Chapter

Thin Films for Medical and Environmental Applications

Ana P. Piedade, Francisco Romeu, Rita Branco and Paula V. Morais

Abstract

A material that presents both the appropriate set of bulk properties in conjunction with an optimal surface performance is hardly found. For this reason, there is the need of modifying its surface. This is a standard procedure in many application fields but particularly important in the medical and environmental research. In this chapter, we describe the use of sputtering, as the chosen technology for the deposition of thin films. The use of the modified surfaces in the medical and environmental fields will be highlighted by two case studies in each one. In biomedicine, the surface modification of medical invasive devices for orthopedic and neural applications will be presented. For the environmental aspect, the results of two bioremediation tools, for arsenic and uranium removal, based on the immobilization of bacterial cells will be discussed.

Keywords: thin films, sputtering, orthopedic implants, neural implants, bioremediation, biotools, arsenic, uranium

1. Introduction

Although genetic engineering and tissue culture are very promising fields for the regeneration of tissues and organs of the human body, these areas are in the early stages of their development and far from a systematic and reliable application for the majority of the population. Therefore, it is still urgent to develop/modify nonbiological materials to be used as medical invasive devices.

The field of application of thin films in Biomedicine is very broad, but the main objective is always to modify the surface of a medical invasive device in order to confer to its surface a set of properties that the bulk material fails to present. These properties include antibacterial action [1], improving the adhesion and proliferation of specific eukaryotic cells [2, 3], inhibiting the immunological reaction of the organism [4], enhancing the compatibility of mechanical properties between invasive device and biological tissue [5], among others. Nowadays, due to the problem of bacterial resistance to antibiotics, there has been an enormous development in thin films that are able to reduce or even prevent bacterial adhesion/proliferation. However, for some applications, the development of thin films that allows the formation of bacterial biofilms is very important. One example is their use in bioremediation, which comprises the use of organisms, for example, bacteria, algae and plants, to alter or reduce the toxic impact of contaminants.

Arsenic (As) is a natural metalloid widely distributed in the air, water and soil and is considered as a major public health concern. Arsenic occurs in the environment as the structural analogue of phosphate, arsenate [As(V)] and as the most toxic form of arsenic oxyanions, arsenite [As(III)] [6, 7].

A large number of microorganisms have been described as arsenic resistant and different strategies have been connected to their ability to grow in environments contaminated with this metalloid. Based on this remarkable capacity, the use of microorganisms to remove arsenic from polluted environments has been seen as a promising solution for arsenic remediation. The arsenic bioaccumulation or biosequestration abilities exhibited by some strains have been used or enhanced in order to obtain efficient organisms to deal with arsenic-contaminated waters [8, 9].

On the other hand, former uranium mines are a source of environmental contamination, since the leaching of acid water resulting from mining activity can transfer heavy metals and radioisotopes to the surrounding environmental compartments. U in the environment occurs mainly as three different isotopes (238 U, 235 U and 234 U), all of them being radioactive; however, it is its chemical toxicity that is of greatest ecological risk. U at contaminated sites has two major oxidation states stable in aqueous media: U(VI), the most common oxidation state of the uranyl ions, $(UO_2)^{2+}$, which is highly soluble and mobile; and U(IV), which is extremely insoluble and usually precipitates as uraninite, UO_2. U(VI) is therefore considered to be more toxic since it is highly mobile in the environmental systems.

Microorganisms can use different mechanisms to immobilize U: (1) biosorption, by establishing nonspecific interactions between the U and the extracellular surfaces of microorganisms [10]; (2) bioaccumulation of U within the cells; (3) oxidation-reduction processes which transform soluble U forms, U(VI), into more stable forms, U(IV) [11] and (4) biomineralization by U precipitation as minerals [12]. Among the previous mechanisms, under aerobic conditions, biomineralization is the process that can be more feasible since it implies the immobilization of U(VI) soluble species with enzymatically-generated ligands like phosphate and sulfides.

2. Production of thin films

There is a myriad of technologies that can be used for the deposition of thin films. The choice of deposition process is dependent upon several factors, such as substrate structure and operating temperature. Several attempts have been made to classify deposition process, but they differ from the perspective of the authors that make these efforts. It is not the goal of this chapter to exhaustively list all the technologies. For that excellent books and reviews can be consulted, such as those of references [13–15].

Considering only the vacuum technologies, more recently, it is generally accepted that there are four general categories of thin film deposition: atomistic growth, particulate deposition, bulk coating and surface modification. Within these technologies especially emphasis will be given to sputtering, which is the technology used for the production of the thin films that are used for the medical and environmental applications described in the next section. Sputtering is one of the techniques of physical vapor deposition (PVD).

The properties of the films deposited by this sputtering depend on the material of the target, the gas used for the discharge and deposition parameters such as pressure, target-distance, polarization of the substrate and the chemical composition of the discharge gas. In fact, in addition to the nonreactive noble gas, the discharge gas, others can be added such as oxygen, nitrogen or methane. In this case, the sputtering is said to occur in a reactive mode. The use of a magnetron associated with

the cathode creates a magnetic field that imposes compulsory trajectories to the electrons ejected by the target, increasing the bombardment density.

The use of different power sources enables not only the deposition of electric conductive materials, direct current (DC) power supply, but also the deposition of ceramic and polymers, when radio frequency (RF) generators are used. Therefore, any material can have its surface changed by the deposition of any other type of material, similar or dissimilar, by sputtering. In order to better understand the enormous advantages of the use of sputtering, many excellent reviews have been published, namely a very recent one with an overview from the 1800s to 2017 [16].

3. Application of thin films

3.1 Medical applications

The use of thin films for medical applications arises from the fact that most bulk biomaterials that are currently used as invasive devices were not developed for this specific purpose. In fact, most bulk materials used as biomaterials can be found in our garages because they were developed for different purposes. Examples are light titanium alloys used in cars parts or carbon reinforced composites in bicycles. The experience has proven that the body more or less tolerates these materials and their overall set of properties/characteristics (mechanical, electrical, etc.) is appropriated for a specific use inside the body. However, in the majority of the cases the surface is not optimized for biological environment and, no matter how good and appropriate the bulk material is, if the surface does not present the adequate combination of chemical composition, wettability, surface charge and morphology/topography, the abiotic/biotic interface is never going to reach its full potential. Consequently, the bulk material is often modified by the deposition of a thin film.

The first example of the use of thin films for medical applications concerns work developed in our laboratories, never published before. This study arose from the fact that in most of the recently published literatures concerning the use of Ti6Al4V alloy for medical invasive devices in contact with hard tissue, such as for orthopedic and orthodontic applications, refer the need to substitute vanadium (V) by another element. This fact, from the biochemical point of view, has no sustainability. In fact, V is a key element in some of the metabolic pathways of our organism and is being considered in some pharmaceutical drugs, due to its antidiabetic effect [17]. Moreover, there are no metabolic pathways that use titanium (Ti) or aluminum (Al) and these elements are present in much higher contents than V. Therefore, our study had the goal to access the difference between an unmodified bulk Ti6Al4V femur implant and the sputter modified implant. For the surface modification, two chemical compositions were produced: Ti6Al4V and Ti6Al. With this approach, it would be possible to study the effect of vanadium in the Ti-based thin film and also compare the same chemical composition (Ti6Al4V) in bulk and thin film form.

Both thin films presented a thickness of 750 nm, and from the X-ray diffractograms, it was possible to calculate the crystallite size of the thin films: 17.5 and 20 nm for Ti6Al and Ti6Al4V, respectively. This indicated that, in opposition to the microscopic grain size of the bulk material, the thin films were nanostructured. The hardness of both bulk and thin films was determined by ultra-micro hardness test and, due to the nanometric crystallite size, the hardness of the thin films was higher than the bulk material (**Table 1**). This was expected according to Hall-Petch relation [18]. Nevertheless, the presence of vanadium in the thin film reduced the hardness value in comparison with the Ti6Al thin film.

Surfaces	Hv (GPa)	Lc1 (N)	Lc2 (N)
Bulk Ti6Al4V	4.0 ± 0.5	—	—
Ti6Al thin film	7.3 ± 0.6	35	44
Ti6Al4V thin film	4.9 ± 0.7	>70	>70

Table 1.
Hardness and adhesion of the bulk and thin films Ti-alloys.

Another important characterization was the evaluation of the adhesion of the thin films to the Ti6Al4V bulk substrate. In fact, the presence of an interface between the substrate and the thin film is often related with the development of stress that result in the detachment of the thin film when in use. The characterization was performed by a scratch indentation test and the results (**Table 1**) allow to determine both cohesive (Lc1) and adhesive failures (Lc2) values. Due to the perfect chemical compatibility between the Ti6Al4V thin film and bulk material, both cohesive and adhesive failures are higher than 70 N (the maximum value determined by the equipment). For the Ti6Al thin film, despite the similar chemical composition, the adhesive failure (detachment of the thin film from the substrate) occurred at 44 N.

The wettability was accessed by static contact angle measurements between 10 μL of water and the three surfaces (**Figure 1**).

The results showed that the bulk material presented the highest contact angle (78°), followed by Ti6Al thin film (62°) and finally Ti6Al4V thin film which presented a zero-contact angle revealing a completely wettable surface. This fact indicates that the ion exchange between the Ti6Al4V thin film and the biological fluid is enhanced, indicating that the formation of an apatite-like layer on this surface will be easier to occur than on the other two materials and, therefore, a better osseoinductive behavior is expected from Ti6Al4V thin film.

In fact, when the unmodified and sputter modified bulk Ti6Al4V were immersed for 60 days in Hanks' simulated body fluid, at 37°C with 100 rpm, the previous expectations were confirmed (**Figure 2**). The analysis of chemical distribution of calcium on the surface of the materials (**Figure 2**) unequivocally showed a higher surface coverage on the surface of the Ti6Al4V thin film.

Therefore, the surface of this specific thin film demonstrated, by the *in vitro* tests, to be the most promising for the osseointegration of medical invasive devices that contact with hard tissue. The presence of V in the thin film seems to be essential for the better performance of the Ti alloy, when in comparison with the Ti6Al thin film. Also, due to its nanostructured structure, Ti6Al4V thin films outperform the chemical mimetic bulk material.

The second example of applications of thin films for medical applications is also based on work developed in our laboratory and never published before and is

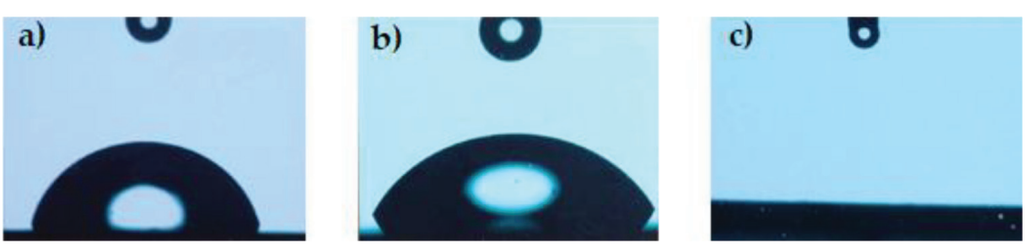

Figure 1.
Contact angle between a drop of water and Ti6Al4V bulk material (a), Ti6Al thin film (b) and Ti6Al4V thin film (c).

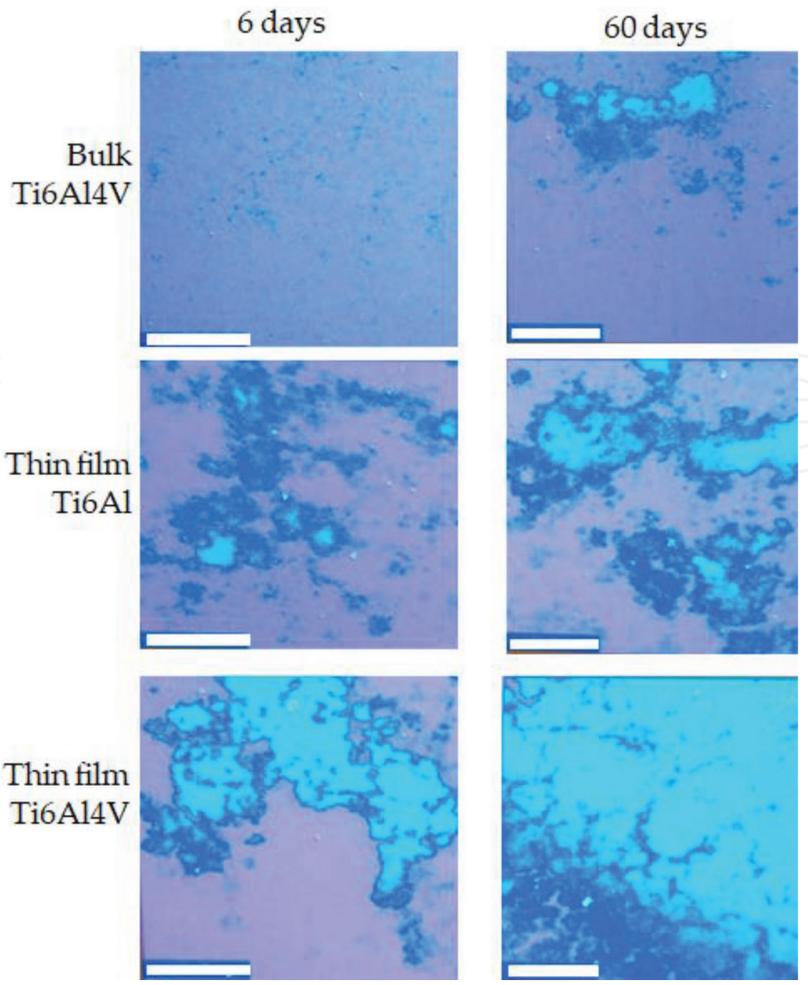

Figure 2.
Elemental map distribution of calcium (light blue) on the surface of the modified and unmodified bulk Ti6Al4V bulk implant after 6 and 60 days of immersion in Hanks' solution (bar = 80 μm).

related with neural implants. Some examples of the use of materials in neurosciences include the monitoring of intracranial pressure, the regeneration of nervous tissue of the medulla region, for controlled drug delivery systems and microelectrodes for the nervous system. In the latter case, the devices may be used to increase or decrease a certain neurological function. Focusing of this type of application, one of the major problems in this field of Biomedicine is the lack of mechanical and biological compatibility between the tissue and the materials used as implants: silicon, tungsten, gold, iridium and stainless steel. In fact, although tests carried out in accordance with ISO 10993 have demonstrated the noncytotoxicity of the above-mentioned materials, the results of *in vitro* and *in vivo* experiments show a very different reality. The development of a glia scar around the nervous tissue inhibits, after few weeks, the stimulation and recording of neurons. Several examples are present in the literature of research addressing this problem by modifying the surface of neural electrodes by different strategies [19–22], but mostly with polymeric materials.

Our work addressed this issue by modifying silicon neural implants by sputter depositing hybrid nanostructured thin films of silica, gold and silver (SiO_2/Ag/Au) [23]. SiO_2 was chosen due to its chemical compatibility with the base material thus preventing the lack of adhesion between substrate and thin film. The nanocomposite system was designed to induce the release of one metal, which has been described as having antimicrobial properties, silver. In order to ensure that silver was oxidized, gold was added.

Several chemical compositions were tested and characterized in order to select the best one for the in vitro tests with prokaryotic and eukaryotic cells. If was confirmed, by transmission electron microscopy (TEM) characterization, the nanocomposite nature of the hybrid deposited thin films (**Figure 3**). They all consisted of an amorphous silica layer with dispersed nanometric size metal grains.

All the films presented a mean surface roughness, determined by atomic force microscopy, lower than 20 nm and hardness values, determined by nanoindentation, of around 2 GPa. The thin film with the chemical composition (in atomic %) of Si = 25.8; O = 53.4; Ag = 9.6 and Au = 11.2, was chosen for the remaining characterizations and the biological tests. This surface presents a static contact angle with water of 26° and a zeta potential value of −70 ± 4 mV.

The first in vitro tests were made with *Pseudomonas aeruginosa* bacteria in order to test the antimicrobial activity of the developed hybrid nanostructured tin film. After 24 h of incubation, the halo inhibition test (**Figure 4**) demonstrated that neither silicon (result not shown), nor SiO_2 presented any antimicrobial effect. On the contrary, the SiO_2/Ag/Au thin film presented a 4 mm inhibition halo indicating that the Si modified surface was able to inhibit the growth of these strain of bacteria, which is considered one of the most harmful in nosocomial related infections.

The tests with rat embryo cortex cells, within the conditions usually used for this type of tests in neurosciences, and after 15 days of incubation, the cells were prepared for the immunocytochemistry tests. The observation in an optical fluorescent microscope (**Figure 5**), where the neurons nuclei are marked in blue, showed that unmodified silicon presents a very poor cell density, while SiO_2/Ag/Au presented

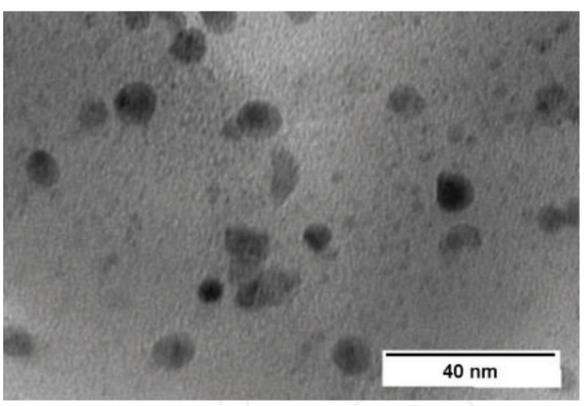

Figure 3.
Bright field TEM micrograph of a SiO_2/Ag/Au hybrid nanocomposite thin film.

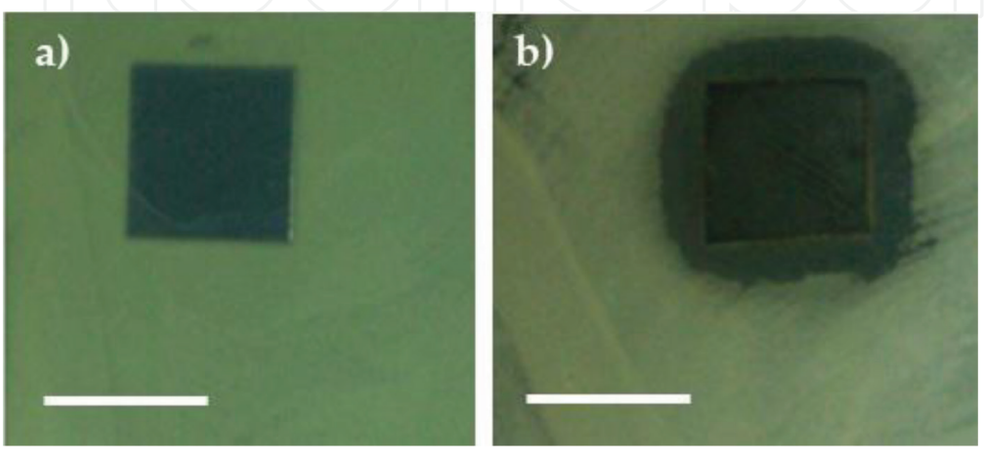

Figure 4.
Optical micrographs of the inhibition growth halo tests, with P. aeruginosa, of silicon modified with SiO_2 (a) and SiO_2/Ag/Au (b) thin films (bar = 10 mm).

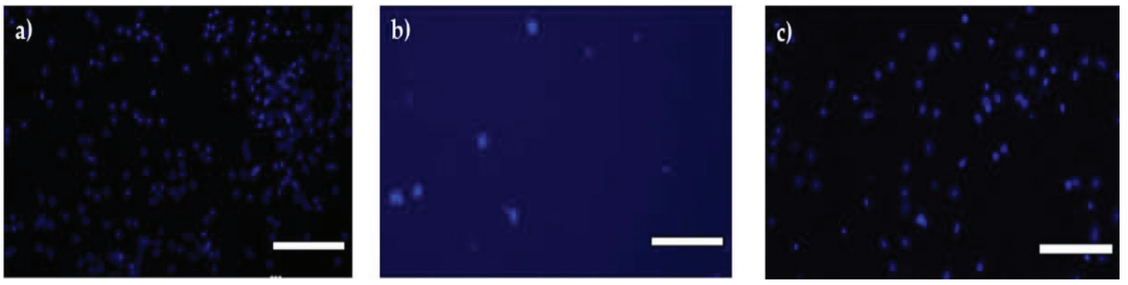

Figure 5.
Optical fluorescent micrographs of rat embryo cortex cells after 14 days of incubation with polylysine (a), silicon (b) and SiO_2/Ag/Au (c) surfaces (bar=200 μm).

a higher cell density, almost similar do the one observed on the "gold standard" of these type of tests, polylysine.

3.2 Environmental applications

Environmental degradation is a very challenging field. Natural resources are being enormously harnessed and their stock is exhausted every day. Moreover, the ecological balance is disturbed and the natural resources are being polluted.

For the removal of contaminants from the environment, several strategies are available. In the past, classic remediation strategies were mainly used. An example of innovation for sustainable development is to use alternative remediation strategies as bioremediation. Although the Romans already used organisms to clean their wastewater in 600 BC, it was only after 1972 that the first official bioremediation strategies were tested.

Microbial responses to metals with potential for bioremediation include chelation, compartmentalization, exclusion and immobilization [24]. The use of these bacterial abilities is an important strategy for the development of the bioremediation tools. Often, their application to the environment requires the immobilization of the cells to obtain an effective bioremediation. Bacterial cells can be immobilized in different supports. Briefly, cells can be immobilized by covalent coupling, affinity immobilization, adsorption, confinement in liquid-liquid emulsion, capture in a semipermeable membrane and entrapment in polymers [25].

When genetically modified organisms are used, their covalent immobilization is mandatory in order to prevent their release into the environment. However, the covalent immobilization of the cells always requires that the biological entities keep their metabolic abilities, useful for bioremediation. Moreover, immobilization of microorganisms presents several major advantages such as: (1) continuous supply of nutrients without competition with other microorganisms [26]; (2) increase of biomass of specialized microorganisms with specific desired metabolic activity [27, 28]; (3) protection of the cells against the environmental stress, toxic chemicals and UV irradiation [27, 29]; (4) easier control of the bioprocess [29] and (5) easy, cheap and feasible technique to be used since the immobilized microorganisms can be used several times without significant loss of activity [30].

The use of thin films in the design of biotools for environmental application is here illustrated by two case studies. The first describes the development of an arsenic biofilter [31]. The environmental contamination with arsenic is a worldwide problem, and the development of new strategies for arsenic removal is an ever ongoing research. In this study, genetically modified bacteria able to accumulate arsenic were generated by modification of the arsenic resistant strain *Ochrobactrum tritici* SCII24T [32]. The *O. tritici* As5 mutant cells, exhibiting a high percentage of arsenite accumulation, were immobilized on a commercial polyethylene (PE) net after sputtered

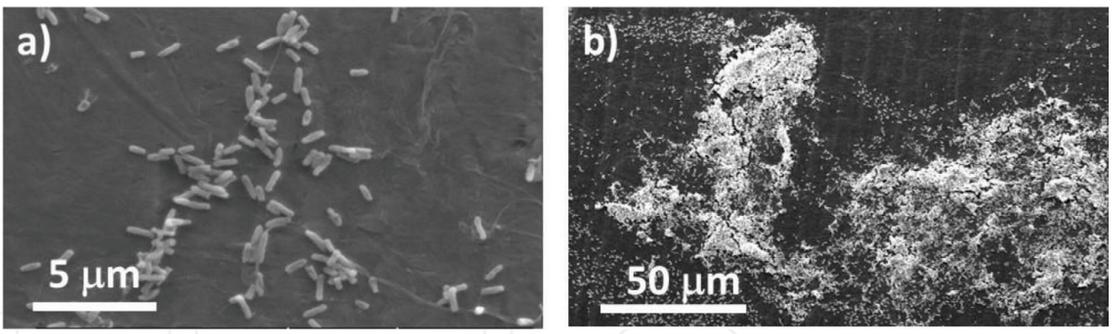

Figure 6.
SEM micrographs of immobilized cells of O. tritici mutant As5 onto PTFE thin films showing an early stage after inoculation (a) and the development of the biofilm (b).

modified by the deposition of poly(tetrafluoroethylene) (PTFE) thin films, with approximately 500 nm thick. Different PTFE thin films were tested regarding their ability to immobilize cells in an active form that enabled the microorganisms to form a biofilm and work as a biofilter for arsenic. The surface that exhibited a mild zeta potential value, hydrophobic characteristics, the lowest surface free energy but with a high polar component and the appropriate ratio of chemical reactive groups showed to be the optimal for bacteria development (**Figure 6**). The immobilized cells maintained their ability to accumulate the surrounding arsenite.

The second case study describes the application of thin films for bacterial cells immobilization in the construction of a biofilter for the removal of uranium from contaminated water. The biofilter was constructed by the immobilization of the strain *Rhodanobacter* A261 onto the PTFE thin films. Under batch conditions, low nutrient content, pH 5 and different uranium concentrations, this strain was able to remove approximately 120 µM of U(VI) when grown aerobically in the presence of 500 µM U (**Figure 7**). As described earlier, the strain was inoculated in PTFE thin films deposited with different deposition parameters, which led to surfaces with distinct values of zeta potential, hydrophobic characteristics, surface free energy, polar component and ratio of chemical reactive groups.

After being evaluated for their ability to immobilize uranium, the optimized PTFE thin film was used to modify PE commercial nets and, after bacterial cells immobilization, the filters were applied in a wastewater treatment plant from a uranium closed mine (Urgeiriça, Portugal). The filters were submerged during 3 months in the last tank of the wastewater treatment station. After 1 and 3 months, they were removed, and their ability to be colonized (biomass in the filter) and uranium detection (**Figure 8**) was evaluated.

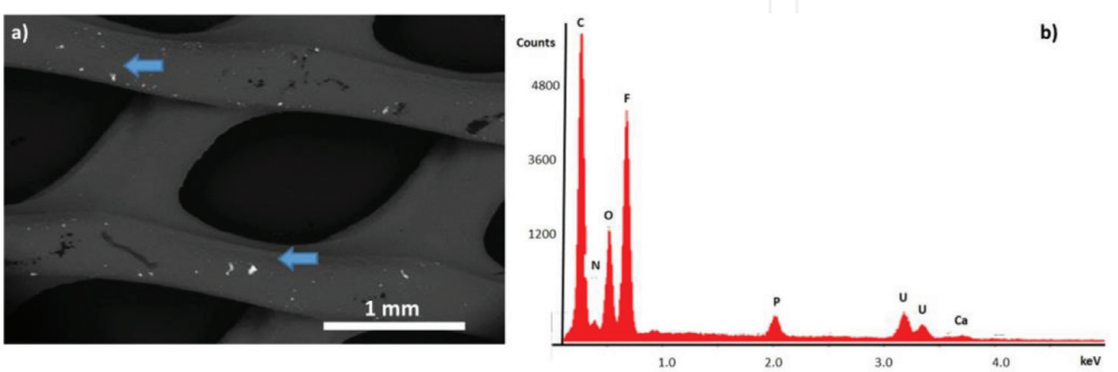

Figure 7.
The backscattered mode SEM micrograph (a) showed the presence of U (brighter spots) and the qualitative energy dispersive spectroscopy (EDS) (b) confirmed uranium detection in PTFE thin film of Rhodanobacter A2-61 microcolonies after being in contact with a 500 µM uranium solution.

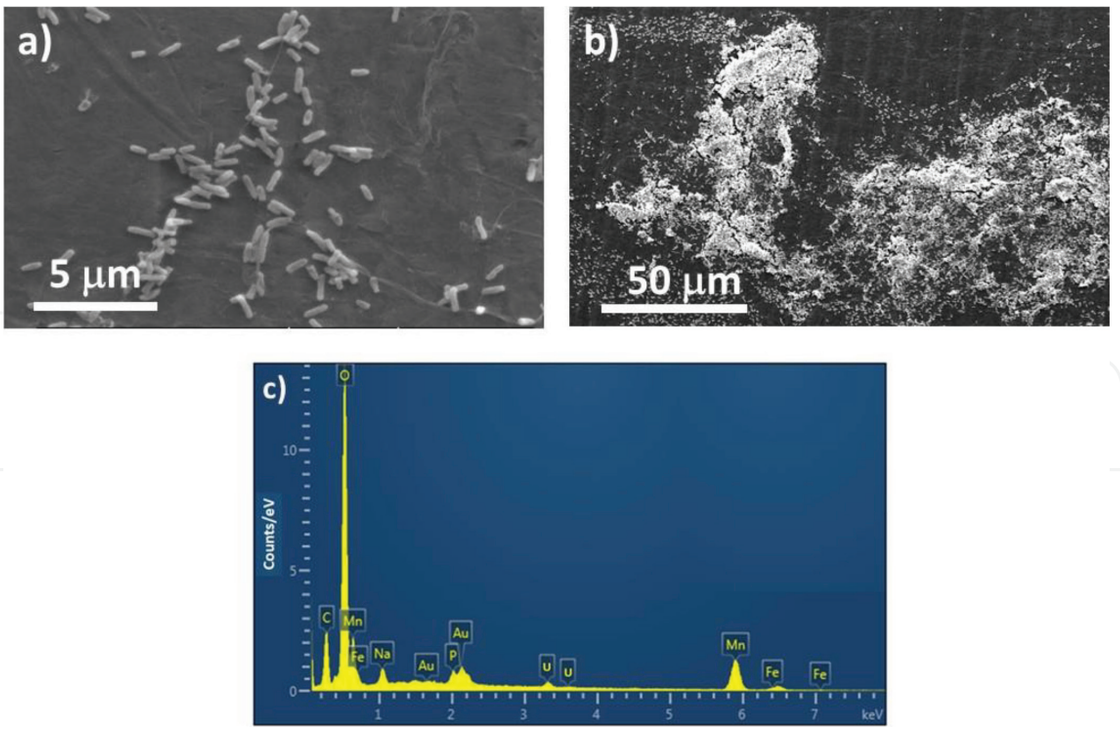

Figure 8.
SEM micrographs of noninoculated (a) and inoculated (b) PTFE thin films with Rhodanobacter A2-61 after 3 months submerged in a mine wastewater passive treatment plant; EDS analysis (c) inoculated surface of the region marked with blue arrow in (b).

The thin films demonstrated its ability to immobilize the cells and stabilize a thick biofilm which was able to be metabolic active in the remediation of metals. Although after 3 months all filters showed microbial colonization, from native species, a difference in thickness and structure was visible between inoculated and noninoculated biofilters. The presence of the thin film showed to be essential for the immobilization of *Rhodanobacter* A2-61 that, in turn, was mandatory for U accumulation. In fact, only these biofilters showed U immobilization. Besides the uranium, other metals were detected in the biofilm, such as Mn and Fe.

4. Conclusions

In this chapter, the production of thin films by sputtering and their uses in medical and environmental fields were exemplified. The ability of the sputtering technique to produce metastable materials, which are not predicted by the traditional chemistry thermodynamic, allows to produce tailor-made surfaces that present the optimized set of properties for specific applications.

A nanostructured Ti6Al4V thin film showed to have better osseointegration properties than in its bulk form. Also, the presence of vanadium proved to be essential to achieve the best performance.

The modification of silicon, used as neural implant, with a hybrid nanocomposite thin film of SiO_2/Au/Ag demonstrated to induce a surface that provides a better adhesion and proliferation of rat embryo cortex cells concomitantly with an antibacterial effect against some of the bacteria responsible for nosocomial infections.

The deposition of PTFE thin films, with optimized surface properties, onto the surface of a commercial PE net allowed to develop biotools for the bioremediation of two elements considered very hazardous both for humans and environment: arsenic and uranium.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Ana P. Piedade[1*], Francisco Romeu[2], Rita Branco[2] and Paula V. Morais[2]

1 CEMMPRE—Department of Mechanical Engineering, University of Coimbra, Coimbra, Portugal

2 CEMMPRE—Department of Life Sciences, University of Coimbra, Coimbra, Portugal

*Address all correspondence to: ana.piedade@dem.uc.pt

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