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Comparison of Two Different Plasma Surface-Modification Techniques for the Covalent Immobilization of Protein Monolayers

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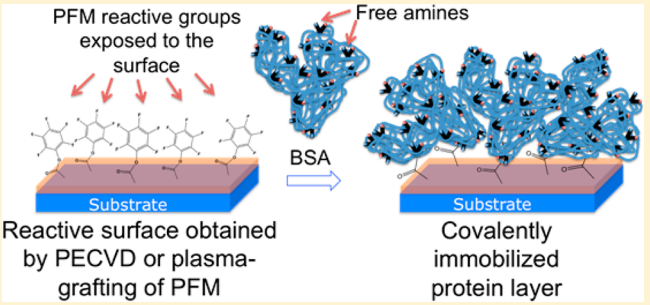
ABSTRACT: The immobilization of biologically active species is crucial for the fabrication of smart bioactive surfaces. For this purpose, plasma polymerization is frequently used to modify the surface nature without affecting the bulk properties of the material. Thus, it is possible to create materials with surface functional groups that can promote the anchoring of all kinds of biomolecules. Different methodologies in protein immobilization have been developed in recent years, although some drawbacks are still not solved, such as the difficulties that some procedures involve and/or the denaturalization of the protein due to the immobilization process. In this work, two different strategies to covalently attach bovine serum albumin (BSA) protein are developed. Both techniques are compared in order to understand how the nature of the surface modification affects the conformation of the protein upon immobilization.



INTRODUCTION

The biocompatibility of materials for food packaging, bioengineering, or biomedical applications is closely related to the interaction between the material surface with the biological medium where it is applied.[1−3] Therefore, it is essential that the used material presents the proper conditions in order to develop its general function, but it also needs to have the optimal surface conditions to enhance the biointeraction with the surrounding media.[4,5] Thanks to the possibility of the material surface to be tuned in order to obtain a grater response on the biointerface whatever the final application is, surface engineering becomes a crucial tool for fabricating novel biomaterials.[2]

Because it is widely accepted that all biomaterials need a surface able to interact with the biological media surrounding it, a previous treatment has to be used. This unique technology is known as surface modification and involves physical or chemical binding of different biomolecules such as proteins, enzymes, growth factors, viruses, and bacteria or genetic material.[2,4] After this process, the obtained surfaces are known as smart, bioactive surfaces on which can be carried out all kinds of sophisticated interactions with the biological entities, including biologically triggered release, selective adhesion, and conformational control.[2,5]

From all of the existing surface-modification techniques, the ones that allow a covalent immobilization of the biomolecule of interest are the most convenient thanks to the high selective capacity of binding that these techniques can offer. Thus, the nonspecific immobilization of undesired biomolecules and uncontrolled interactions can be avoided.[4,6]

To successfully immobilize the desired biomolecules to fabricate these bioactive surfaces, a previously used treatment of the surface material is frequently required. During the last few decades, plasma polymerization approaches have been optimized as a previously used surface treatment to achieve functionalized surfaces, thanks to its capacity to modify all kinds of materials, even inert ones, and its ability to do so to all different geometries.[7−9] However, a majority of these techniques lead to a polymer thin film that needs prior activation before the biomolecule of interest can be immobilized, resulting in a multistep modification.[10,11] In our group, the expertise in PECVD surface modification allowed the development of polymer thin films by using a monomer that, when polymerized, leads to a reactive group exposed on the surface.[8,12] This monomer, pentafluorophenyl methacrylate (PFM), is activated by plasma excitation and it is able to form thin films on the desired surface. Its exposed ester groups are highly reactive toward amines, which makes it an optimal candidate for achieving extremely active surfaces for selected species.[8,12−14] Thanks to both parameters, the polymerization technique and the monomer used, we obtained a versatile approach to fabricating all kind of bioactive surfaces capable of covalently immobilizing proteins in a tunable way. In this work, we optimized two techniques, PECVD and plasma grafting of PFM, in order to attach biomolecules in a specific way where their activity is maintained. To do so, a widely known protein such as bovine serum albumin (BSA) was used, and its conformation, when anchored on the surface, was carefully analyzed. Each approach differs in the resulting bioactive surface properties and the behavior of the proteins interacting with them, which make them an extremely interesting tool for

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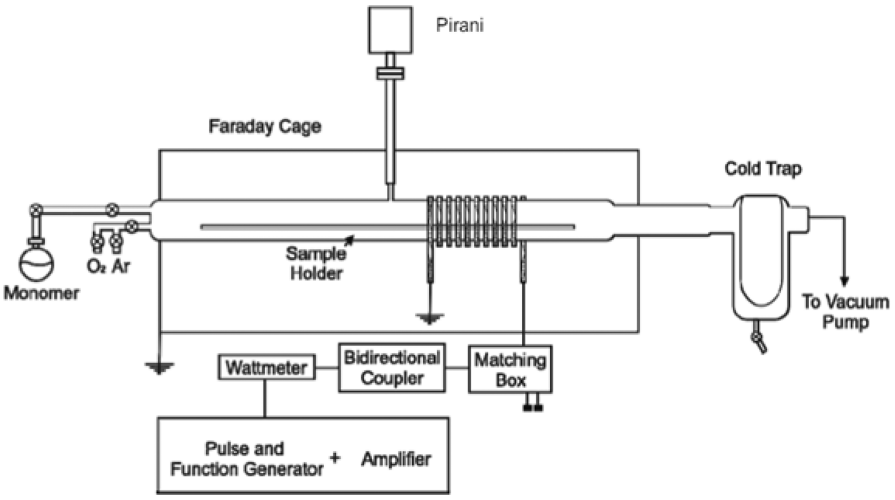

Figure 1. Schematic diagram of the plasma reactor and its electrical components.

immobilizing proteins in a desired way regarding the final application of the material.

EXPERIMENTAL SECTION

Plasma Reactor. Both types of plasma modification were carried out in a homebuilt plasma reactor (Figure 1). Plasma treatment was performed by using an excitation frequency of 13.56 MHz. Gases that were fed through the system pass through a glass, CO_2/acetone-cooled trap for the collection of excess reactant before reaching the pump (Edwards RV12 903). An analogical Pirani-type vacuum meter (MKS, USA) was connected near the middle of the reactor chamber to monitor the reaction pressure. In a homebuilt system, the pulse generator controlled the pulsing of the radio frequency signal, which was amplified by a 150 W amplifier and passed via an analogue watt meter and a matching network to a 10-cm-long coil located around the exterior of the reactor. The typical base pressure prior to all experiments was 2×10^{-2} mbar. PFM monomer vapor was introduced at a constant pressure of 1.5×10^{-1} mbar via a needle valve. The reactor's inner volume is approximately 3 L, and the effective plasma volume is about 1.7 L.

Plasma Polymerization. Polystyrene strips (Servei Estació, Barcelona; 10 × 10 mm^2 area; PS) were modified with pentafluorophenyl methacrylate (Apollo Scientific, PFM) by plasma enhanced chemical vapor deposition (PECVD). When the substrates were placed in the previously described plasma reactor, the PFM monomer flask was opened until reaching the desired pressure. The continuous rf power was fixed at 15 W, and pulsed plasma polymerization (duty cycle 10/20) was carried out for 5 min. Plasma discharge was then turned off, and the PFM vapor flow was kept constant for 15 min more. After the polymerization process, samples (pPFM) were carefully removed from the reaction chamber and stored until further use.

The micropatterning process was carried out by the modification of the substrate with a cooper grid (G50P, Gilder Grid) previously colocated on its surface. The cooper grid was carefully placed on the desired part of the substrate guaranteeing full contact between the surface and the grid. The sample was then treated with either PECVD or plasma grafting.

Plasma-Grafting Modification. Polystyrene strips (Servei Estació, Barcelona; 10 × 10 mm^2 area; PS) were modified with PFM by plasma grafting in the same plasma reactor described previously. The substrates were first activated by argon plasma (Carburos Metálicos 5.0), which entered the reactor at a constant flow of 45 sccm at a pressure of 7.5×10^{-2} mbar. The continuous rf power setting was 15 W and was carried out for 15 min. After surface activation, the plasma was turned off and the Ar gas was closed. The PFM monomer flask was then opened until reaching the desired pressure for 15 min. When the grafting procedure was finished, samples were removed from the reaction chamber and stored for subsequent characterization.

Modified Surface Characterization. The modified surfaces were characterized by the water contact angle (DSA100, Krüss; WCA), atomic force microscopy (XE 100 Park System; AFM), and fluorescence microscopy (AxioVs40, Zeiss Imaging Solutions). To analyze the surface modification by WCA and AFM, both modified PS substrates were directly analyzed after polymerization. The fluorescence assay was performed on the modified PS strips by using fluorescein-5-tiosemicarbazide (Fluka, FTSC) as a fluorescent dye. All analytical techniques were also carried out on nonmodified surfaces to confirm the changes achieved with respect to its behavior (data not shown).

Quartz Crystal Microbalance with Dissipation (QCM-D). QCM-D technology (Q-Sense E1, Sweden) was used to characterize the immobilization process of bovine serum albumin (Sigma-Aldrich, BSA).

This technique has been proven to be suitable for in situ dynamic studies of both mass and mechanical properties by monitoring the frequency (f) and dissipation (D) of an oscillating sensor.[6,15,16] The sensor used consists of a piezoelectric quartz crystal sensor (Q-Sense) with polished gold electrodes that has an rms roughness of less than 3 nm, a diameter of 13 mm, and an effective area of 5 mm diameter. An increase in the attached mass on the sensor's surface leads to a decrease in the frequency signal (Δf) thanks to the Sauerbrey relation (eq 1), which shows that the adsorbed mass (Δm) is directly proportional to the frequency shift, where the mass sensitivity constant is known as C (17.7 $ng \cdot cm^{-1} \cdot Hz^{-1}$ for a 5 MHz sensor) and n (1, 3, 5...) is the overtone number.

$$\Delta m = -\frac{C\Delta f}{n} \tag{1}$$

Moreover, insight into the viscoelastic properties of the film can be inferred from the dissipation signal obtained from the oscillating sensor, and it is defined as the ratio of the dissipated to the stored energy (eq 2).

$$D = \frac{E_{\text{dissipated}}}{2\pi E_{\text{stored}}} \tag{2}$$

In this work, the QCM-D technology was used to carry out a real-time study of the interactions between the polymerized PFM film and the BSA and measure the mass and structural properties of the protein film covalently attached to the surface and achieve deeper insight into the viscoelastic properties of the resulting protein layer. This information can be used to examine the protein monolayer that is formed and how the immobilization affects the BSA conformational form.

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The sensors purchased were coated with a thin film of gold or PS and were modified with PFM by the two different techniques analyzed in this study, PECVD and plasma grafting, respectively. When the sensor was correctly placed in the QCM-D chamber, a baseline with phosphate-buffered saline (PAA Laboratories, PBS) was obtained for 5 min, and the 20 $\mu g \cdot mL^{-1}$ BSA solution in PBS was pumped through the sensor for 25 min. The excess free BSA was removed by PBS flow for 10 min, and a final cleaning with 10 mM SDS ensured that the BSA remaining on the sensor surface was covalently attached.[17] The BSA affinity toward the nonmodified gold and PS sensors was also checked as a comparative control (data not shown). All QCM-D analysis was performed at 25 °C whereas the previously degassed solution was pumped at a flow rate of 50 $\mu L \cdot min^{-1}$.

RESULTS AND DISCUSSION

Plasma-Modified Surface Characterization. Plasma grafting of PFM to PS substrates led to a considerable decrease in the contact angle of the original PS. The nonmodified PS had a contact angle of 83°, which decreased to 52° after plasma-grafting modification (Figure 2). This was an unexpected value

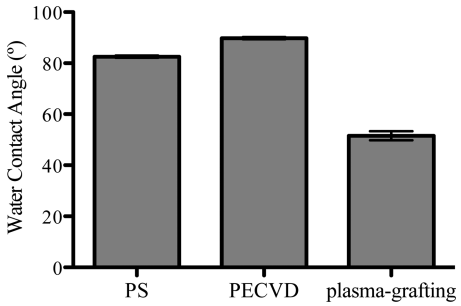

Figure 2. Water contact angle of the bare PS in contrast to the same substrate but modified by the two different plasma techniques: PECVD and plasma grafting.

considering the hydrophobicity observed on the PFM films polymerized by PECVD shown in other work and confirmed here in Figure 2 (90°).[8,12,18] These studies have proven that the fluorinated ring from the PFM remains exposed to the surface, giving the hydrophobic behavior noticed when it is polymerized under such conditions. Then, when a PFM thin film is obtained by PECVD, the resulting surface is highly reactive because of the large number of exposed PFM groups achieved and thus the surface acquires hydrophobic properties.

Nevertheless, when PFM is grafted on the polymeric surface by plasma grafting it forms a polymer brush layer, as can be interpreted from the AFM images taken (Figure 3). When the surface is activated by plasma in the presence of Ar, an etching effect is produced together with the creation of free radicals.[10] These are needed for the formation of the PFM brushes when the monomer comes into contact with the activated surface and are responsible for the obtaining of a highly hydrophilic surface. However, the PFM grafting was not enough to achieve the hydrophobicity observed for the PFM films polymerized by PECVD. The polymeric brushes did not cover the total surface as confirmed by the AFM data; therefore, the smaller number of PFM reactive groups present on these types of surface were not enough to decrease substantially the hydrophilicity achieved during the plasma treatment.

During the activation procedure in the first step of the plasma-grafting modification, active sites are generated on the surface, and when PFM is exposed, the polymer chain starts growing from the surface.[19] This is why by this technique polymer brushes of PFM were formed according to the AFM image shown in Figure 3C. Thus, as has been stated before, the grafted surface may then provide less and more spread out active sites for the binding of protein molecules but in a more hydrophilic environment, which may avoid remarkable conformational changes of the protein structure.[20] This fact is especially interesting when an active biomolecule, such as an enzyme, is immobilized on a surface given the importance of maintaining its conformation and ultimately its activity.[1]

The AFM analysis performed for all samples showed different topography from the original PS strip to the modified samples.

When PFM polymerization was carried out by PECVD, a homogeneous thin film was obtained, as can be seen in Figure 3B and as our group has reported before.[8,12] The roughness of these modified substrates was typically around 25 nm. However, as already described, the PFM brushes formed during the plasma-grafting modification technique can be inferred from the AFM image shown in Figure 3C. Surfaces obtained by this method had a roughness of between 7 and 11 nm, just slightly higher than that of bare PS (4 nm) as expected from the low coverage of the PFM brushes. Briefly, as shown by the values, the grafted surfaces were flatter than the PECVD films. These results can be explained by the existing interaction between the groups' PFM during polymerization, which causes the formation of clusters, increasing the final surface roughness.[21]

Surface Functionalization. The modified substrates were also characterized by fluorescence microscopy by using FTSC, which is a fluorescent molecule with a free amine on its structure and is able to react with the PFM group on the surface.[8,13] This method provides qualitative tracking of the reaction between the activated surface and the amine of interest and enables the confirmation of PFM reactivity toward primary amines and shows the homogeneity of the coatings achieved by these techniques. Before the plasma modification was carried out, a Cu grid was placed on the surface of the substrate in order not only to confirm the modification but also to test the micropatterning viability that this approach may offer (Figure 4).

After several cleaning steps with Milli-Q water, the fluorescence signal remains and thus the covalent union of FTSC on the PS surface is confirmed. Although the Cu grid was not able to avoid the modification of some areas of the surface under it, especially in Figure 4A, the major fluorescence signal observed was on the parts exposed to the previous modification, meaning that the FTSC molecules are able to react with the polymerized (Figure 4A) or grafted PFM (Figure 4B) on the desired surface. As a result, this approach opens a wide range of possibilities to achieve easily micropatterned surfaces for biomedical and biosensor applications.[2,3]

Protein Immobilization. At this point, the reactivity of the surfaces was tested only in front of amines present in small molecules, such as the fluorescent FTSC dye; however, the attachment of bio- and macromolecules on the surface was needed to achieve bioactive surfaces.[3] Thus, not only did the capacity to immobilize proteins on the modified surfaces by both techniques have to be evaluated but also how these biomacromolecules are covalently attached to the surface needed to be studied.

QCM-D is a well-suited technique for this purpose and is the one chosen in order to develop this study.[6,16] Then, the immobilization of BSA was monitored in situ using the QCM-

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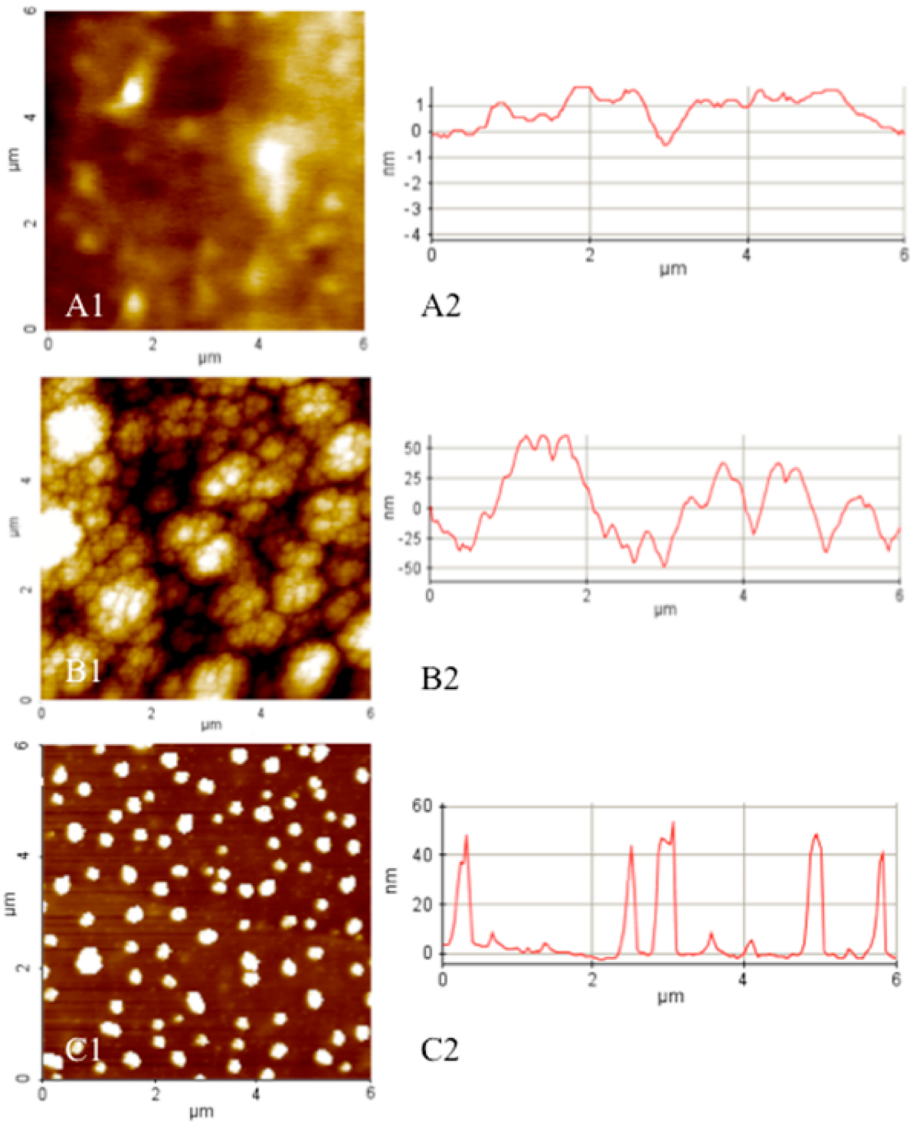

Figure 3. (1) AFM 2D image and (2) topographic profile of the (A) nonmodified PS samples, (B) PFM polymeric thin films obtained by PECVD, and (C) polymer brushes of the grafted PFM by argon plasma grafting.

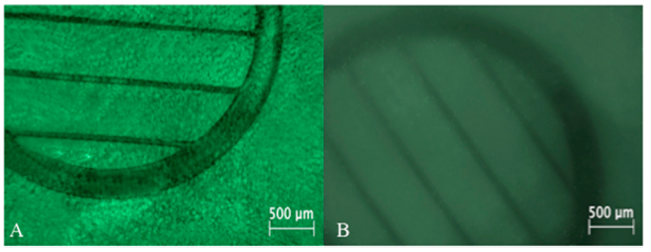

Figure 4. PS samples functionalized with FTSC after (A) PFM polymerization by PECVD and (B) PFM grafting.

D technology. Only subtle changes in ΔD were observed on BSA-coated surfaces via both PECVD and grafting-modification techniques. However, it indeed caused a significant frequency shift (Δf), which can be directly related to the increase in mass on the sensor's surface as a result of the attachment of the BSA (Figure 5). Nevertheless, this frequency profile varied depending on the surface modification previously carried out. This is the main reason that deeper insight into the frequency signal is obtained.

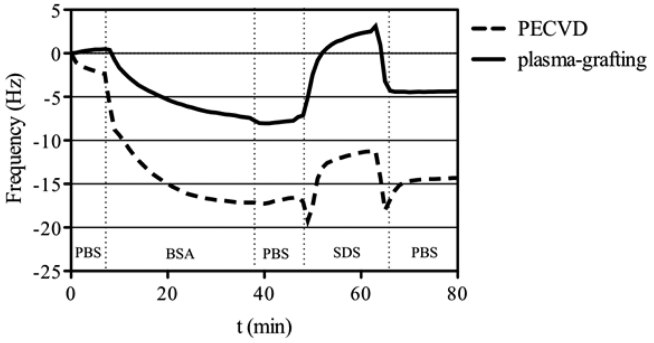

Figure 5. QCM-D data of the frequency shift (Δf) observed during the BSA immobilization and subsequent PBS and SDS cleaning on both types of surfaces. The stabilization with PBS (baseline) of the PFM film on the PECVD samples caused a small drift that was taken into account when the final quantification was carried out.

Initially, a baseline was obtained with PBS for 5 min in order to stabilize the surface-coating polymer.

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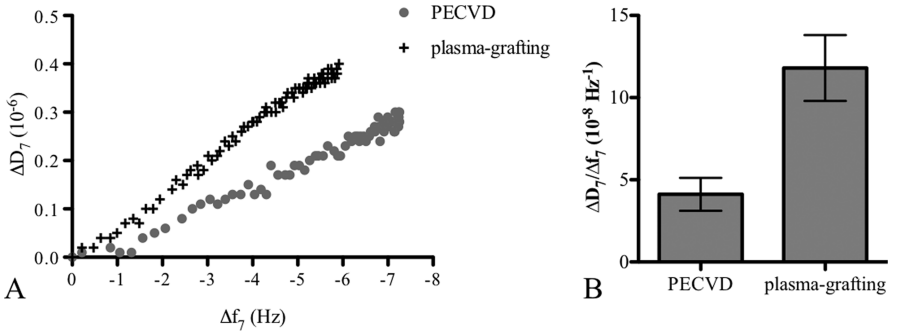

Figure 6. (A) Plot of the variation of the dissipation (ΔD) vs the frequency shift showing a higher increase in the viscoelastic properties per mass unit attached on the gPFM-modified surface. (B) The final value of the $\Delta D/\Delta f$ ratio confirmed that the protein layer formed by PECVD was more rigid and thus had lower water content.

When the BSA comes into contact with both modified quartz crystal sensors, the result is expressed as a shift in their resonance frequencies. It can be seen how the frequency decreases rapidly in the PECVD-modified sensor and how, after 30 min, the signal is stabilized at around −17 Hz (Figure 5). In contrast, the frequency variation on the crystal modified by plasma grafting is much slower, suggesting that it is capable of immobilizing less than a half of the protein (−7 Hz approx.) when compared to PECVD. These results confirm that greater amounts of active PFM and thus higher reactivity are achieved when PECVD is used. Therefore, the final application of the modified surface will indicate which of the plasma treatments should be carried out. This has to be pointed out, as will be explained later, because the different reactivity of the surfaces toward proteins may in some cases cause a conformational change on the protein structure, probably affecting its final activity.[20]

A final cleaning with SDS and PBS removed all of the unbounded BSA, which ensures that a protein monolayer has been obtained and the nonspecific interaction has been removed from the surface. The frequency data obtained was then analyzed and converted to the consequent mass value by using eq 1, which is valid only for rigid layers as is the case for the study carried out after obtaining an almost negligible variation in the dissipation signal for both surfaces. As a result, the surface coated with pPFM by PECVD was able to immobilize 210 ng·cm^2 and the grafted PFM can covalently bind only 115 ng·cm^2. This difference can be attributed to the different densities of active sites present on the modified surfaces, as has been previously described (Figures 2 and 3). During the plasma-grafting process, when the polymer brushes are formed, fewer molecules of free PFM are available to react with the proper amine. Instead, the polymeric film obtained via the PECVD process is much more reactive, resulting in a greater number of covalent linking sites to react with the protein. Thus, the protein layer formed by PECVD modification is more dense than the obtained when the plasma-grafting technique is carried out. However, this greater attachment to the surface achieved with the pPFM films and the high hydrophobicity may affect the protein structure and cause its denaturalization, which will consequently have a negative influence on its activity. This fact is particularly undesirable when a smart, bioactive surface is required.

Protein-Layer Characterization. An alternative way to analyze the data obtained by QCM-D and plotted in Figure 6A is to study the variation of the dissipation versus the frequency shift.[6,15,22] By this method, the parameter time is eliminated from the plot and a direct comparison of the energy dissipation (ΔD) per mass unit attached to the surface (Δf) is possible. Thanks to this relation, the influence that the protein absorption on the surface has on the damping of the sensor oscillation due to viscoelastic effects can be immediately determined. Then, not only the viscoelastic properties of the protein layer formed but also the conformation of how the protein is immobilized on the surface can be deduced.

The variation of the viscoelasticity per mass unit of adsorbed protein is higher when the BSA reacts with the PFM brushes obtained by the plasma-grafting modification. This behavior would suggest that the formed protein layer in these surfaces is less rigid than the BSA linked on the pPFM, resulting in a more viscoelastic film.

To gain a deeper understanding of the viscoelastic properties of the protein layer obtained by these two techniques, its water content needs to be analyzed. The trapped water on the formed protein layer can be determined by the final value of the $\Delta D/\Delta f$ relation, which decreases with the rigidity of the protein film. In other words, the lower this value is, the lower water content the BSA adsorbed layer has, thus offering less viscoelastic behavior. However, it has to be considered that this tool does not give quantitative values of the water present on the film but still gives satisfactory information about the system in order to make the appropriate approximations in terms of viscoelastic properties.

By plotting the two values corresponding to both modification techniques (PECVD and plasma grafting) discussed here, it can be seen that the rigidity of the BSA layer formed on the gPFM sensors is significantly lower than when the protein is attached to the pPFM film (Figure 6B). Thanks to that data, the more viscoelastic behavior of the protein layer obtained by plasma grafting can be confirmed whereas that formed on the PECVD-modified sensors is more dense, rigid, and flat.[22] Moreover, the lack of water retention in the BSA structure achieved on the pPFM-modified surfaces may induce a conformational change of the protein, as has been previously discussed.

Study of the BSA Conformation Acquired When Attached to the Modified Surfaces. As previously mentioned, protein immobilization on a surface can cause a conformational change in its structure.[20] It is known that BSA has a high affinity for hydrophobic surfaces, which can induce a deformation because of the exposure of its hydrophobic domains located inside its structure.[23] As a result, BSA's structure can evolve from the native form (N form), which corresponds to the folded form and is found under normal

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conditions, to the expanded form (E form), which is totally denaturalized and inactive. However, between these two, there is a third form called fast (F form) in which the two main domains of the protein are partially unfolded, but it preserves part of its activity.[23,24]

Given the differences in hydrophobicity seen on both surfaces studied here, it is particularly interesting to analyze the final conformational structure by which BSA is attached to each type of surface. To do so, a calculus of a protein monolayer by using its footprint is needed (Table 1). Each possible form adopted by the BSA has known dimensions described elsewhere,[23,24] including the native form, which has been measured by molecular dynamics (Gromacs v.4.0) and dynamic light scattering (DLS). Both measurements include the water content of the protein by giving the hydrodynamic radius.

Table 1. Both Calculated Footprints and Theoretical Protein Mass Expected To Be Immobilized for Each Type of BSA Conformation

	N form	F form	E form
footprint (nm^2)	80	43	36
theoretical mass ($ng{\cdot}cm^{-2}$)	140	258	302

It has to be considered that it is unlikely to obtain a full coating of the protein on the surface because of the fact that the first contact of the biomolecule with the surface is totally random. Moreover, because it is an irreversible interaction, some dead spots might be produced, where any protein would be able to access. However, to avoid that, the BSA concentration used in this experience was high enough to ensure the maximum coverage of protein on the modified surfaces.

Having said that, it is statistically difficult to obtain a protein layer formed mainly by BSA in the E form because it would mean that the immobilization yield obtained on both surfaces regarding the theoretical mass calculated is much lower than what would be expected given the highly reactive PFM chemistry shown in previous work.[8,12]

On the PECVD-modified surfaces, the difference between the theoretical mass obtained if the BSA attached to the surface and the actual mass detected by the QCM-D technology is only in its N form is around 30%. That would suggest that the rest of the mass that is quantified experimentally could be attributed to the water content of the protein layer. This amount of water trapped on a protein coating is typically expected when viscoelastic proteins such as a mussel adhesive protein (Melp-1) is used.[25] This is not the case when using BSA for immobilization, which usually forms rigid layers with low water contents.[6] Thus, these results suggest that BSA undergoes a conformational change when it reacts with the pPFM deposited by PECVD, most likely to the F form. The large number of accessible lysines, the amino acid in charge of reacting with the PFM labile group and present in a large quantity in the BSA structure, may cause the partial unfolding of the protein as a result of the different tensions created around its structure during the immobilization process. This phenomenon is also favored by the hydrophobic nature of the pPFM coating previously discussed, which may induce a conformational change.[20] Both effects are especially crucial when protein units are first immobilized on the surface, but as soon as different BSA molecules cover it, the hydrophobic forces decrease together with the free PFM groups able to interact with Lys. In this environment, the interaction of BSA with the surface is weaker and the unfolding of its structure is less probable, so in the end the N form can be immobilized as well. The average mass considering the theoretical mass calculated by assuming that half of the protein will be immobilized in its F form and the other half will be immobilized in its native structure (N from) is 200 $ng{\cdot}cm^{-2}$, which differs from the experimentally obtained mass by only 5%. This percentage of extra mass monitored by the QCM-D technology can be attributed to the water content of the layer, meaning that the protein coverage of the surface is close to totality.

As has been said, the obtained mass of immobilized BSA on the surfaces modified by plasma grafting is approximately 115 $ng{\cdot}cm^{-2}$. This value is lower than the three calculated by the different footprints depending on the form in which BSA is attached to the surface (Table 1), which confirms that the density of active sites is lower than that formed by PECVD. For that reason, the amount of immobilized protein does not cover the entire surface. However, the attachment of the BSA in the F or E form is ruled out by the low yield that the gPFM reactivity would offer. In that case, it can be assumed that BSA is immobilized on the surface in its native conformation. The less hydrophobic environment that the plasma grafting confers to the modified surface may favor this behavior, also causing a more viscoelastic layer capable of trapping a greater amount of water on its structure, as has been previously demonstrated. However, because the value of the native BSA radius was obtained by DLS and so the solvating water around the molecule was also measured (hydrodynamic diameter), the difference could be due to the real amount of trapped water on the protein layer, which seems to be higher considering the viscoelasticity shown. However, it has to be considered that BSA might suffer a deformation of its native structure without resulting in a notable conformational change when it approaches to the modified surface. If so, the footprint used to calculate the theoretical mass would not fully adjust to the real dimensions during the immobilization process.

Therefore, the use of each surface-modification technique can be used depending on its final application, regarding the amount of protein immobilized on it and its conformational structure (Table 2)

Table 2. Summary of the General Characteristics of the Obtained Protein Layers for Both Modification Techniques Studied

	modification properties	immobilized BSA ($ng{\cdot}cm^{-2}$)	BSA conformation
PECVD	dense pPFM	210	F and N form
plasma grafting	gPFM brushes	115	N form

CONCLUSIONS

We have developed two different approaches to the immobilization of biomacromolecules, depending on the final application of the functionalized material. Both techniques, PECVD and plasma grafting, allow the covalent union of BSA proteins to a highly reactive surface in different ranges (200 and 100 $ng{\cdot}cm^{-2}$, respectively). Moreover, the differences in the properties of the modified surfaces lead to a conformational

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change in the protein structure. By PECVD, a more hydrophobic surface is achieved thanks to the PFM film layer, which caused a greater conformational change. However, the greater number of exposed PFM groups obtained by this method and compared to the PFM brushes formed when plasma grafting is carried out results in a higher capacity of BSA immobilization.

We have demonstrated that each technique offers different properties of the final protein monolayer, depending on the crucial function that the biomaterial will need to develop. If full coverage of the surface is needed and the activity of the biomacromolecule is not important, then PECVD would be the preferred option. However, plasma grafting would be more suitable when bioactive proteins or molecules such as enzymes have to be immobilized without suffering any conformational changes.

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interests.

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ABBREVIATIONS

PECVD, plasma-enhanced chemical vapor deposition; PFM, pentafluorophenyl methacrylate; BSA, bovine serum albumin; QCM-D, quartz crystal microbalance with dissipation

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