces, which complicate the results interpretation;
- The alignment has to be perfect in order to insure the uniform loading across the interface;
- This tests are limited by the strengths of the available adhesives or solders;
- There’s always the possibility of the adhesive or solvent penetrates and affects the film-substrate interface; of production of stresses during the setting of cement or adhesive; of distribution of non-uniform stress or stress concentrations over the contact area during the pulling process.

When performing this test is also important to take into account that.
The cohesive strength of the adhesive should be more than the adhesion between
the film and the adhesive, and between the film and the substrate;
- The adhesion between the adhesive and the film should be more than the one
  between the film and the substrate;
- The adhesive used should not alter the properties of the film-substrate interface.

A standard method for the application of this test is available in ASTM D4541
“Standard Test Method for Pull-Off Strength of Coatings using Portable Adhesion
Testers” and ISO 4624.

6. BIOLOGICAL CHARACTERIZATION

Evaluation of tissues biocompatibility is a fundamental step in the development
process of biomaterials and implants for short- or long-term applications. This
evaluation must examine biomaterial’s performance under conditions that reproduce the
ones existent in the biological environment, their end-use application and the period of
exposure to these circumstances. It must involve both “biosafety” and
“biofunctionality” areas. While “biosafety” deals with the exclusion of the injurious
effects of the biomaterial, “biofunctionality” deals with the “ability to perform with an
appropriate host response in a specific application”. Biocompatibility evaluation
consists of a sequence of tests which include in vitro tests and in vivo tests (animal
models and clinical trials) and for this purpose have been developed several guidelines
and procedures that must be followed. This information can be obtained from national
and international standards organizations (for instance the American Society for Testing
and Materials - ASTM), from international organizations (for example the International
Organization for Standardization – ISO), federal agencies (such as the Food and Drug
Administration or FDA, and the National Institutes of Health or NIH).

6.1. IN VITRO TESTING

In vitro assessment of bone grafts may be performed through the use of cell cultures
or of cell-free solutions with ionic concentrations similar to body fluid. While in vitro
tests that employ cell cultures are performed in order to investigate the biological
response of the cells, researches in cell-free solutions with ionic concentrations similar
to body fluid allow the study of chemical and mineralogical changes of the implant under a simulated physiological environment.96

**Cell cultures**

*In vitro* biocompatibility tests, using cell cultures, are made in order to reproduce the biological reactions to materials at the time they are in contact with body tissues.97 They are usually static don’t taking into account the dynamics to which the implant is subjected *in vivo*. Thus, the buffering capacity of complex cellular and humoural systems in the organism are missing, so that a biomaterial may not perform well in the *in vitro* test, but be biocompatible *in vivo*.98 Factors such as anatomical position of the material, length of the device, load, fatigue of the material, fretting of the material, changes in the material in response to the biological fluid plus changes in the surrounding tissue, and age of the patient are all important concerns *in vivo* tests. However, when *in vitro* tests are performed, all these factors are irrelevant or uncontrollable.99

In order to evaluate biocompatibility, three cell cultures assays are used: direct contact, agar diffusion and elution. These three assays differ from each other in the manner that the material is exposed to the cells.100

- In direct contact, the cells are exposed directly to the testing material;
- In agar diffusion, the cells contact the material through a layer of agar;
- In elution assays, the material is extracted in an appropriate solution and then placed on the cells.

The choice of the method should take into account the material test characteristics, the foundation for doing the test and the application of the data for evaluating biocompatibility.100

In a first stage of the cytocompatibility evaluation, cytotoxicity tests, which consist of morphology, viability, adhesion to the surface and degeneration or lysis of the cells assessment, are performed. Then, in a second stage, functional evaluations are assessed. The adopted spectrum of functional parameters will vary from cell type to cell type.101 It is important to study functional activity not only at the gene product level, but also at the level of transcription, since the combination of both levels of gene expression is essential for understanding gene control and signal transduction within cells.98
As biomaterials are intended for clinical use, cells of human origin should be used for in vitro tests since various comparative studies demonstrate that human cells can react in a very different manner from cells of other mammals.

The choice of cell types for biofunctionality assays in vitro depends on the biomaterial application. Thus, for bone implants, human osteoblasts and osteoclasts, responsible for bone formation and degradation, respectively, are of major interest. However, since is unequivocal that inflammation is a constant response to implantation, cells of the monocyte/macrophage lineage can also be used. It is recognized that the process of new blood vessel formation, or angiogenesis, is coupled to the development and maturation of bone. The close association of the vascular endothelium with bone and osteogenic cells, suggests that endothelial cells could be prime sources of modulators of bone development and function. Not only they process new blood vessels and capillaries that support bone tissue, but also produce a number of local and systemic mediators that could influence the recruitment, proliferation, differentiation and function of various cells. Thus, analysis of the growth of endothelial cells on bone grafts biomaterials is essential in determining the suitability of its use for implant use.

In the soft tissue field, as well as in most forms of implantation, macrophages and fibroblasts are two central figures in the host response.

Different cell culture systems are used in vitro tests such as cell lines and primary cultures. Cell lines exhibit patterns of gene expression, modes of adhesion, or signals transduction pathways that are based on a particular stage of differentiation. The majority of cell lines don’t display a complete pattern of in vitro differentiation. These are transformed cells that display an aberrant genotype, have an uncoupled proliferation/differentiation relationship, demonstrate a substrate independent attachment and exhibit phenotypic stability in long-term culture. They do not reflect the normal phenotype of primary cells. Therefore, the results obtained must be interpreted with caution because the response of these cells to the material, may not represent a “normal” cell response. Nevertheless cell lines, which have the advantage of reducing experimental costs, improving intra-and interlaboratory variation and permitting more rapid advancement of a research field by facilitating more experiments in a unit of time are the mainly cells used in studying cytotoxicity. Primary isolated human cell cultures are extremely difficult to culture and have the disadvantage that experimental reproducibility can be difficult to achieve, as individual donors can differ
widely with respect to functional status. Nevertheless, the use of primary cells is recommended in evaluating functional cytocompatibility of the biomaterials.

A more complex _in vitro_ model that has recently explored is the application of co-culture assays to study cell–cell interactions, since the _in vivo_ state practically never involves a single cell type in an isolated biological microenvironment. In addition the simulation of the three-dimensionality (3D) which characterises our tissue structures has also been an experimental system adopted instead of the popular 2D assays mostly used for assessing biocompatibility.\(^ {98, 109} \)

### 6.2. _In vivo_ testing

_In vivo_ testing involves the use of animals for testing biomaterials in order to reproduce the environment that might be found in humans, making possible the prediction of clinical behavior, safety and biocompatibility of medical devices in humans.\(^ {110, 111} \) They allow the evaluation of the implanted materials in both loaded and unloaded situations over long time durations, in different tissue qualities (e.g. normal healthy and osteopenic bone) and age. Besides, they allow the assessment of tissues in the immediate neighborhood of the implant as well as in locations more distant. Although animal models represent both mechanical and physiological human clinical situation, they still only an approximation with each animal model having distinctive advantages and disadvantages.\(^ {112} \) Therefore, it’s important to take into account the fact that the animal anatomy, physiology and biochemistry are different from the ones of the humans.\(^ {110} \)

The selection of the animal model is a very important feature in these tests. It must take into account the advantages and disadvantages of each animal model on the prediction of biomaterials clinical behavior in humans.\(^ {111} \) For example, for studies involving bone-implant interactions is necessary an understanding of the specific bone characteristics of the different animals in order to extrapolate the results obtained to the humans. These characteristics include bone microstructure and composition, bone modeling and remodeling properties.\(^ {112} \) Besides it’s important to use a reproducible model in which implant dimensions are comparable to the ones used in humans. The choice of the animal model must take into account the number and size of the implants as well as the implantation site.\(^ {112} \) International standards established that dogs, sheep, goats, pigs and rabbits are suitable species for testing bone-implant interactions.\(^ {112} \)
7. **Market Trends**

Biocompatible materials U.S. market is valued at $22.2 billion in 2007 and is estimated to reach $30.9 billion in 2012.\(^{113}\) Biomaterial’s market is constituted by the following segments: tissue replacements, implantable medical devices and surface coatings. In 2007, tissue replacements constitute the largest segment with a value of nearly $11.7 billion, representing 52% of the total biomaterials U.S. market value; implantable medical devices represent 35.6% of the total market, with a value of $7.88 billion; biocompatible surface coatings constitute the smallest segment with a value of $2.6 billion. In the year of 2012 is expected that tissue replacements segment reach over $16 billion, implantable medical devices segment is expected to exceed $11.5 billion and biocompatible surface coatings is expected to reach $3.4 (Figure 15).\(^{113}\)

Concerning hip implant markets, U.S. and Europe have the largest markets for hip arthroplasty worldwide. The increase of life expectancy and the number of younger patients undergoing replacement procedures together, led to the growth of this market. These increased demands impose an improvement of the implant materials used and of the surgical techniques. In the year of 2005, revenues for hip implants were of $2 billion in US and $1.4 billion in Europe. From 2004 to 2005, the hip prostheses average prices grew by 2-3%.\(^{114}\)

![Figure 15](image-url) - U.S. market forecast for biocompatible materials by segment, through 2012 ($Millions) (adapted from reference 113).
8. REFERENCES


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CHAPTER 2

SURFACE COATING’S OPTIMIZATION
1. **INTRODUCTION**

The main aim of this work consists in the evaluation of an approach to deposit on UHMWPE a coating made of commercial pure Titanium (cpTi) and of commercial pure Titanium and Hydroxyapatite (cpTi/HA). This approach may, then, be useful on the development of a novel acetabular component in which the metallic shell is substituted by a coating material and, therefore, being made of just one piece, thus having the great advantage of the reduction of the implant costs. Moreover, the coating of the implants’ surface also contributes for the enhancement of their performance by promoting bone ingrowths and supplying improved fixation.¹

Today’s technology presents us a wide range of coating techniques whose selection must take into account the following factors: the material that is deposited, rate of deposition, limitations that are imposed by the substrate (maximum deposition temperature, for example), the adhesion of the film to the substrate, throwing power, purity of target material (this will influence the impurity content in the film), apparatus required as well as its availability, the cost, ecological considerations and the abundance of the deposition material in the world.²

Before the selection of the coating technique most suitable for the accomplishment of this work, several coating methods were assessed. The major difficulty on this assessment was related with the substrate, which was made of Ultra High Molecular Weight Polyethylene (UHMWPE). Being a polymer, this material has a low melting temperature of 137ºC.³ Therefore, the deposition techniques chosen had to be able to deposit films at room temperature. This feature was not so determinant for the Titanium deposition as it was for the Hydroxyapatite one. The deposition of calcium phosphate materials must have into account the changes that occur on their structure during the deposition process. Instead of having a crystalline structure, the calcium phosphate coatings may become amorphous. This feature is not very favorable since a coating with a higher degree of crystallinity presents a lower dissolution rate and ideally, Hydroxyapatite layer’s dissolution rate should be in consonance with the bone ingrowths. It should be gradually reabsorbed, allowing the surrounding tissue to be in direct contact with the implant and form a strong attachment with its interface layer.⁴ Usually, this issue is solved with a post-annealing treatment that improves ceramic’s dissolution properties by crystallizing the film. However, it could not be done in these samples because of the high temperatures achieved during it (above 600ºC).⁵ Thus, it
was necessary to guarantee a thicker layer in order to prevent its fast dissolution when implanted. The lack of crystallinity was attenuated by the increase of the layer’s thickness. However, attention should be paid to the fact that thick films may possibly delaminate or fracture over the time. Thus, it was necessary a compromise between the layer’s thickness and its dissolution rate.

Besides the assessment of the coating’s morphology, thickness and degradation rate, the evaluation of its adhesion to the substrate is also a very important parameter that should be evaluated. A good adhesion is a fundamental requirement of any film-substrate system. It requires strong chemical bonding between different atoms, close contact between different materials, a high fracture toughness of the materials in contact, low residual stress in the interfacial region and no degradation mechanism operating. Even if the chemical bonding between the different atoms involves a weak bond, the adhesion can still be good if these atoms are in good atomic contact. The measurement of the apparent adhesion is usually done through the application of an external force to the thin film structure, to a level that causes failure between the film and substrate, or in the material near the interface.

Titanium deposition was performed by DC magnetron sputtering. Concerning Hydroxyapatite, deposition techniques such as RF magnetron sputtering and Electron Gun, were tested. DC/RF magnetron co-sputtering was also assessed in order to deposit a multi-component layer made of commercial pure Titanium and Hydroxyapatite.

In this chapter are going to be presented the results obtained from these depositions techniques, and discussed which of them is the most appropriated one to accomplish this work’s objectives.

2. MATERIALS AND METHODS

2.1. MATERIALS

Ultra high molecular weight polyethylene (UHMWPE) discs (diameter of 16.1mm and thickness of 5.1mm) were used as substrates. They were cut from an UHMWPE cylindrical bar and grinded with silicon carbide papers of P400, P600, P1200, P2500 and 1200/4000 grits. Following this, these substrates were cleaned ultrasonically for 10 minutes in ethanol and then were rinsed with deionised water and dried.
Commercial pure Titanium (Ti) and Hydroxyapatite were used as the sputtering target materials.

Phase pure Hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2, \text{HA}]$ preparation required the precipitation between calcium hydroxide, $\text{Ca(OH)}_2$, and orthophosphoric acid, $\text{H}_3\text{PO}_4$, according to the following chemical reaction:

$$10 \text{Ca(OH)}_2 + 6 \text{H}_3(\text{PO})_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 18 \text{H}_2\text{O}$$

Filtered and dried HA precipitate was ground into a fine powder, with a granulometry less than 75 $\mu$m. Afterward the obtained powder was uniaxially pressed to a disc and sintered at 1300$^\circ$C for 1 hour.

### 2.2. Commercial pure Titanium deposition by DC Magnetron Sputtering (cpTi-UHMWPE)

Commercial pure Titanium deposition on UHMWPE substrates (cpTi-UHMWPE) was performed in a sputter deposition chamber at room temperature. This chamber was evacuated to a base pressure in the range of $10^{-7}$ mbar. Subsequently, high purity Argon (Ar) gas was back-filled into the chamber till a working pressure of $5\times10^{-3}$ mbar maintained fixed during all the deposition time. UHMWPE substrates were placed on the sample holder facing the magnetron source at a distance of approximately 10 cm. The coating deposition rate was controlled by the power supplied to the cpTi target: 0.06Kw direct current (DC). The sputtering deposition time was of 3 hours.

### 2.3. Hydroxyapatite Deposition (HA/cpTi-UHMWPE)

#### 2.3.1. RF Magnetron Sputtering

Hydroxyapatite deposition on cpTi-UHMWPE samples was performed in a sputter deposition chamber at room temperature. The chamber was evacuated to a base pressure in the $10^{-7}$ mbar range. Subsequently, a mixture of high purity Argon (Ar) gas and Oxygen (O$_2$) (70% Ar/30% O$_2$) were introduced into the chamber reaching the $5\times10^{-3}$ mbar deposition pressure. The substrates were placed on the sample holder facing the Hydroxyapatite target at a distance of approximately 10 cm. The film deposition was
controlled by the power supplied to the Hydroxyapatite target: 150W radio frequency (RF) at 13.56MHz. The sputtering deposition time was of varied in order to control the heating of the substrates with the time.

2.3.2. **Electron Gun Deposition**

The substrates used in this technique were the same used in RF magnetron sputtering technique and were prepared in the same way. The chamber base pressure was $10^{-7}$ mbar and during the deposition process the working pressure became $1.2 \times 10^{-4}$ mbar. The deposition time was varied in order to control the heating of the substrates with the time.

2.4. **Commercial Pure Titanium and Hydroxyapatite Deposition by DC/RF Magnetron Co-sputtering (cPTi/HA-UHMWPE)**

In this technique two sputtering targets were used, made of commercial pure Titanium and Hydroxyapatite, at the same time. This deposition was performed in a sputter deposition chamber at room temperature. This chamber was evacuated to a base pressure in the $10^{-7}$ mbar range. A working pressure of $5 \times 10^{-3}$ mbar was reached introducing high purity Argon (Ar) gas into the chamber. The substrates were placed on the sample holder facing both targets at a distance of approximately 10 cm. The film composition was controlled by the power applied to the cPTi and HA targets: 0.06Kw direct current (DC) for the cPTi and 90W radio frequency (RF) at 13.56MHz for HA. The sputtering deposition time was of 1h:30min.

2.5. **Coatings Characterization**

The film thickness was measured by scanning electron microscopy (SEM) view of the coatings’ cross-section. Coatings’ surface morphology was also observed by SEM and the presence of titanium, calcium and phosphor on the samples was determined by X-ray Spectroscopy (EDS). X-ray diffraction (XRD) was used to identify the deposited Hydroxyapatite phases. Data was collected from 10-50° 2θ, with a step size of 0.05° and a counting time of 1 sec per step in both, bragg-brentano and glancing incidence configuration.
3. RESULTS AND DISCUSSION

3.1. AS-DEPOSITED COMMERCIAL PURE TITANIUM FILMS BY DC MAGNETRON SPUTTERING (cpTi-UHMWPE)

Titanium surface layer morphology was observed by SEM (Figure 1). The deposited film presents a good coverage of the substrate surface area.

As it can be seen from Figure 1, the morphology of the as-deposited Titanium layer seems very smooth. Besides, the cross section of the cpTi layer (Figure 2) shows a tendency to a columnar-like structure broadening from the substrate to the top of the coating. As it can be seen in Figure 2, the as-deposited layer presented a uniform thickness of approximately 1 micrometer.

In order to infer about the possible damages that the coating process could cause to the UHMWPE, it was performed an X-ray diffraction to the substrates before and after the deposition of the film (Figure 3). From the XRD patterns it’s possible to conclude that no significant changes in the UHMWPE samples were observed.
Figure 3 – XRD pattern of UHMWPE samples before (PE) and after (PETIC) the deposition of the coating.

3.2. AS-DEPOSITED HYDROXYAPATITE FILMS (HA/cpTi-UHMWPE)

3.2.1. RF MAGNETRON SPUTTERING

Hydroxyapatite layer deposited by RF magnetron sputtering on cpTi-UHMWPE samples was observed by SEM (Figure 4). As it can be observed from its surface morphology analysis the deposited layer presents many cracks and other defects, which may be mainly due to the samples manipulation.

In order to control the heating of the substrates with the time, the sputtering deposition time was varied (Table 1). The coatings thickness was measured by SEM.
Table 1 - Description of the many attempts to control the heating of the substrates with the time.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Deposition Time</th>
<th>Average Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP3</td>
<td>1h 50 min</td>
<td>0.2µm</td>
</tr>
<tr>
<td>OP5</td>
<td>10h</td>
<td>1.7 µm</td>
</tr>
<tr>
<td>OP7</td>
<td>5h</td>
<td>1.27 µm</td>
</tr>
<tr>
<td>OP8</td>
<td>3h</td>
<td>0.8 µm</td>
</tr>
<tr>
<td>OP10</td>
<td>3h</td>
<td>0.8 µm</td>
</tr>
</tbody>
</table>

3.2.2. ELECTRON GUN TECHNIQUE

Hydroxyapatite layer deposited by electron gun technique on cpTi-UHMWPE samples was observed by SEM (Figure 5). The deposited film presented a good coverage of the substrate surface, presenting no cracks or defects. Contrasting coated samples by RF magnetron sputtering technique, these coatings present some crystal like structures.

Figure 5 – SEM morphology of the as-deposited Hydroxyapatite layer on UHMWPE/Ti substrates by Electron gun technique.

In order to control the heating of the substrates with the distance of the substrate to the target, the distance substrate-target was varied (Table 2). The coatings thickness was measured by SEM.

Table 2 - Description of the many attempts to control the heating of the substrates with the time.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Deposition Time</th>
<th>Distance substrate-target</th>
<th>Average Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egun_2</td>
<td>1 hour</td>
<td>10cm</td>
<td>0.6µm</td>
</tr>
<tr>
<td>Egun_3</td>
<td>1 hour</td>
<td>12cm</td>
<td>0.2µm</td>
</tr>
</tbody>
</table>
In order to assess the alterations occurred to the Hydroxyapatite during these two deposition processes (RF magnetron sputtering and Electron gun), it was performed an X-ray diffraction to the Hydroxyapatite before and after the coating processes (Figure 6).

![XRD pattern of Hydroxyapatite before and after the deposition by RF magnetron sputtering (OP5, OP7) and electron gun (Egun 2 and 3).](image)

**Figure 6** – XRD pattern of Hydroxyapatite before and after the deposition by RF magnetron sputtering (OP5, OP7) and electron gun (Egun 2 and 3).

Comparing Hydroxyapatite’s XRD pattern with the other patterns of the Figure 6, which correspond to the deposited Hydroyapatite using RF magnetron sputtering (OP5, OP7) and Electron gun (Egun 2 and 3) techniques, it is possible to conclude that they are not similar. The deposited calcium phosphate materials had no longer the composition of the initial target, absenting the foremost peaks of HA (31.74º, 32.868º and 32.179º, according to JCPDS 72-1243 file). Through this analysis it was possible to conclude that the crystal like structures present on the surface of HA/cpTi-UHMWPE samples coated by Electron gun technique (Figure 5) did not correspond to any of the phases of the Hydroxyapatite. Therefore, this technique was no longer studied.

Concerning RF magnetron sputtering technique more studies had to be performed in order to assess its possible use on the Hydroxyapatite deposition. In order to assure coating durability under physiological conditions, it was carried out a dissolution test in which a sample was immersed in desionized water during 24 hours. In figure 7 are presented SEM and EDS analysis performed after and before the immersion test, respectively.
Figure 7 – SEM morphology and EDS analysis of the as-deposited Hydroxyapatite layer on cpTi-UHMWPE substrates by RF magnetron sputtering: (a) before the 24 hours dissolution test; (b) after the 24 hours dissolution test.

Results from the dissolution test revealed that the deposited film was not thick or crystalline enough to avoid its fast dissolution. After 24 hours of immersion the Hydroxyapatite layer disappeared. Thus, it was still necessary to find out another coating technique which could assure the maintenance of the film in a biological environment for a reasonable time.

3.3. AS-DEPOSITED TITANIUM AND HYDROXYAPATITE FILM BY DC/RF MAGNETRON CO-SPUTTERING (cpTi/HA-UHMWPE)

Commercial pure Titanium and Hydroxyapatite were co-deposited onto the UHMWPE substrates. Its surface’s morphology was observed by SEM and the presence of titanium, calcium and phosphor was analyzed by EDS (Figure 8). These analyses revealed that the deposited film presented a good coverage of the UHMWPE samples. EDS analysis revealed that the film presented a much higher content in titanium than in calcium and phosphor, due to the higher sputtering rate supplied to the commercial pure
Titanium sputtering target than to the Hydroxyapatite one. The deposited film presented a thickness of approximately 1 micrometer, which was measured by SEM.

After the deposition of this multi-component coating, a dissolution test was also performed, immersing a sample in desionized water during 24h. In Figure 8 are presented SEM and EDS analyzes performed before and after the immersion test.

![Figure 8](image)

**Figure 8** – SEM morphology and EDS analysis of cpTi/HA-UHMWPE samples: (a) before the 24 hours dissolution test; (b) after the 24 hours dissolution test.

Analyzing SEM and EDS results before and after the dissolution test is possible to conclude that Hydroxyapatite deposited by this technique remained in the samples after 24h immersed in the water.

4. **CONCLUSION**

DC/RF magnetron sputtering technique proved to be the best process to accomplish the purpose of this work. The substrate material used, as well as the calcium phosphate ceramic material deposited, presented a set of difficulties in the achievement of the best deposited film. Being a polymer, the substrate presented limitations concerning the maximum deposition temperature. Because UHMWPE has a low melting point, the depositions had to be made at room temperature. This excluded coating methods such as
plasma spraying which are the most popular for the deposition of calcium phosphate ceramics.9

Despite of obtaining a coating with some crystal like structures with the Electron gun technique, it led to hydroxyapatite decomposition into different phases that we weren’t able to identify, which guide to the rejection of this technique. The ceramic materials deposited by the other processes (RF magnetron sputtering and DC/RF magnetron sputtering) presented an amorphous structure rather a crystalline one. Because an amorphous film dissolves faster than a crystalline film, and ideally the dissolution rate should be in consonance with the bone ingrowths, the ceramic film had to be thicker. The dissolution test performed at the samples coated by RF magnetron sputtering revealed that the film wasn’t thick enough and it dissolved completely after 24h immersed in water. The same didn’t happen with the Hydroxyapatite deposited by DC/RF magnetron co-sputtering. All these factors contributed to the preference of this technique as the best for the accomplishment of the aim of this work. There was, however, a set of tests that had to be made and which will be analyzed in the following chapters.
5. REFERENCES


CHAPTER 3

PHYSICOCHEMICAL CHARACTERIZATION
PHYSICOCHEMICAL CHARACTERIZATION OF cPTI AND cPTI/HA COATINGS ONTO UHMWPE MATERIAL

M. A. Silva¹,², M. Vila³, C. J. Tavares⁴, P. S. Gomes⁵, M. H. Fernandes⁵, J. D. Santos¹,², R. F. Silva³ and M. A. Lopes¹,²

¹ Instituto de Engenharia Biomédica (INEB), Laboratório de Biomateriais, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal
² Faculdade de Engenharia, Universidade do Porto (FEUP), Secção de Materiais, DEMM, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
³ CICECO, Departamento de Engenharia Cerâmica e do Vidro., Universidade de Aveiro, 3810-193 Aveiro, Portugal
⁴ Departamento de Física, Universidade do Minho, 4800-058 Guimarães, Portugal
⁵ Laboratório de Farmacologia e Biocompatibilidade Celular, Faculdade de Medicina Dentária, Universidade do Porto (FMDUP), Rua Dr. Manuel Pereira da Silva, s/n 4200-392 Porto, Portugal

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Abstract

The main aim of this work consists in developing an approach to deposit commercial pure Titanium (cPTi) or a multi-component coating, made of commercial pure Titanium and Hydroxyapatite (cPTi/HA) onto UHMWPE, by DC Magnetron sputtering and DC/RF Magnetron co-sputtering, respectively. This approach is useful on the development of a novel acetabular component in which the metallic shell is substituted by a coating and, therefore, made of just one piece. Aiming to improve Ti osteointegration, it was associated HA to cPTi in a multicomponent coating. Therefore, cPTi-UHMWPE coatings were compared with cPTi/HA-UHMWPE coatings regarding its physicochemical properties. cPTi/HA-UHMWPE samples revealed to be less negative and more hydrophilic than cPTi-UHMWPE ones. Determined total surface tension is very similar for both coated surfaces. However, while the dispersive component of the surface tension was found to be the predominant one on the cPTi-UHMWPE coating, the polar component was the predominant one on the cPTi/HA-UHMWPE coating. Concerning to the adhesion, cPTi-UHMWPE coating presents a smaller adhesion than the cPTi/HA-UHMWPE coatings.

The results attained for both cPTi-UHMWPE and cPTi/HA-UHMWPE materials indicate their potential in its future application as a substitute of the metallic shell currently used on acetabular components of hip prostheses.
1. **INTRODUCTION**

The advances made in the field of medical sciences, contributed greatly for the increase of life expectancy. Because people live more, their joints will start wearing out, requiring their replacement in order to maintain their life quality.\(^1\) In the case of the hip joint, which is subjected to high levels of cyclic mechanical stress, it’s expected that after 50 or more years of activity, or because of degenerative or rheumatologic disease, it starts wearing out, having to be replaced.\(^2\) Nowadays, the hip prosthesis available in the market, presents a wide range of materials and geometries varieties. Usually, hip prosthesis are made of a titanium or cobalt-chromium alloy femoral stem (cemented with poly(methyl methacrylate), PMMA, or press fit into place), connected to a “modular” cobalt-chromium alloy or ceramic head that articulates on a ultrahigh-molecular-weight polyethylene (UHMWPE) or ceramic acetabular cup fitted into a titanium or cobalt-chromium cup liner cemented, screwed, or press-fit into place.\(^3\)

Because the chance for revision of the implant is minimal for elderly persons (>80 years old) when compared to younger ones (<60 years old), there are some general guidelines as to which type of implant should be used. Therefore, the implants used in older persons are typically cemented into place with PMMA bone cement. Because at the time of the revision surgery performance, the removal of this cement is complicated and may compromise the availability of the individual’s bone stock, implants used in younger persons are screwed or pressed fit into place.\(^3\)

Titanium and titanium alloys are considered biocompatible materials, because of their relatively inertness and good corrosion resistance, which is due to their thin oxide surface. However, titanium surfaces find difficulties on the achievement of a good chemical bonding with bones as well as forming new bone on its surface at the early stage after its implantation. In fact, proper adhesion of titanium to bones has not been observed yet. Instead, its bond is attributed to the mechanical interlocking of the titanium asperities and pores in the bones.\(^4,5\) Therefore, in order to improve titanium and its alloys bioactivity, biocompatibility, blood compatibility, wear and corrosion resistance, many surfaces modifications methods have been attempted. These, are classified into mechanical, chemical and physical methods, according to the formation mechanism of the modified layer on the material surface. Mechanical methods involve physical treatment, shaping, or removal of the materials surface in order to obtain specific surface topographies and roughness, remove surface contamination, and/or
improve adhesion in subsequent bonding steps. Chemical methods may comprise chemical and electrochemical treatment, sol-gel, chemical vapor deposition (CVD), and biochemical modification. Physical methods, like thermal spraying and physical vapor deposition (PVD) include those in which the formation of the surface modified layer, films or coatings are mainly attributed to the thermal, kinetic and electrical energy.4

The deposition of bioactive materials on titanium surfaces can be classified as chemical or physical methods, according to the deposition technique used. Calcium phosphates materials, such as hydroxyapatite, accelerate the process of bony growth in the surrounding area of an implant, because of its similarity with the chemical and mineral components of teeth and bone. However, its clinical applications are largely limited to non-major-load-bearing parts of the skeleton, because of their poor mechanical properties. Therefore, it has being used as coating materials on metallic implant substrates, such as commercial pure titanium and its alloys.6

The main aim of this work consists in developing an approach to deposit commercial pure Titanium (cpTi) or a multi-component coating, made of commercial pure Titanium and Hydroxyapatite (cpTi/HA) onto UHMWPE by DC Magnetron sputtering and DC/RF Magnetron co-sputtering, respectively. This approach may be useful on the development of a novel acetabular component in which the metallic shell is substituted by a coating and, therefore, made of just one piece, thus having the great advantage of the reduction of the implant costs. Moreover, the coating of the implants’ surface also may contribute for the enhancement of their performance by promoting bone ingrowths and supplying improved fixation.7

Despite the novelty associated to the coating of UHMWPE, its application in the development of a novel acetabular component would have some restrictions. In cases that a revision surgery is necessary, the acetabular component would have to be totally replaced.

In this work, commercial pure Titanium coatings on UHMWPE substrates by DC Magnetron Sputtering were compared with deposited multi-component coatings, made of commercial pure Titanium and Hydroxyapatite, on UHMWPE substrates by DC/RF Magnetron co-sputtering technique. The coated materials were physicochemical characterized, namely its morphology, topography, thickness, surface chemistry, surface energy, and adhesion.
2. MATERIALS AND METHODS

2.1. MATERIALS

Ultra high molecular weight polyethylene (UHMWPE) discs (diameter of 16.1mm and thickness of 5.1mm) were used as substrates. They were cut from an UHMWPE cylindrical bar and grinded with silicon carbide papers of P400, P600, P1200, P2500 and 1200/4000 grits. Following this, these substrates were cleaned ultrasonically for 10 minutes in ethanol and then were rinsed with deionised water and dried.

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\[
10 \text{Ca(OH)}_2 + 6 \text{H}_3\text{PO}_4 \rightarrow \text{Ca}_{10}\text{(PO}_4)_6\text{(OH)}_2 + 18 \text{H}_2\text{O}
\]

Filtered and dried HA precipitate was ground into a fine powder, with a granulometry less than 75 µm. Afterward the obtained powder was uniaxially pressed to a disc and sintered at 1300°C for 1 hour.

2.2. COMMERCIAL PURE TITANIUM DEPOSITION BY DC MAGNETRON SPUTTERING (cpTi-UHMWPE)

Commercial pure Titanium deposition was performed in a sputter deposition chamber at room temperature. This chamber was evacuated to a base pressure in the range of 10^{-7} mbar. Subsequently, high purity Argon (Ar) gas was back-filled into the chamber till a working pressure of 5x10^{-3} mbar maintained fixed during all the deposition time. UHMWPE substrates were placed on the sample holder facing the magnetron source at a distance of approximately 10 cm. The coating deposition rate was controlled by the power supplied to the cpTi target: 0.06Kw direct current (DC). The sputtering deposition time was of 3 hours.
2.3. **Commercial Pure Titanium and Hydroxyapatite Deposition by DC/RF Magnetron Co-sputtering (cPTi/HA-UHMWPE)**

In this technique two sputtering targets were used, made of commercial pure Titanium and Hydroxyapatite, at the same time. This deposition was performed in a sputter deposition chamber at room temperature. This chamber was evacuated to a base pressure in the $10^{-7}$ mbar range. A working pressure of $5 \times 10^{-3}$ mbar was reached introducing high purity Argon (Ar) gas into the chamber. The substrates were placed on the sample holder facing both targets at a distance of approximately 10 cm. The film composition was controlled by the power applied to the cPTi and Hydroxyapatite targets: 0.06Kw direct current (DC) for the cPTi and 90W radio frequency (RF) at 13.56MHz for the Hydroxyapatite. The sputtering deposition time was of 1h30min.

2.4. **Coatings Physicochemical Characterization**

2.4.1. **Assessment of Coating Morphology**

Coatings’ surface morphology was observed by SEM and the presence of titanium, calcium and phosphor in the samples was analyzed by X-ray mapping (FEI Quanta 400FEG scanning electron microscope equipped with X-ray EDS microanalysis capability, EDAX Genesis X4M). Data was collected with a dwell time of 200µs by point from a sample matrix of 512x400µm, calibrated in the x axis by 1.152µm per pixel and in the y axis by 1.167152µm per pixel.

2.4.2. **Assessment of Coating Thickness**

The film thickness was measured observing as-coated samples cross sections through scanning electron microscopy (SEM) technique (FEI Quanta 400FEG scanning electron microscope).

2.4.3. **Zeta Potential Measurement**

Zeta potential (ZP) was determined from streaming potential measurements, which were performed in Anton Paar EKA – Electro Kinetic Analyzer equipment, using a rectangular cell in which the samples were paralleled placed from each other, leaving a
small gap through which the liquid was forced to flow. The potential measured by the
flow, was then measured by the two AgCl electrodes placed at each end of the
rectangular cell.

The measurements were performed using 0.001 M KCl as electrolyte solution at pH
7.4. Zeta potential, $\zeta$, was calculated from:

$$\zeta = \frac{V_s}{\Delta p} \cdot \frac{\eta}{\varepsilon \varepsilon_0} \cdot \frac{L}{A} \cdot \frac{1}{R}$$

where, $V_s$ is the streaming potential, $\Delta p$ the hydrodynamic pressure difference across
the sample, $\eta$ is the viscosity, $\varepsilon$ the permittivity of the liquid, $\varepsilon_0$ the permittivity of free
space, $L$ and $A$ are the length and cross sectional area of the sample, and $R$ corresponds
to the electrical resistance across it.\(^8\) Fairbrother and Mastin’s approach\(^9\) was used to
determine the term $L/A$.

### 2.4.4. Wettability and Surface Tension Determination

For the determination of the total solid surface tension and its components, distilled
and deionised water, glycerol and diiodomethane (Merk Schuchardt, > 99%) were used
as testing liquids.

Contact angles were measured using the sessile drop technique in a videobase
system DATA Physics Contact Angle System OCA 15. The drops were deposited with
a micrometric syringe directly on the samples surface from the metallic needle. The
ellipse method was used to fit a mathematical function (Laplace-Young Fitting) to
measure the drop contour line.

The measurements were carried out at 25 °C inside a thermostatted stainless steel
chamber, with glass windows of optical quality and saturated with a pool of the liquid in
analysis. For each experiment, were analyzed at least 8 drops.

The surface tension of a liquid in equilibrium with its vapor, $\gamma_{LV}$, and the contact
angle of a liquid drop resting on a solid surface, $\theta$, are related through Young’s
equation:\(^10\),\(^11\)

$$\gamma_{LV} \cos \theta = \gamma_{SV} - \gamma_{SL}$$

\(^2\)
where $\gamma_{SV}$ is the solid-vapor surface tension and $\gamma_{SL}$ is the solid-liquid interfacial tension. Thus, from the experimental values $\gamma_L$ and $\theta$, is possible to obtain the difference $\gamma_{SV} - \gamma_{SL}$. According to Owen and Wendt’s approach\textsuperscript{12}, the total surface tension can be expressed as a sum of disperse ($\gamma_d$) and polar components ($\gamma_p$). According to Owen and Wendt, the solid-liquid interfacial tension, $\gamma_{SL}$, can be calculated from the following equation:\textsuperscript{13}

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2(\gamma_{SV} \cdot \gamma_{LV})^{1/2} = 2(\gamma_{SV} \cdot \gamma_{LV})^{1/2} \quad (3)$$

Using Young’s equation and equation (3) the components of the solid’s surface tension can be determined taking the values of contact angles measured with three testing liquids whose dispersive and polar surface tension components are known.\textsuperscript{13}

### 2.4.5. ASSESSMENT OF COATING ADHESION

Adhesion measurement was performed using a Sebastian V Tester, which has the same functional principle as the pull-off test. In this measurement, several pull studs of 5.9mm$^2$ and a length of 12.5mm were fixed to the samples using epoxy glue. After the fixation of the studs, they were pulled off perpendicularly from the substrate (Z-axis pull test) allowing the measurement of the force required to the failure of the coating. The detached area was observed by SEM and optical microscopy.

### 3. RESULTS

#### 3.1. ASSESSMENT OF COATING MORPHOLOGY

*As-deposited Titanium films by DC Magnetron Sputtering (cpTi-UHMWPE)*

cpTi-UHMWPE coating’s morphology was observed by SEM (Figure 1). The deposited film presents a homogeneous coverage of the substrate surface. As it can be seen from Figure 1, the morphology of the coating seems very smooth.
Figure 1 – SEM morphology of the as-deposited commercial pure Titanium layer on UHMWPE substrates.

As-deposited Titanium and Hydroxyapatite films by DC/RF Magnetron Co-Sputtering (cpTi/HA-UHMWPE)

Commercial pure Titanium and Hydroxyapatite were co-deposited onto the UHMWPE substrates. Its surface’s morphology was observed by SEM (Figure 2a) and the presence of titanium, calcium and phosphor was analyzed by X-ray mapping (Figure 2b).

Figure 2 – a) SEM morphology of the as-deposited commercial pure Titanium and Hydroxyapatite by DC/RF magnetron co-sputtering. b) X-ray mapping of the as-deposited Ti/HA-UHMWPE.

Figure 2a revealed that the deposited material presented a uniform coverage of the UHMWPE samples. X-ray mapping analysis allowed concluding that, at the observing
scale, the distribution of the elements calcium, phosphorus and titanium was very homogenous. These maps represent the distribution in weight percentage of these three elements, and Figures 2b didn’t reveal any inconsistency on their distribution.

3.2. **Assessment of Coating Thickness**

In order to access the coating thickness, a cross section of the coating was observed by SEM (Figure 3). cpTi-UHMWPE coatings presented a uniform thickness of approximately 1.61 ± 0.65 micrometers. cpTi/HA-UHMWPE coatings presented a uniform thickness of 0.22±0.04 micrometers.

![SEM morphology of a cross section of cpTi-UHMWPE sample.](image)

3.3. **Zeta Potential Measurement**

The measurement of the samples’ zeta potential, revealed slight differences in the electrokinetic interactions at the interface between the electrolyte and the coatings surfaces (Table 1).

<table>
<thead>
<tr>
<th>Coatings</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpTi-UHMWPE</td>
<td>-55.1±5.2</td>
</tr>
<tr>
<td>cpTi/HA-UHMWPE</td>
<td>-45.4±3.7</td>
</tr>
</tbody>
</table>

![Figure 3 – SEM morphology of a cross section of cpTi-UHMWPE sample.](image)
3.4. **Wettability and Surface Tension Determination**

cpTi/HA-UHMWPE samples are more wettable than the cpTi-UHMWPE ones (average contact angle of 84.0°±14.4° and 93.5°±7.4° for water, respectively). Based on the contact angles achieved for water, glycerol and diiodomethane, surface tension values of both surface’s coatings were determined. The surface tension of cpTi-UHMWPE coating was found to be 38.2mN/m, and the one found at the cpTi/HA-UHMWPE coating was of 26.6mN/m (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Surface Tension (mN/m)</th>
<th>Dispersive component (mN/m)</th>
<th>Polar component (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>71.5</td>
<td>21.4</td>
<td>50.1</td>
</tr>
<tr>
<td>Glycerol</td>
<td>63.4</td>
<td>37.0</td>
<td>26.4</td>
</tr>
<tr>
<td>Diiodomethane</td>
<td>50.5</td>
<td>50.5</td>
<td>0</td>
</tr>
<tr>
<td>cpTi-UHMWPE</td>
<td>38.2</td>
<td>37.6</td>
<td>0.5</td>
</tr>
<tr>
<td>cpTi/HA-UHMWPE</td>
<td>26.6</td>
<td>0.5</td>
<td>26.1</td>
</tr>
</tbody>
</table>

While the dispersive component of the surface tension was found to be the predominant one on the cpTi-UHMWPE coating material (37.6mN/m), the polar component was the predominant one on the cpTi/HA-UHMWPE coating (26.1mN/m).

Both dispersive and polar components had a contribution of about 98% to the total surface tension on cpTi-UHMWPE coating and cpTi/HA-UHMWPE coating, respectively.

3.5. **Assessment of Coating Adhesion**

The adhesion of the as-deposited films, measured using a Sebastian V tester, presented an average value of 3.26±1.19MPa, for the cpTi-UHMWPE coating material, and an average value of 5.00±1.61MPa, for the cpTi/HA-UHMWPE one. The detached area was observed by SEM (Figure 4) and from the images obtained is possible to
conclude that both coatings were completely pulled off and, therefore, the coated samples showed an adhesive failure.

![Figure 4 – SEM of cpTi/HA-UHMWPE sample after Sebastian V testing.](image)

4. DISCUSSION

Materials characterization forms the basis for understanding the potential of a new medical device material in order to have success when the device is put into use in a specific application. Therefore, in order to evaluate if the coated UHMWPE prepared (cpTi-UHMWPE by DC Magnetron Sputtering and cpTi/HA-UHMWPE by DC/RF Magnetron co-Sputtering) will serve the main propose of this work, coated samples were characterized with respect to its morphology, topography, thickness, surface chemistry, surface energy, and adhesion.

SEM analysis revealed that both coatings presented a homogeneous coverage of the substrate with few cracks and defects. X-ray maps revealed a uniform distribution of calcium, phosphor and titanium elements on cpTi/HA-UHMWPE samples.

With reference to the coatings thickness, experimental conditions used during the coating of the samples, led cpTi-UHMWPE coating materials to present a uniform thickness greater than cpTi/HA-UHMWPE ones. The firsts presented a thickness of approximately 1.61 ± 0.65 micrometers and the lasts presented a thickness of 0.22±0.04 micrometers. This is due to the fact that the deposition time used for cpTi-UHMWPE samples was of 3 hours and for cpTi/HA-UHMWPE was of 1h30. Commercial pure titanium has a greater deposition rate than Hydroxyapatite. Therefore, in order to equilibrate the cpTi and HA deposition, cpTi deposition was decreased and HA deposition was increased. If the deposition time was the same for both sample varieties (cpTi-UHMWPE and cpTi/HA-UHMWPE), the thickness of cpTi/HA-UHMWPE
would still being smaller. Thus, in order to obtain a coated sample with the same thickness, the deposition time would have to be greater than the one used to coat cpTi-UHMWPE.

The electric charge of a material surface is one of the main physical factors involved in the biological evolution of the tissue around an implant. Together with surface’s wettability affect biomaterial’s protein adsorption and the subsequent cell attachment and proliferation, which are decisive on the osteointegration of the implant. Generally, it is recognized that while hydrophilic surfaces are more expected to resist protein adsorption, hydrophobic ones usually will adsorb a monolayer of tightly adsorbed protein. Exist, however, exceptions to these generalizations.

Surface samples’ zeta potential measurement revealed slight differences in the electrokinetic interactions at the interface between the electrolyte and the coated surfaces. cpTi/HA-UHMWPE samples presented an average zeta potential greater than cpTi-UHMWPE materials (-45.4±3.7mV and -55.1±5.2mV, respectively). This is due to the fact that HA zeta potential values presented in the literature are greater than the ones of cpTi. Therefore, these zeta potential values were expected. The fact that both coating surfaces present a negative zeta potential is in accordance with published results.

cpTi/HA-UHMWPE samples revealed to be more wettable than the cpTi-UHMWPE ones. cpTi/HA-UHMWPE and cpTi-UHMWPE coatings presented an average contact angle of 84.0°±14.4° and 93.5°±7.4° for water, respectively. This is indicative that both surfaces are hydrophobic, which is in accordance with published results.

Despite the mechanisms involved in the long term biological response are not entirely understood, it’s acknowledged that some surface properties, such as surface energy, have an influence on the protein adsorption process and, consequently on cell response. According to the Owens and Wendt approach, the total surface tension is of the same order of magnitude for both coated surfaces. Whereas cpTi-UHMWPE samples presented a surface tension of 38.2mN/m, cpTi/HA-UHMWPE materials presented a surface tension of 26.6mN/m. However, while the dispersive component of the surface tension was found to be the predominant one on the cpTi-UHMWPE coating, the polar component was the predominant one on the cpTi/HA-UHMWPE coating. Because cpTi/HA-UHMWPE samples revealed to be more wettable than cp-Ti-
UHMWPE samples, it is expected that their polar component will predominate over the dispersive one.

Concerning the adhesion, which is a fundamental requirement of any film-substrate system\textsuperscript{22}, cpTi-UHMWPE samples presents a smaller adhesion than the one of the cpTi/HA-UHMWPE samples (3.26±1.19MPa and 5.00±1.61MPa, respectively). There are no minimum requirements for this kind of coatings in the medical field, but authors believe they are strong enough to be used in the applications envisaged.

The use of this approach on the development of an acetabular component which metallic shell is substituted by a coating material will require a new prostheses’ design, because the space aforetime occupied by the metallic shell, will be empty. Therefore, the accomplishment of biomechanical studies becomes imperative.

5. CONCLUSIONS

The evaluation of an approach to deposit on UHMWPE a coating made of commercial pure Titanium or a multi-component coating, made of commercial pure Titanium and Hydroxyapatite, may have potential in its future application on hip prostheses. The coating of UHMWPE with commercial pure Titanium and commercial pure Titanium and Hydroxyapatite seem suitable for the accomplishment of this work’s main aim. The use of Hydroxyapatite combined with Titanium on the coating of UHMWPE samples, brought some positive changes to the physicochemical features of the coatings, attending to the recognized needs for the biomaterial’s osteointegration. However, it’s still necessary to validate these results with both \textit{in vitro} and \textit{in vivo} tests.
6. REFERENCES


CHAPTER 4

IN VITRO BIOLOGICAL CHARACTERIZATION
**IN VITRO BIOLOGICAL CHARACTERIZATION OF NOVEL cpTi AND cpTi/HA COATINGS ONTO UHMWPE FOR NONCEMENTED ACETABULAR CUPS IN TOTAL HIP ARTOPLASTY**

M. A. Silva\(^{1,2}\), P. S. Gomes\(^3\), M. Vila\(^4\), M. A. Lopes\(^{1,2}\), J. D. Santos\(^{1,2}\), R. F. Silva\(^4\), M. H. Fernandes\(^3\)

\(^1\) Instituto de Engenharia Biomédica (INEB), Laboratório de Biomateriais, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal

\(^2\) Faculdade de Engenharia, Universidade do Porto (FEUP), Secção de Materiais, DEMM, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

\(^3\) Laboratório de Farmacologia e Biocompatibilidade Celular, Faculdade de Medicina Dentária, Universidade do Porto (FMDUP), Rua Dr. Manuel Pereira da Silva, s/n 4200-392 Porto, Portugal

\(^4\) CICECO, Departamento de Engenharia Cerâmica e do Vidro, Universidade de Aveiro, 3810-193 Aveiro, Portugal

**Tissue Engineering Part C: Submitted**

**Abstract**

The development of hip joint materials is one of the most challenging problems to prostheses technology in this millennium. Ultra high molecular weight polyethylene (UHMWPE) has been a favorite material for the acetabular component and, regarding the cementless approach, several novel coating options may be considered in order to contain and stabilize UHMWPE bearing surfaces and establish a direct interface with the bone tissue. In this work, newly developed UHMWPE substrates coated with either commercial pure Titanium (cpTi-UHMWPE), by DC Magnetron Sputtering, or with commercial pure Titanium and Hydroxyapatite (cpTi/HA-UHMWPE), by DC/RF Magnetron co-Sputtering, have been prepared and biologically evaluated by the establishment and characterization of human bone marrow-derived osteoblastic cultures. cpTi-UHMWPE samples allowed a high cell growth rate and the expression of the complete osteoblastic phenotype with high alkaline phosphatase activity and evident cell-mediated mineralization of the extracellular matrix. In comparison, cpTi/HA-UHMWPE samples reported lower cell proliferation but increased osteogenic differentiation as shown by the anticipation of the cell-mediated matrix mineralization. Accordingly, both newly developed systems may constitute adequate approaches in the development of an acetabular component made of just one piece, reporting induced biological behavior which can favor long-term survivability of total arthroplastic applications.
1. INTRODUCTION

The increase of life expectancy, due to the advances occurred in the field of medical sciences contributed to enlarge the number of the elderly demanding replacing of failed tissues with regenerative medicine strategies, which include the implantation of biomaterials and artificial implants\(^1\). In the case of the hip joint, which is subjected to high levels of cyclic mechanical stress, it's expected that after 50 or more years of activity it starts wearing out having to be replaced, because of degenerative or rheumatologic diseases\(^2\). In this way, the selection of adequate components for joint replacement must consider biological, as well as, physical and mechanical issues\(^3\). Currently, and in a general way, hip prostheses are made of a titanium or cobalt-chromium alloy femoral stem (cemented or press fit into place), connected to a cemented ultrahigh-molecular-weight polyethylene (UHMWPE) acetabular insert, or to a press-fitted modular metal-backed UHMWPE acetabular component\(^4\).

UHMWPE has gained widespread support as a material of choice for bearing surfaces routinely used in several arthroplastic applications (including total shoulder, knee and hip replacements), due to its adequate elastic properties, corrosion resistance and ability to achieve a smooth surface through machining techniques\(^5,6\). Despite the clinical success attained with this material, definitive long-term function has not been established, especially within cemented applications\(^7\). Major limitations include deleterious biological effects due to the exothermic reaction during the cement curing process\(^8\), and mechanical failure of the bone-cement interface, cement-implant interface, or the cement mantle itself, generally leading to implant revision due to aseptic loosening\(^9\). In fact, long-term failure rate (combining clinical and radiographic failure) of cemented polyethylene cups has been reported in the range of 22 to 49\(^\%\)\(^10-12\).

Consequently, cementless acetabular components were developed with the introduction of a modular metal liner, aiming to contain and stabilize UHMWPE bearing surfaces and establish a direct interface with the bone tissue. This direct union has the ability to provide a dynamic and durable interface which is expected to be resistant to late aseptic loosening\(^12\). Other advantages of cementless techniques include the ease of insertion and the increased surgical options afforded by modularity, which all contribute to rank this application as the most popular option for acetabular component fixation in the USA\(^12\). Even so, the metal-backed system has been reported to generate new complications including liner dissociation, expansile osteolysis and late
debonding of the coatings\textsuperscript{12}, most of the time associated with reduced polyethylene thickness required by component modularity and suboptimal designs related to locking mechanisms and tridimensional geometry\textsuperscript{13,14}.

In this way, the development of a new approach that allows the control of surface properties and maintains the thickness and design of the UHMWPE component, at the same time that favors long-time integration with the bone tissue, may improve the clinical outcome of total hip replacements\textsuperscript{15}. Several coating options may be considered in order to control physical and mechanical properties of the bulk material, enhancing, at the same time, the biological response. Either way, and depending on strategy, the coatings should either provide a stable, nondissolving interface with tissues (achieved, for instance, with a titanium coating), or should dissolve gradually being substituted by newly formed bone (which can be achieved with a calcium phosphate-based coating)\textsuperscript{15}. Titanium is a bioinert material that does not induce adverse reactions following tissue implantation\textsuperscript{16}. In fact titanium and titanium alloys have reported adequate biocompatibility both for \textit{in vitro} and \textit{in vivo} function and are currently used in diverse orthopedic applications\textsuperscript{17,18}, including arthroplastic fixative surfaces\textsuperscript{19}. Furthermore, bioactive calcium phosphate coatings have also been used to provide a superficial environment that induces bone growth at the materials’ surface, promoting a high strength interfacial bonding between the implant and the tissue\textsuperscript{20-22}.

Accordingly, and to the best of the authors’ knowledge, the development and comparison of titanium and titanium plus hydroxyapatite coatings over UHMWPE substrates have not been previously reported, although these novel approaches are expected to significantly enhance the biological and biomechanical behavior of total arthroplastic applications. In this way, the purpose of this study consists in the evaluation of the \textit{in vitro} biological performance of deposited commercial pure Titanium (cpTi) and commercial pure Titanium and Hydroxyapatite (cpTi/HA) onto UHMWPE substrates, by DC Magnetron sputtering and DC/RF Magnetron co-sputtering, respectively. These techniques allow the deposition of thin, dense, fine-grained and uniform coatings with strong adhesion and compact structure that can survive without delamination in body fluids and can withstand high surface pressures\textsuperscript{15,23}. \textit{In vitro} biological performance of cpTi-UHMWPE and cpTi/HA-UHMWPE was evaluated by the establishment and characterization of human osteoblastic cell cultures.
2. MATERIALS AND METHODS

2.1. MATERIALS

Ultra high molecular weight polyethylene (UHMWPE) discs (diameter of 16.1mm and thickness of 5.1mm) were used as substrates. They were cut from an UHMWPE cylindrical bar and grinded with silicon carbide papers of P400, P600, P1200, P2500 and 1200/4000 grits. Following this, substrates were cleaned ultrasonically for 10 minutes in ethanol and then rinsed with deionised water and dried.

Commercial pure titanium (Ti) and hydroxyapatite were used as the sputtering target materials.

Phase pure hydroxyapatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2, \text{HA}]\) preparation required the precipitation between calcium hydroxide, \(\text{Ca(OH)}_2\), and orthophosphoric acid, \(\text{H}_3\text{PO}_4\), according to the following chemical reaction:

\[
10 \text{Ca(OH)}_2 + 6 \text{H}_3\text{PO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 18 \text{H}_2\text{O}
\]

Filtered and dried HA precipitate was ground into a fine powder, with a granulometry less than 75 µm. Afterwards, the obtained powder was uniaxially pressed to a disc and sintered at 1300ºC for 1 hour.

UHMWPE substrates were coated, at room temperature, with commercial pure Titanium by DC Magnetron Sputtering and with commercial pure Titanium and Hydroxyapatite by DC/RF Magnetron co-Sputtering.

The deposition of cpTi and cpTi/HA was performed in a sputter deposition chamber, which was evacuated to a base pressure in the range of \(10^{-7}\) mbar. Afterwards, high purity Argon (Ar) gas was back-filled into the chamber till a working pressure of \(5\times10^{-3}\) mbar that was maintained fixed during all the deposition time.

On DC Magnetron Sputtering, UHMWPE substrates were placed on the sample holder facing the magnetron source at a distance of approximately 10 cm. The coating deposition rate was controlled by the power supplied to the cpTi target: 0.06Kw direct current (DC). The sputtering deposition time was of 3 hours.

On DC/RF Magnetron co-sputtering, the substrates were placed on the sample holder facing both targets (cp Ti and HA) at a distance of approximately 10 cm. The film composition was controlled by the power applied to the cpTi and HA targets:
0.06Kw direct current (DC) for the cpTi and 90W radio frequency (RF) at 13.56MHz for the HA. The sputtering deposition time was of 1h30min.

Physicochemical characterization of cpTi-UHMWPE and cpTi/HA-UHMWPE coated samples was studied in detail in a previous study (24), i.e. the coatings exhibited slight differences in the wettability (contact angle of 93.5°±7.4 and 84.0°±14.4 for water, respectively), surface tension (38.2 mN/m, and 26.6 mN/m, respectively) and zeta potential (-55.1±5.2 mV and -45.4±3.7 mV, respectively). On scanning electron microscopy (SEM; FEI Quanta 400FEG scanning electron microscope) both coatings presented a homogeneous coverage of the substrate surface, as showed on Figure 1.

Prior to the \textit{in vitro} biological study, all samples were sterilized with ethylene oxide (EO) gas.

\textbf{2.2. Cell cultures}

Human bone marrow cells were collected from orthopaedic surgical procedures. Informed consent to use this biological material, that would be otherwise discarded, was obtained. Bone marrow was cultured in $\alpha$-Minimal Essential Medium ($\alpha$-MEM) supplemented with 10% of fetal bovine serum, 50 $\mu$g/ml of ascorbic acid, 100 IU/ml of penicillin, 100 $\mu$g/ml of streptomycin and 2.5 $\mu$g/ml of fungizone. The culture was incubated at 37°C in a humidified atmosphere of 95% air and 5% CO2, and the culture medium was changed twice a week. This primary culture was maintained until near confluence, which took 10-15 days. At this time, enzymatic release of the adherent cells was performed (0.05% trypsin, 0.25% EDTA) and the obtained cellular suspension was seeded at a density of $10^4$cells/cm$^2$ in control conditions (standard polystyrene culture plates) and onto the cpTi-UHMWPE and cpTi/HA-UHMWPE coated samples, in 12-well dishes. Cultures were maintained for 21 days. In order to infer about the needing of
a pre-incubation period, cpTi-UHMWPE and cpTi/HA-UHMWPE samples were divided into two groups, A and B. Samples of group A were seeded in the “as-prepared” condition (i.e., not submitted to a pre-incubation treatment) and samples of group B were incubated with fully supplemented culture medium over 24 hours. Cells were seeded and cultured under the same conditions used in the primary culture. The culture medium, in addition to the other components referred above, was supplemented with 10mM β-glycerophosphate and 10nM dexamethasone. Seeded samples of both groups, as well as control cultures, were characterized at days 7, 14 and 21 for cell viability/proliferation (MTT assay), total protein content and alkaline phosphatase (ALP) activity. In addition, samples were observed by confocal laser scanning microscopy (CLSM, days 3 and 14) and scanning electron microscopy (SEM, days 14 and 21).

2.3. CELL VIABILITY/PROLIFERATION

Evaluation of the cellular viability/proliferation was performed through the MTT assay at days 7, 14 and 21. In this assay, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide) is reduced, by the living cells present in the culture, originating purple formazan crystals. Cultures were incubated with 0.5mg/ml of MTT at 37°C in a humidified atmosphere of 95% air and 5% CO₂, during 4 hours. After this period, the medium was decanted, the formazan crystals were dissolved with dimethylsulphoxide and the absorbance was measured at 600nm using a spectrophotometer ELISA (Denley Wellscan). Results were expressed as absorbance per square centimetre (A/cm²).

2.4. PROTEIN CONTENT AND ALKALINE PHOSPHATASE ACTIVITY

At days 7, 14 and 21, cultured samples were washed twice with phosphate-based solution (PBS) and total protein content was determined according to Lowry method, using bovine serum albumin as a standard.

Activity of ALP was determined in cell lysates (obtained by treatment of the cultures with 0.1% triton) and assayed through the hydrolysis of p-nitrophenyl phosphate, carried out for 30 minutes at 37°C, in an alkaline buffer solution (pH 10.3). Colorimetric determination of the obtained product, p-nitrophenol, was carried out at 405nm. Results were expressed as nanomoles of p-nitrophenol produced per min per microgram of total protein (nmol.min⁻¹/µgprotein).
2.5. IMMUNOSTAINING OF F-ACTIN CYTOSKELETON

At days 3 and 14 of the culture, cells were fixed with 4% formaldehyde (methanol free), permeabilized with 0.1% triton and incubated in 10mg/ml bovine serum albumin (BSA) with 100µg/ml RNAse. F-actin filaments were stained with Alexafluor-conjugated phalloidin and nuclei were counterstained with 10µg/ml propidium iodide. Afterwards, samples were washed with PBS and mounted with Vectashield®. The images were obtained on a Leica TCP SP2 AOBS confocal microscope.

2.6. MATRIX MINERALIZATION

At days 14 and 21, cultures were fixed (1.5% of glutaraldehyde in 0.14M of sodium cacodilate buffer, 10 minutes), dehydrated in graded alcohols (70, 80, 2 x 90 and 99.8%), critical-point dried and sputter-coated with gold. Samples were observed by scanning electron microscopy (SEM) and analyzed by X-ray Spectroscopy (EDS) spectra, in FEI Quanta 400FEG scanning electron microscope equipped with X-ray EDS microanalysis capability, EDAX Genesis X4M.

2.7. STATISTICAL ANALYSIS

The results are presented as arithmetic means ± standard deviation of three replicates. Analysis of the results was carried out with the use of the Student’s t test, with a significance level of $P<0.05$.

3. RESULTS

3.1. CELL VIABILITY/PROLIFERATION

Cell viability/proliferation, evaluated through the MTT assay, is presented in Figure 2. Cells grown on the surface of the culture plate (control cultures) proliferated through the incubation time, with a stationary phase during the third week. Regarding cpTi-UHMWPE samples, MTT reduction was lower at days 7 and 14 and similar at day 21. The pre-incubation treatment did not have a significant effect on the cell viability/proliferation of the seeded cpTi/UHMWPE samples. Comparatively, seeded cpTi/HA-UHMWPE presented lower MTT reduction values through the culture time. The outcomes of the pre-incubation revealed to be significant on this material. At days
7, 14 and 21 values were significantly higher than those observed on the non pre-incubated samples.

![Graph](image)

**Figure 2** – Cell viability/proliferation of human bone marrow osteoblastic cells cultured for 21 days on the culture plate (control), and on the surface of cpTi-UHMWPE and cpTi/HA-UHMWPE. A: non pre-treated samples; B: pre-treated samples. *Significant different from control. **Significantly different from cpTi-UHMWPE

### 3.2. IMMUNOSTAINING OF THE CYTOSKELETON

CLSM appearance of the material samples labelled for F-actin and nuclei is shown in Figure 3. On cpTi-UHMWPE (both not submitted and submitted to the pre-incubation treatment), at day 3, cells were well distributed on the material surface and presented an elongated morphology and cell-to-cell contact (Figure 3 A,B); at day 14, the cell layer covered a significant area of the material surface (Figure 3 E,F). Non-treated cpTi/HA-UHMWPE presented a poor performance; the few cells attached to the surface displayed a disrupted morphology, which further deteriorates throughout the culture time (Figure 3 C,G). However, on pre-incubated samples, cells appeared well spread and, at day 14, disperse cell aggregates were found throughout the materials’ surface (Figure 3 D,H). At a high magnification, the organization of the actin cytoskeleton of both pre-treated cpTi-UHMWPE and cpTi/HA-UHMWPE appeared similar to that of control, Figure 3 I-L.
Chapter 4 - In vitro biological characterization

3.3. CELL FUNCTION

Figure 4, regarding alkaline phosphatase activity, shows that cpTi-UHMWPE samples both non-pre-incubated and pre-incubated samples, as well as the pre-incubated cpTi/HA-UHMWPE samples presented a profile similar to that of control cultures, i.e. a significant increase in the enzyme activity during the third week of culture. By contrast, ALP was practically absent on cpTi/HA-UHMWPE samples seeded in the “as-prepared” condition (i.e. not submitted to the pre-treatment with culture medium).
Figure 4 – ALP activity of human bone marrow osteoblastic cells cultured for 21 days on the culture plate (control) and on the surface of cpTi-UHMWPE and cpTi/HA-UHMWPE. A: non pre-treated samples; B: pre-treated samples.

SEM observation of cpTi-UHMWPE samples at days 14 and 21 (Figure 5) showed an excellent proliferation profile with the surface completely covered by a cell layer.

Figure 5 – SEM appearance of cpTi-UHMWPE samples cultured with human bone marrow osteoblastic cells for 14 (A - D) and 21 (E - H) days. High magnification of the mineralized deposits in 21-day samples (I and L) and the respective EDS-spectra (J and M). A: non pre-treated samples; B: pre-treated samples.
In addition, 21-day cultures displayed discrete mineralized deposits which contain calcium and phosphorus. The pre-incubation treatment did not affect the cell behavior. On the other hand, cpTi/HA-UHMWPE seeded in the “as prepared” condition showed few attached cells that displayed a disrupted structure (Figure 6 A-B, 14-day cultures).

![cpTi/HA-UHMWPE samples](image)

**Figure 6** – SEM appearance of cpTi/HA-UHMWPE samples cultured with human bone marrow osteoblastic cells for 14 days. A: non pre-treated samples; B: pre-treated samples. F: EDS-spectrum of the mineralized deposits present on pre-treated cp-Ti/HA-UHMWPE.

However, pre-incubated cpTi/HA-UHMWPE samples presented cell clusters with an evident formation of cell-mediated mineralized deposits at day 14 (Figure 6 C-E). For comparison, Figure 7 shows the SEM appearance of human bone marrow cells cultured on the tissue culture plate (control cultures), at days 14 and 21. The presence of calcium phosphate deposits was observed by day 21.

![human bone marrow cells](image)

**Figure 7** – SEM appearance of human bone marrow osteoblastic cells cultured on standard tissue culture plates (control cultures) for 14 (A) and 21 (B, C) days. D: EDS-spectrum of the mineralized deposits.
4. DISCUSSION

The development of UHMWPE samples coated with commercial pure Titanium (cpTi-UHMWPE) or commercial pure Titanium and Hydroxyapatite (cpTi/HA-UHMWPE), by DC Magnetron Sputtering and DC/RF Magnetron co-Sputtering respectively, aimed the conception of a novel acetabular component made of just one piece, in which the metallic shell is substituted by a coating material. Cellular biocompatibility of the coated UHMWPE samples was assessed with human bone marrow cells cultured in experimental conditions known to favor osteoblastic differentiation\textsuperscript{25}. Material samples were tested “as-prepared” and after a pre-incubation treatment with fully supplemented culture medium for 24 hours.

Results showed that seeded cpTi-UHMWPE samples exhibited the complete proliferation/differentiation sequence of human bone osteoblastic cells, with a phase of exponential cell growth, the synthesis of high ALP levels and the formation of a calcium-phosphate containing mineralized matrix by day 21. Compared to control, fewer cells attached to the material surface, as suggested by the lower values in the MTT assay at day 7. This is most probably related with the seeding procedure in which a cell suspension was poured over the surface of a disc and, as expected, a significant percentage of cells slipped off to the bottom of the culture plate. This event was further enhanced by the smooth features of the material surface. However, subsequently, attached cells presented a high proliferation rate forming a cell sheet over the surface, from two weeks onwards. At day 21, MTT reduction values were similar to control suggesting a good performance of this material. Pre-incubation treatment revealed to have no significant influence in the biological behaviour of cpTi-UHMWPE samples, once both cpTi-UHMWPE A and B samples presented no considerable differences regarding the biological parameters evaluated. The present results are in agreement with the known behaviour of titanium surfaces which are reported to be biocompatible, supporting osteoblast cell proliferation and differentiation\textsuperscript{26} and adequate osseointegration \textit{in vivo}\textsuperscript{27}.

Results regarding cpTi/HA-UHMWPE samples showed that the performance of this coating was very sensitive to the pre-incubation treatment. Non-treated samples showed few attached cells with disrupted morphology and low viability. This behaviour might be related with the leaching of the HA component leading to increased ionic concentration on the surface microenvironment impairing cell anchorage and adhesion which compromises the subsequent proliferation events. These local events are favoured
by the in vitro stationary system used which does not allow the renewal of the medium, resulting in accumulation of leaching material on the coating surface. In vivo, this behaviour is expected to be attenuated by the permanent flow of body fluids at the cell/biomaterial interface and, simultaneously, the material surface is conditioned by bioactive molecules, providing a suitable environment to the adhesion of the available osteoprogenitor cells. In this way, pre-incubation of cpTi/HA-UHMWPE samples with complete culture medium before cell seeding might decrease the deleterious effects resulting from the accumulation of leaching material in the cell microenvironment and also provides some surface biological conditioning. Accordingly, results showed that pre-treatment of cpTi/HA-UHMWPE samples improved cell behaviour, i.e. cells exhibited a normal morphology and organized into small cell clusters that expressed high levels of ALP and showed earlier exuberant cell-mediated matrix mineralization compared to cpTi-UHMWPE coating. These observations suggested that cpTi/HA-UHMWPE enhanced osteoblastic differentiation compared to cpTi-UHMWPE. Several works in the literature using CaP sputtered coatings support these results. For instance, Hulshoff JG et al. stated that proliferation of mouse osteoblastic cells was significantly higher on noncoated comparing to CaP-coated Ti samples, although an increased matrix mineralization was established on the coated surfaces. Also, TEM analysis showed that cells were embedded by crystallized needle-shape CaP structures, entrenched in collagen fibres. Further, Perizzolo D et al. reported that osteogenic cells from newborn rat calvaria presented significantly more “bone-like nodules” when cultured over HA-coated surfaces, compared to titanium coated ones. One of the mechanisms associated with the increased differentiation process may be related to the early dissolution of the CaP coating, increasing the availability of calcium and phosphate in the microenvironment, followed by the formation of a bone-like mineral layer by re-precipitation. This mineral layer may favour early protein adhesion that enhances cell behaviour, namely the differentiation process of osteoblastic cells. Also, it has been reported that sputtered CaP coatings facilitate an osteoblast-dependent inhibition of osteoclast formation and that the biological activity of osteoclastic precursors (chemotaxis, proliferation and colony formation) is not supported by CaP sputtered surfaces. Both mechanisms might contribute to an increased early osteoblastic response that could favor and induce material osseointegration.

Surface characteristics of cpTi-UHMWPE and cpTi/HA-UHMWPE coated samples might also contribute to the differences in cell behavior observed between the two
coatings. The coatings presented similar surface topography, as observed by SEM, but the association of HA with cpTi led to a slight increase of the coatings hidrophilicity, as it was determined by contact angle measurements with water, and to a less negative zeta potential, as it was determined by streaming potential measurements. However, the biological significance of these differences are most probably negligible considering that the behavior of cpTi/HA-UHMWPE coating was highly conditioned by the material leaching, hampering a correlation with surface charge and hydrophobicity effects.

5. CONCLUSION

UHMWPE samples coated with commercial pure Titanium favored a high proliferation rate of human osteoblastic cells and allowed the complete phenotype differentiation, reflected by the formation of a calcium phosphate mineralized extracellular matrix. Comparatively, the association of commercial pure Titanium and Hydroxyapatite resulted in a lower proliferation rate but in a significant enhancement of the osteogenic differentiation, as evident by the anticipation of the formation of cell-mediated matrix mineralization, suggesting the possibility of an earlier osteointegration process. These results suggest that cpTi-UHMWPE and cpTi/HA-UHMWPE systems, prepared by DC Magnetron sputtering and DC/RF Magnetron co-sputtering, respectively, might constitute a useful approach to the development of novel acetabular components made of just one piece, in which the coating material establish a direct interface with the bone tissue. These newly developed systems are expected to report reduced substrate wear and induce early biological events which can lead to an increased long-term function of the arthroplastic devices.
6. References


CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS
Due to the increase of life expectancy, one of the current medical challenges is to develop an enhanced hip prosthesis with an improved performance. The development of an acetabular component made of just one piece, in which the metallic shell is substituted by a coating, constitutes one of the mainstream approaches. Therefore, in this work the coating of ultra high molecular weight polyethylene (UHMWPE) with commercial pure Titanium (cpTi) and a mixture of commercial pure Titanium and Hydroxyapatite (cpTi/HA) was attempted by several techniques. The coatings developed were characterized by assessing physicochemical properties as well as their in vitro biological performance.

Titanium and titanium alloys are widely used in biomedical applications because they are considered biocompatible materials. Due to the thin oxide layer that forms at their surface, they are relatively inert and have good corrosion resistance. Under biological conditions they do not experience significant corrosion and their surfaces can also support cell’s growth and differentiation.1-3 When implanted in close apposition to the mineralized tissues, and under suitable conditions, in spite of healing, there are difficulties getting a good chemical bonding with bone, as well as forming new bone on its surface at the early stage after its implantation. In fact, proper adhesion of titanium to bone has not been observed yet. Instead, its bond is attributed to the mechanical interlocking of the titanium asperities and pores in the bone.1,3 Titanium’s association with a bioactive material, such as Hydroxyapatite, was attempted in order to improve its osteointegration, promoting the acceleration of bone growth in the surrounding area of an implant.

The coating techniques most suitable to deposit onto UHMWPE, commercial pure Titanium (cpTi-UHPWPE) and commercial pure Titanium and Hydroxyapatite together (cpTi/HA-UHMWPE) were found to be DC Magnetron Sputtering and DC/RF Magnetron co-Sputtering, respectively. The substrate material used, as well as the calcium phosphate ceramic material deposited, gathered a set of difficulties to the accomplishment of the best coating technique. The selection of these techniques had to take into account the substrate’s low melting point, which required the deposition to be made at room temperature and, therefore, the existence of a refrigeration system in order to avoid the substrate’s heating. This excluded coating techniques such as plasma spraying which is the most commonly used in the deposition of calcium phosphate ceramics.4 During the selection of the coating techniques, parameters such as the coating’s composition, morphology, thickness and dissolution rate were taken into account. During Hydroxyapatite deposition several changes
may occur on its structure. Instead of maintaining its crystalline structure, Hydroxyapatite coatings may suffer phase transformation and become amorphous affecting the coating’s dissolution rate, which ideally should be in consonance with the bone growths rate. The dissolution test performed on samples coated by DC/RF Magnetron co-sputtering revealed that the multi-component coating made of commercial pure Titanium and Hydroxyapatite still remained after 24 hours in contact with deionized water. This factor, together with the coating’s morphology and thickness, contributed to the selection of this technique for the simultaneously deposition of these two materials onto UHMWPE. Concerning commercial pure Titanium deposition, DC Magnetron sputtering was the only technique assessed since the characterization of the coatings achieved revealed to be appropriated since the beginning.

The assessment of coating’s physicochemical properties required the evaluation of its morphology, topography, thickness, surface chemistry, surface energy and adhesion.

For both coatings, the scanning electron microscopy (SEM) analysis revealed a homogeneous coverage of the substrate. Besides, X-ray mapping exposed a uniform distribution of calcium, phosphorus and titanium elements on the coated cpTi/HA-UHMWPE samples.

Concerning the thickness of the coatings, the experimental conditions used led to cpTi coatings thickness to be greater than cpTi/HA ones (1.61 ± 0.65 µm and 0.22±0.04 µm, respectively).

In relation to the adhesion of the coatings to the UHMWPE substrate, cpTi coatings presented a smaller adhesion than the cpTi/HA ones (3.26±1.19MPa and 5.00±1.61MPa, respectively). Regardless not existing minimum requirements for this kind of coatings in the medical field, authors believe they are strong enough to be used in the applications envisaged.

Analysis to the surface chemistry revealed that the combination of Hydroxyapatite with Titanium in a multi-component coating caused an increase of the surface charge, still remaining negative, and reduced hydrophobicity of coated samples. Concerning the surface energy, opposing cpTi-UHMWPE samples, the polar component of cpTi/HA-UHMWPE samples was predominant over the dispersive one. These properties, as it will be mentioned below, will influence the adsorption of proteins and the cell spreading, when evaluating these samples biological performance.
Direct *in vitro* cell culture assessment was performed by growing human osteoblastic bone marrow cells onto the coated samples. In order to infer about the needing of a pre-incubation treatment, cpTi-UHMWPE and cpTi/HA-UHMWPE samples were divided into two groups, A and B. While the samples of group A weren’t submitted to a pre-incubation treatment, the samples of group B were incubated with fully supplemented culture medium over 24 hours.

Pre-incubation treatment revealed to have no significant influence in cpTi-UHMWPE samples. Seeded cpTi-UHMWPE A and B coated samples presented a similar behaviour and cultured cells exhibited the complete proliferation/differentiation sequence, including the expression of alkaline phosphatase activity (ALP) and the formation of a mineralized matrix, suggesting a good performance of this material.

Concerning cpTi/HA-UHMWPE, coated samples pre-incubated with fully supplemented culture medium over 24 hours, revealed a much better performance than the samples which were not subjected to this pre-incubation treatment. This fact is associated to the presence of poor crystalline HA and the deleterious effects of excessive material leaching during the culture period, which are supposed to be more noteworthy at the beginning of the incubation, prejudicing cell adhesion and the subsequent cellular activity. Consequently, few cells were able to attach onto these samples, particularly on the samples that were not submitted to a pre-incubation treatment. However, despite the number of cells present in the material surface being relatively low, these exhibited a normal morphology and formed small cell groups over the surface which expressed high levels of ALP (comparable to those observed in control culture and cpTi-UHMWPE) and demonstrated exuberant cell-mediated matrix mineralization at earlier incubation time. These facts suggest that cpTi/HA coating may enhance osteoblastic differentiation compared to cpTi coating.

Other surface features, besides chemical properties, of cpTi-UHMWPE and cpTi/HA-UHMWPE samples might also have contributed to the discrepancies in cell behavior observed in the two coatings. As mentioned above, the association of Hydroxyapatite with commercial pure Titanium led to an increase of coatings hydrophilicity and to a less negative zeta potential. The higher hydrophobicity and magnitude of the negative charge of cpTi-UHMWPE may have influenced the adsorption of proteins and the cell spreading. Once the
biological behavior of cpTi/HA-UHMWPE samples was conditioned by the excessive leaching, it dificulted the correlation with its surface physicochemical characteristics.

Attending to the recognized needs for the biomaterial’s osteointegration, the use of Hydroxyapatite combined with Titanium on the coating of UHMWPE samples, brought some positive changes to the physicochemical and biological features of the coatings. UHMWPE samples coated with commercial pure Titanium allowed the complete proliferation/differentiation osteoblastic sequence, including the formation of a mineralized extracellular matrix. Under the in vitro closed system used, these samples performed better than the UHMWPE samples coated with commercial pure Titanium and Hydroxyapatite, due to the extensive material leaching to the surrounding medium, delaying the cell adhesion and the subsequent cellular activities. Thus, a pre-incubation treatment with culture medium increased the biological performance with an enhanced osteoblastic differentiation, as evidenced by an earlier matrix mineralization. Considering the in vivo dynamic environment with a continuous fluid flow and permanent availability of osteoblast precursor cells, the results suggest that the association of Titanium and Hydroxyapatite might result in an earlier osteointegration process.

The developed approach to deposit on UHMWPE a coating made of commercial pure Titanium or a multi-component coating, made of commercial pure Titanium and Hydroxyapatite, may have potential to be used in medical devices such as hip prostheses. According to the attained characterization results, the coatings achieved in this work seem suitable to accomplish the latter application.
REFERENCES


