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EFFECT OF CHOLESTEROL ON THE DEPOSITION OF BETA-AMYLOID 1-40 AND 1-42 FILMS

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We report the pressure vs area isotherms of Langmuir films of pure and doped with cholesterol (Ch) amyloid beta, Aβ (1-40) and (1-42) at the air-water interface and, scanning electron microscopy (SEM) and atomic force microscopy (AFM) studies of Aβ (1-40) and (1-42) layers deposited from solution by drop cast and/or dipping and/or Langmuir–Blodgett on substrates of quartz and Si. The particularity of the substrate surface morphology determines the adhesion forces and plays an important role in the formation and stability of the multilayer films which are deposited on these substrates. The effect of cholesterol on the morphology of amyloids films and appearance of fibril structures is also analysed. AFM investigations have revealed the particularities of the aggregation process of the strongly hydrophobic Aβ(1-42) on cholesterol films deposited by Langmuir-Blodgett on solid support of quartz and Si.

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1. Introduction

The major components of the senile plaques associated with the development of progressive neurodegenerative disorder like Alzheimer disease are peptides containing 39-43 amino acids. The aggregates, including fibrils, of Aβ(1-40) and (1-42) are the most important responsible for neuronal cell disrupt. Amyloids can organize in nanoscale highly ordered structures determined by the ability of polypeptides molecules to form hydrogen bonding in the backbone [1]. Therefore amyloids show not only important biological functions, but also have a great potential for being used as a nanomaterial in nanotechnology and biotechnology for a large scale of applications from molecular electronics to tissue engineering [2]. Despite the intense research and large amount of experimental data, no clear conclusions have been obtained till now about the aggregation of Aβ.

Amyloid is usually an α-helical form [3] but it can misfold into other forms like β-sheets and aggregates determining the formation of oligomers or fibrils and these intermediate oligomers may have higher neurotoxicity than the mature inert form of amyloid fibrils [4, 5]. Previous research have evidenced that the interaction Aβ with lipid membrane is the initial step in the formation of amyloid fibrils in brain affected by Alzheimer disease [6].

The aggregation processes are very important because they determine the morphology which influences the properties of amyloids materials. Beta-amyloid is a protein which can self-assemble into fibrillar nanostructures which are rich in β-sheet secondary structures. The process of amyloids aggregation is determined by the supramolecular assembly of β-sheet structures. Because the release of Aβ takes place near the plasma membrane of the cell it is possible to interact with many membrane lipids affecting the process of oligomerization and determining sometimes, by interaction with gangliosides, conformational changes from random coil to a

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protective α-helical structure or β-structures [7,8]. Moreover the microdomains of the membrane enriched in cholesterol named lipid rafts favour the aggregation and fibrogenesis of Aβ [9].

Amyloids have a specific cross-β-sheet structure with individual β-strands which are perpendicular to the fibril axis connected by a dense network of hydrogen bonding and leading to β-sheet parallel to the fibril axis extended over thousands of molecular units [2]. In β-sheet structures the bonds are between groups of different stands which can be from different parts of the same peptide or from different peptides being stabilized by aggregation while in α-helical structure the hydrogen bonds stabilizing the structure are between groups in the same stand [10]. The formation of amyloid fibrils implies many intermediate steps such as the generation of partially folded intermediates from soluble native proteins, followed by self-assembly into oligomers and monomer addition [11,12].

Cholesterol the most abundant and well-known precursor of steroids is a component of all cells of mammals and interact with many compounds including the lipids from the cellular membrane and proteins [13]. The lipid monolayers and bilayers are often used to mimic the cellular membranes and study their interaction with different types of biomolecules [14].

The study of the interaction at the molecular level between cholesterol and a special class of peptides, amyloids, is relevant for the investigation of some neurodegenerative disorders [7]. A way to investigate these interactions is to use the Langmuir-Blodgett layers of cholesterol as a simplified model of the cell membrane. It is supposed that Aβ (1-42) is a major constituent of neuronal plaques having a greater tendency than Aβ (1-40) to form aggregates because of its increased hydrophobicity and having a greater pathogenic potential [13,15].

The interaction with the surfaces can control the process of β-sheet aggregation and formation of fibrils and therefore it is important to investigate the deposition of Aβ on surfaces showing different topographies [16].

This paper presents a study about the preparation from solution of Aβ (1-40) and (1-42) thin films on different substrates such as quartz and Si covered with a native layer of SiO_2, both showing hydrophilic behaviour. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) measurements have been used for morphological characterisation. The effect of the deposition conditions correlated with the deposition method (drop cast, dipping, Langmuir-Blodgett) on the aggregation process was investigated. We also analysed amyloid aggregation process in the presence of cholesterol and amyloid deposition on lipid thin film used as the simplest cellular membrane approximation.

2. Experimental

We have used three methods for the deposition of amyloid films from solution: Langmuir-Blodgett, drop cast [17,18] and dipping. Amyloids beta (1-40) and (1-42) were purchased from Aldrich and used without further purifications. The peptides layers are formed using a Langmuir-Blodgett KSV 2000 System with moving Delrin barriers, a Wilhelmy plate for calibration and a Teflon trough. The experiments were carried out at room temperature using substrates of quartz and single crystal silicon, Si, with area of 10 mm x 10 mm. The Si (100) substrates have been cleaned by successive boiling in n-butyl acetate for 30 sec, acetone for 30 sec and isopropylic alcohol for 30 sec followed by drying in nitrogen flow. The quartz substrates were cleaned by successive ultrasonication in detergent for 5 min, acetone for 5 min and chloroform for 5 min, washed in warm deionised water, followed by ultrasonication in alcohol isopropylic for 5 min and drying in nitrogen flow.

As subphase we used deionised water obtained with a TKA Pacific apparatus (final conductivity 0.055 μS/cm). 2,2,2-trifluoroethanol (TFE) was used as volatile inert spreading solvent. The concentration of the sample solution used for the deposition of Aβ (1-40) and Aβ (1-42) was of 0,1 mg amyloid in 1 ml TFE. Before the spread of the sample solution, the subphase surface is cleaned by moving the barriers across the trough reducing the surface to collect the impurity with a special pipette. This procedure is repeated until the surface pressure at the minimum area is 0.2-0.3 mN/m. Subsequently, droplets of the sample solution are spread on the surface of the deionised water subphase with a Hamilton syringe and let to evaporate for 30 min.

In the first stage of the Langmuir-Blodgett deposition we have drawn the surface pressure versus mean molecular area isotherm for Aβ (1-40), Aβ (1-42) and cholesterol using a barrier symmetrical compression speed of v=5 mm/min with the purpose to evaluate the target surface

pressure. During the compression process the molecule of the sample solution showing amphiphilic behaviour reorganize and reorient in a compact monomolecular film on the surface of the water. The target surface pressure was bellow the surface collapse pressure situated at 25 mN/m for Aβ (1-40) (Figure 1a), 17 mN/m for Aβ (1-42) (Figure 1b), 20 mN/m for cholesterol in TFE (Figure 1c) and bellow the isotherm plateau situated at 40 mN/m for cholesterol in chloroform (Figure 1d) because the monolayer is in liquid state. In the stage of Langmuir-Blodgett film formation, maintaining the surface pressure constant, the cleaned quartz/Si substrate is raised from the dipping well of the KSV System through the liquid/air interface with a speed v=3 mm/min. Each film was prepared using two deposition cycles.

Amyloid films have also been prepared by dipping, the cleaned quartz and Si plates being lowered with the dipping motor of the KSV 2000 System in the a vessel containing the same sample solution as the solution used for Langmuir-Blodgett deposition and then raised at a high constant speed of v=32,57 mm/min.

Amyloids drop cast films have been prepared by depositing three drops of each solutions [Aβ (1-40) in TFE, Aβ (1-42) in TFE, cholesterol in TFE] used in Langmuir-Blodgett deposition with a pipette on the surface of quartz and Si substrates. In 1-2 minutes the solvent evaporated at room temperature and on the surface of substrates we have obtained a dry thin film of amyloid or amyloid mixed with cholesterol. The films obtained are non uniform depending on the way the solutions wet the substrate's surface: the drops easily spread over the quartz substrate while preserve their shape on Si substrates.

Supported lipid monolayer, the simpler approximation for cellular membrane consists in films of lipid successively deposited on a solid substrate. Selected lipid, cholesterol, thin film has been deposited by Langmuir-Blodgett method using solution with concentration c=2 mg/ml of cholesterol in chloroform and a barrier symmetrical compression speed of v=10 mm/min with the purpose to evaluate the target surface pressure. The target surface pressure for cholesterol in chloroform deduced from the isotherm surface pressure vs mean molecular area was around 36-38 mN/m, bellow the isotherm plateau situated at ~40 mN/m (Fig. 1d). Each film of cholesterol was prepared using five deposition cycles starting with the substrate in down position. In the stage of film formation, maintaining the surface pressure constant, the substrate is raised from the dipping well through liquid/air interface with a speed v=3 mm/min. The first layer obtained rising the substrate from the dipping well is unstable as confirmed by a transfer rates >1 and the next layers are stable confirmed by a transfer rates ~ 1. The Aβ (1-42) film was deposited on the film of cholesterol by drop cast at room temperature.

The morphology of the deposited films has been analysed by microscopic methods: SEM and AFM. For SEM images acquisition was used a Hitachi S 3400 apparatus at an acceleration voltage V_{acc}=20kV and different magnifications. The AFM images have been obtained with Nanonics 4000 MultiView System working in tapping mode using a probe with diameter of 10 nm, resonance frequency of approximately 35 kHz and factor of merit of 1700. Evaluating the surface amplitude parameters, root means square (RMS) and roughness average (RA), we have obtained details about the topography of the surface of amyloid pure and amyloid doped with cholesterol films deposited on quartz and Si. The effect of the cholesterol film roughness on the topography of Aβ(1-42) film deposited on top has been also emphasised.

3. Results and Discussion

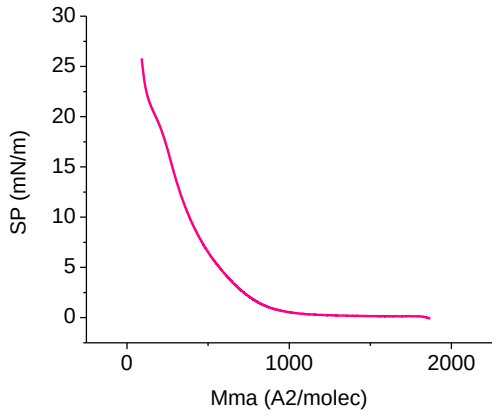
The Langmuir-Blodgett films can be characterised by measuring the isotherms surface pressure (SP)-mean molecular area (Mma) at the air-water interface. When the surface pressure increases the two dimensional film passes through different phases, the molecules showing different orientation and degree of freedom. At the beginning of the deposition cycle, the molecules of amyloid/cholesterol are spread on the water surface, they are not interacting and form a two dimensional gas (G) phase. When the barriers are approaching, the SP rises, the monolayer is compressed and correspond to a two dimensional liquid expanded (LE). Further compression increases the SP and corresponds to liquid condensed phase (LC). Increasing the compression, the film collapses and correspond to the minimum area where the molecules can be crowded. The condensed layer is characterised by a Mma obtained by extrapolating the linear part of the isotherm before collapse to zero SP and the limiting molecular area parameter corresponds to the most closely packed molecules at the interface air-water.

The limiting molecular area is high for the compounds with complex structure and high molecular weight like Aβ (1-40) with M = 4329.9 and Aβ (1-42) with M=4514.1 compared to cholesterol with M=386.65. The limiting molecular area obtained by extrapolating to zero surface pressure is 27 [Å^2/molecule] for cholesterol (Figure 1a) compared to 750 [Å^2/molecule] for Aβ (1-40) (Figure 1b) and 550 [Å^2/molecule] for Aβ (1-42) (Figure 1c). There is no films collapse evident upon compression up to 25 mN/m for Aβ (1-40) (Figure 1a), 17.5 mN/m for Aβ (1-42) (Figure 1b) and 20 mN/m for cholesterol (Figure 1c) corresponding to a mean molecular area of 430 [Å^2/molecule], 400 [Å^2/molecule] and 15 [Å^2/molecule] respectively.

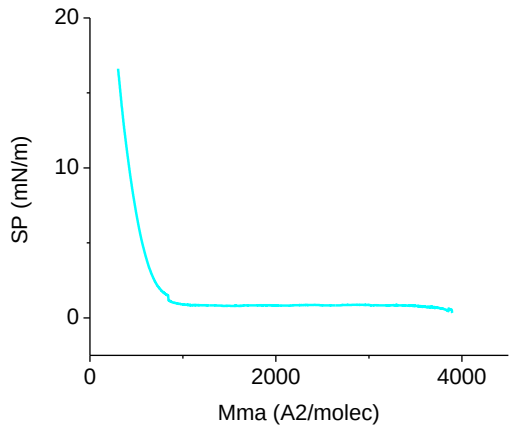
The LE phase starts to appear at a surface pressure around 0.5 mN/m both in Aβ (1-40) (Figure 1a) and in Aβ (1-42) (Figure 1b) while the LC phase starts to appear around at 5.0 mN/m both in Aβ (1-40) (Figure 1a) and Aβ (1-42) (Figure 1b). Slightly higher surface pressure are necessary both for LE (1 mN/m) and LC (7.5 mN/m) phases generation in cholesterol (Figure 1c).

Considering the pressure-area isotherms of cholesterol on a water subphase can be seen that the isotherm starts at low surface pressure which is typical for the gas phase presence and the coexistence with liquid condensed phase. At ~ 40 (A^2/molecule) the isotherm shows a sharp increase in pressure (Figure 1d) and enter in the region of pure liquid condensed phase.

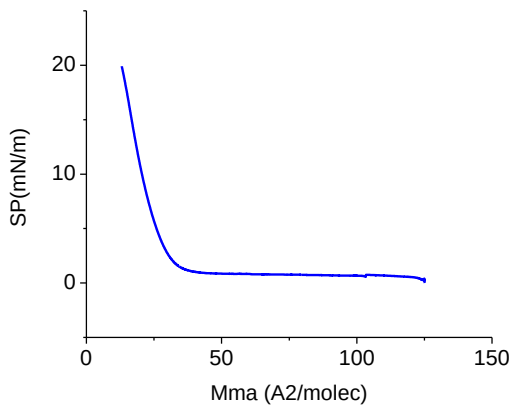
The sharp increase in the pressure means high slope of the isotherm and low compressibility because the compressibility coefficient is inversely proportional to the slope. Therefore the molecules of cholesterol are untitled and show no anisotropy [19]. At a surface pressure of ~42 mN/m it is obtained a plateau and is the point where takes place the onset of the multilayer formation and probably is the surface pressure at which the monolayer collapses [20].



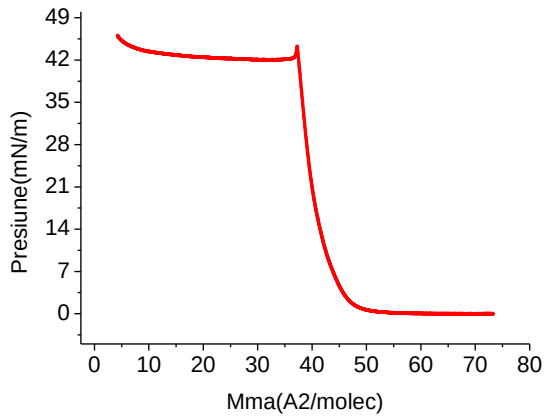
(a) Surface pressure vs mean molecular area isotherm for Aβ (1-40) in TFE c=0,1 mg/ml



(b) Surface pressure vs mean molecular area isotherm for Aβ (1-42) in TFE c=0,1 mg/ml



(c) Surface pressure vs mean molecular area isotherm for cholesterol in TFE c=0,1 mg/ml



(d) Surface pressure vs mean molecular area isotherm for cholesterol in chloroform c=2 mg/ml.

Fig. 1. Surface pressure vs mean molecular area isotherms for Aβ(1-40), Aβ(1-42) and cholesterol Langmuir monolayers deposited on a water subphase.

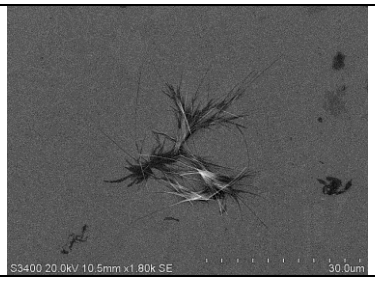
The adhesion forces which depend on the particularity of the substrate surface play an important role in the formation and stability of the multilayer films which are deposited on different substrates [21]. The surface of Si semiconductor wafer is of great importance for the growth and stability of different layers/structures developed on it. The surface of Si completely covered by oxide known as OH-terminated Si surface is hydrophilic in nature and very stable such as the quartz surface [22]. On the other hand the molecule of cholesterol is strongly hydrophobic and therefore the interaction between the hydrophilic substrate and hydrophobic cholesterol is not strong and it is very difficult to obtain by Langmuir-Blodgett the deposition of many successive layers [23]. The transfer is realised by pulling the hydrophilic substrate through the lipid monolayer of cholesterol. Two counteracting processes take place at the interface: some molecules are adsorbed when others are desorbed. Despite the hydrophilicity of the surface we could transfer in our experiments more than 1 layer, more precisely, 5 layers, of hydrophobic cholesterol. This is confirmed by the values of the transfer rates which are ~1. This means that at every upstroke and downstroke of the dipper of the Langmuir-Blodgett system, the amount of adsorbed molecules is higher than the amount of desorbed molecules [24].

We have transferred the cholesterol film from the water-air interface on solid substrate by Langmuir-Blodgett technique at two different surface pressures of 37 mN/m and 54 mN/m. A quantity of 100 μL of cholesterol in chloroform has been spread on the subphase. The experiments for drawing the isotherm, surface pressure vs. area per molecule, were carried out at a temperature of T=25 ^{o}C. At the surface pressure >40 mN/m, the layer approaches the collapse region (Figure 1d) and as a consequence it is not very stable for deposition. Analysing the isotherm we can see a steep region between 40 (A^2/molecule) and 45 (A^2/molecule) which corresponds to the condensed phase. The selected surface pressure of 37 mN/m is in the upper region of the steep part of the isotherm.

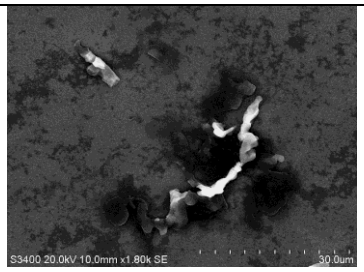
The transfer efficiency is evidenced by the transfer ratio whose values when the transfer takes place on quartz are >1 and confirm the dominance of the adsorption over desorption of the molecules independently of upstrokes or downstrokes and the instability of the multilayer. The interaction between the hydrophobic cholesterol and hydrophilic substrate both quartz and Si, is weak and for this reason it is difficult to deposit the first layer of cholesterol. On the other hand the layer to layer interaction from upstroke to downstroke is weak and therefore can be favoured the generation of defects which can act as nucleation centres for the subsequent desorption of molecules during the upstroke stages. The filling of defects and reorganization of the molecules are mechanisms involved when the number of deposition cycles increases [24]. The interaction cholesterol-cholesterol layers is weaker compared to interaction hydrophobic substrate-first layer of cholesterol [24] and therefore it is difficult the formation of cholesterol multilayer.

SEM images have revealed for Aβ (1-40) deposited on Si wafer by drop cast a morphology characterised by the presence of bundles of wires or thin fibrils (Figure 2a) quite scattered, which became compact clusters like wide strips adding cholesterol (Figure 2b). The layer of Aβ (1-42) deposited by drop cast on Si showed a clear fibril morphology (Figure 2c).

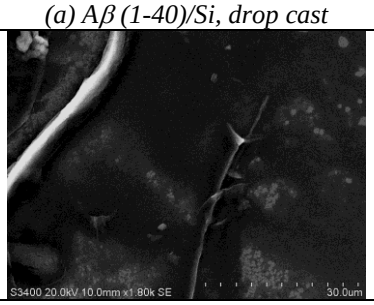
To facilitate the comparison between different layers we have used the same magnification (1.8 K), for all samples deposited by drop cast on Si.



(a) Aβ (1-40)/Si, drop cast



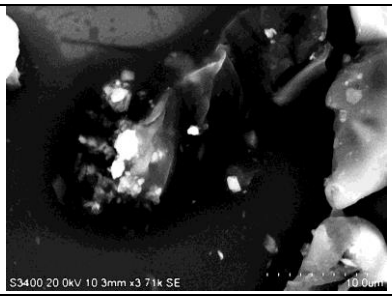
(b) Aβ (1-40)+Ch /Si, drop cast



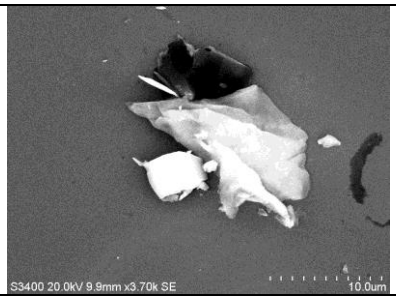
(c) β (1-42)/Si, drop cast

Fig. 2: SEM images of layers prepared from Aβ (1-40), Aβ (1-40)+Ch and Aβ (1-42) by drop cast on Si.

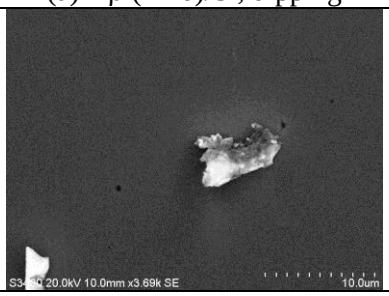
The samples prepared by dipping on Si showed a completely different morphology characterised for Aβ (1-40) by clusters randomly distributed and twisted strips (Figure 3a) and mostly by large twisted strips for Aβ (1-40) with cholesterol (Figure 3b) and significantly smaller twisted strips for Aβ (1-42) deposited on Si (Figure 3c).



(a) Aβ (1-40)/Si, dipping



(b) Aβ (1-40)+Ch/Si, dipping



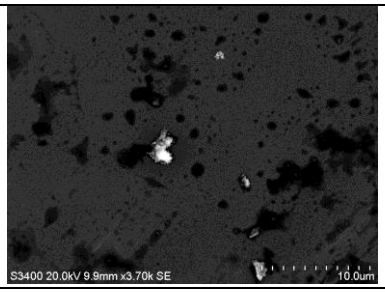
(c) Aβ (1-42)/Si, dipping

Figure 3: SEM images of layers prepared from Aβ (1-40), Aβ (1-40)+Ch and Aβ (1-42) by dipping on Si.

For comparison we have used a higher magnification (3.7 K) compared to the samples deposited by drop cast. This magnification was maintained the same for all the investigated samples deposited by dipping and Langmuir-Blodgett on Si.

The morphology of Aβ (1-40) (Figure 4a) and Aβ (1-42) (Figure 4b) deposited by Langmuir-Blodgett on Si is not very different from that of the same layers deposited by dipping and is dominated by the presence of clusters randomly distributed with smaller dimension for Aβ (1-42).

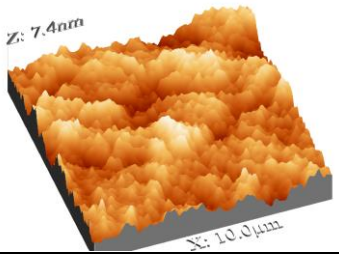
(a) Aβ (1-40)/Si, Langmuir-Blodgett



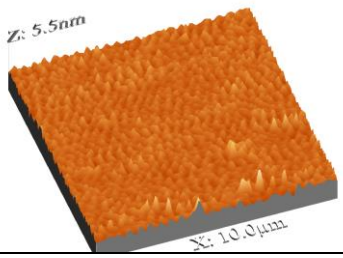
(b) Aβ (1-42)/Si, Langmuir-Blodgett

Fig. 4: SEM images of layers prepared from Aβ (1-40) and Aβ (1-42) by Langmuir-Blodgett on Si.

The roughness of the Aβ (1-40) layer deposited on quartz is low and the surface is smooth independently of the method used for deposition. The layers deposited on Si by the same methods have shown lower roughness and are smoother than the layers deposited on quartz. The roughness of the layer deposited by dipping and Langmuir-Blodgett on Si are comparable. The layer deposited by drop cast on quartz (Figure 6a) showed a slightly lower roughness than the same layer deposited by dipping (Figure 6b) and Langmuir-Blodgett (Figure 6c) on the same substrate. On the contrary, the roughness of the Aβ (1-40) layer deposited on Si by drop cast (Figure 6d) was slightly higher than that of the layer deposited by dipping (Figure 6e) and Langmuir-Blodgett (Figure 6f) on Si. As reference are given the AFM images for quartz (Figure 5a) and Si (Figure 5b) substrates. A weak organisation in short fibrils was identified in Aβ (1-40) layer deposited on quartz by Langmuir-Blodgett (Fig. 6c).

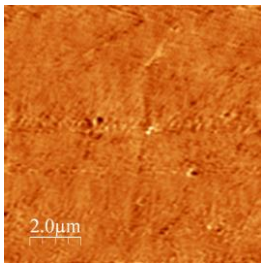


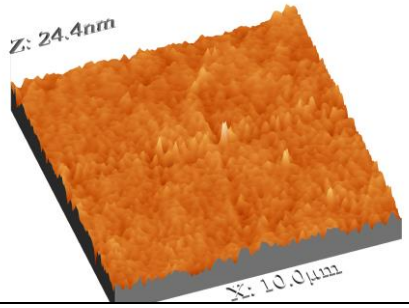
(a) Quartz substrate: RMS=1.0 nm, RA=0.8 nm



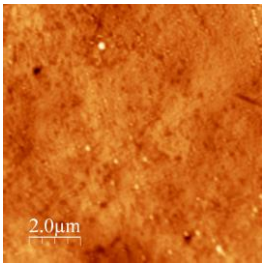
(b) Si substrate: RMS=0.33 nm, RA=0,25 nm

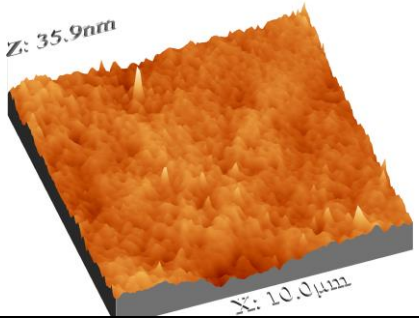
Fig. 5: AFM images of quartz and polished Si (100) substrates.



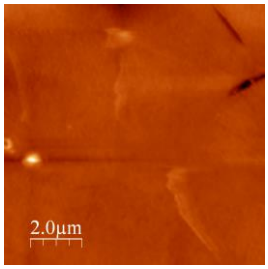


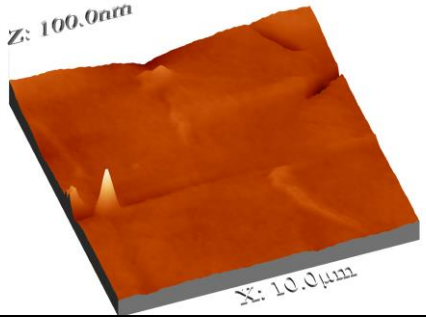
(a) Aβ (1-40)/quartz/drop cast: RMS=1.33 nm, RA=0.98 nm



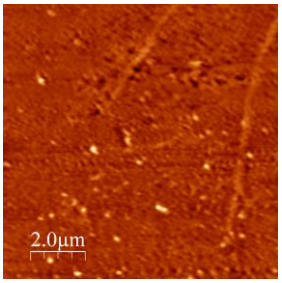


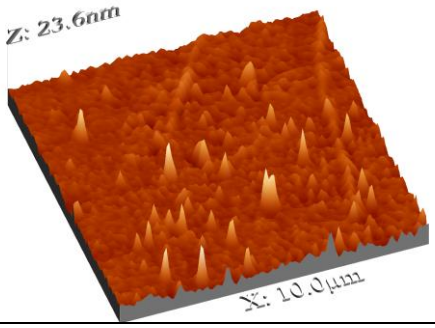
(b) Aβ (1-40)/quartz/dipping: RMS=2.38 nm, RA=1.83 nm



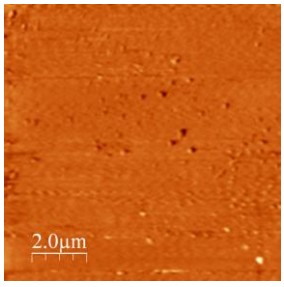


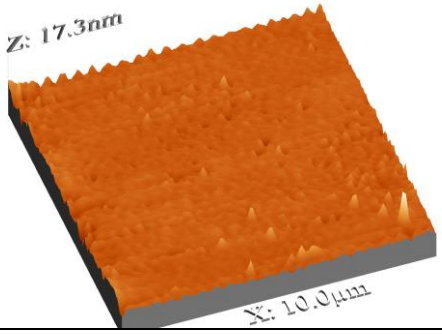
(c) Aβ(1-40)/quartz/Langmuir-Blodgett:RMS=3.50 nm, RA=2.08 nm



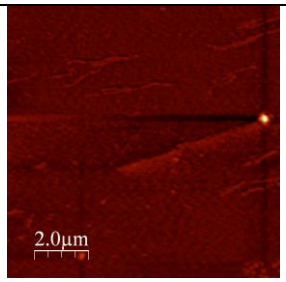


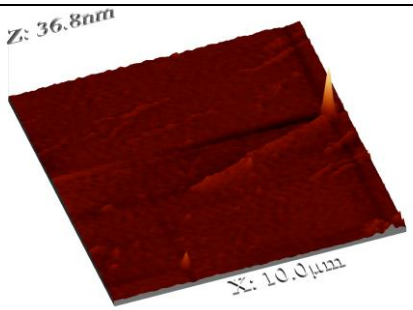
(d) Aβ (1-40)/Si /drop cast: RMS=1.36 nm, RA=0.91 nm





(e) Aβ (1-40)/Si/dipping: RMS=0.68 nm, RA=0.46 nm





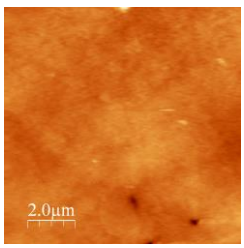
(f) Aβ (1-40)/Si/Langmuir-Blodgett: RMS=0.80 nm, RA=0.40 nm

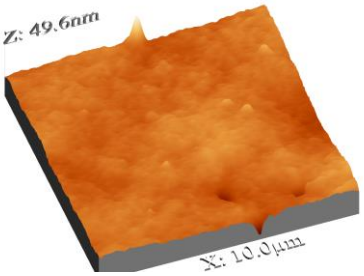
Fig. 6: AFM images of Aβ(1-40) deposited on quartz by: drop cast (a); dipping (b); Langmuir-Blodgett (c). AFM images of Aβ(1-40) deposited on Si by: drop cast (d); dipping (e); Langmuir-Blodgett (f).

The AFM image of Aβ(1-40) film deposited by drop cast on Si (Figure 6d) shows some preferential orientation suggesting the organization in wire or thin fibrils as already identified on SEM image (Figure 2a). The AFM images of Aβ(1-40) film deposited by dipping (Figure 6e) and Langmuir-Blodgett (Figure 6f) on Si show predominantly clusters as identified in SEM images (Figure 3a and Figure 4a) with the tendency to assembly in thin fibrils in Langmuir-Blodgett film evidenced only in AFM image (Figure 6f).

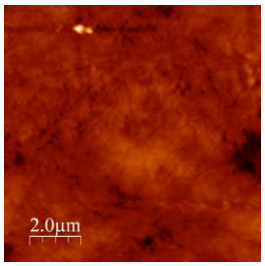
The behaviour of Aβ(1-42) layers deposited by drop cast (Figure 7a), dipping (Figure 7b) and Langmuir-Blodgett (Figure 7c) on quartz is similar to that of Aβ(1-40) deposited on quartz by the same methods (Figure 6a,b,c), the surface of the layers showing a low roughness. The layer of Aβ(1-40) is more compact and homogeneous than the layer of Aβ(1-42).

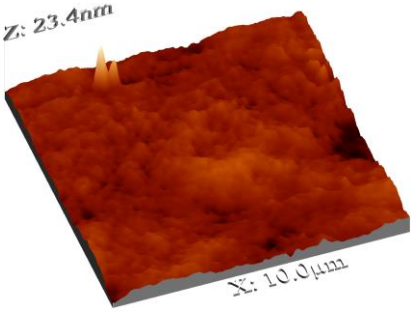
The layer of Aβ(1-42) deposited on Si by drop cast (Figure 7d) show the highest roughness and large aggregates suggesting thick fibrils determined probably by the low wettability of the more hydrophobic Aβ(1-42) [25] on the hydrophobic Si surface. The Aβ(1-42) layers deposited by dipping (Figure 7e) and Langmuir-Blodgett (Figure 7f) show a morphology characterised by droplets showing a preferential orientation in line with the tendency to form fibrils more evident in Langmuir-Blodgett layers (Fig. 7f).





(a) Aβ (1-42)/quartz/drop cast: RMS=2.35 nm, RA=1.80 nm





(b) Aβ (1-42)/quartz/dipping: RMS=1.24 nm, RA=0.93 nm

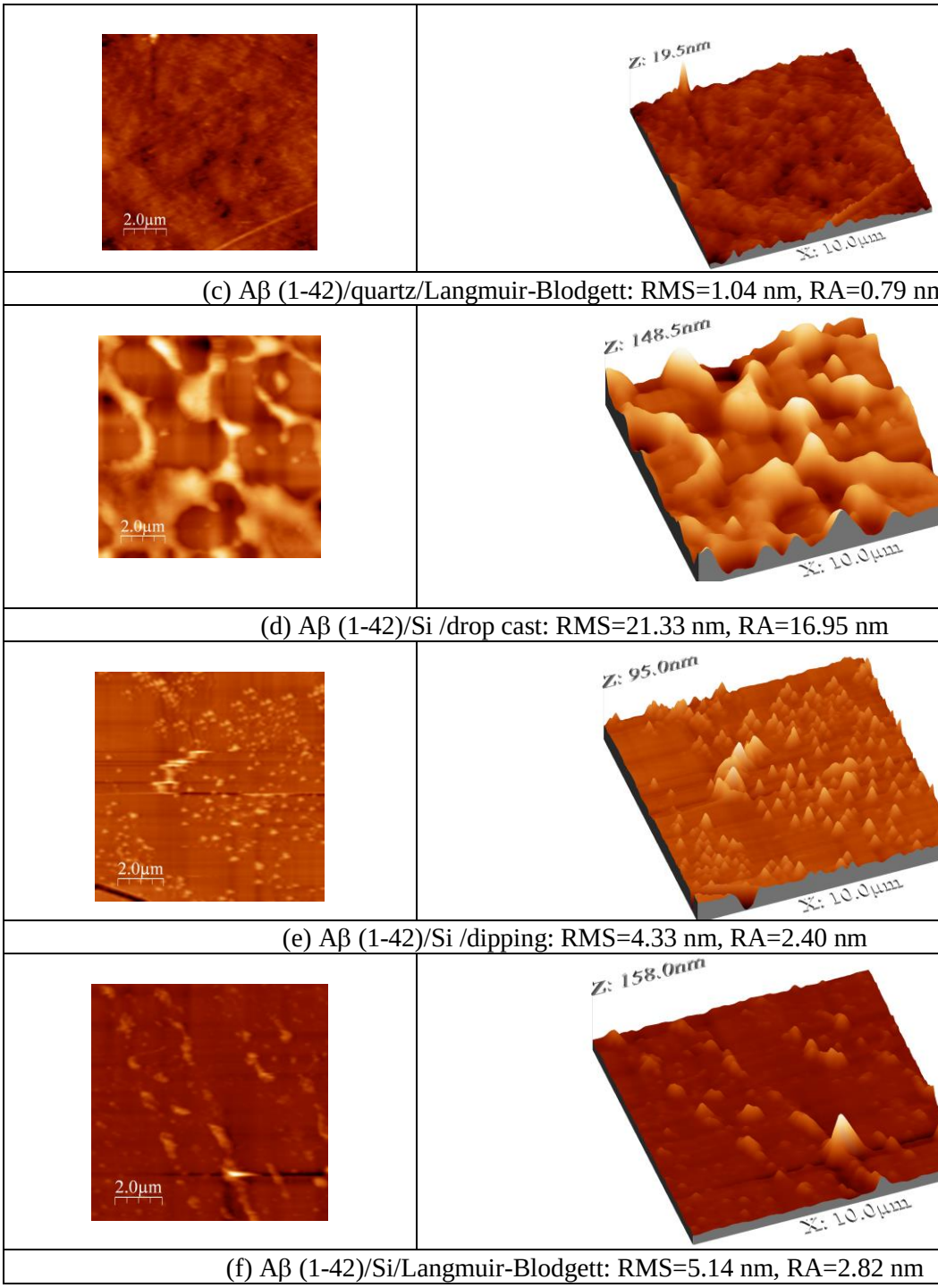


Fig. 7: AFM images of Aβ(1-42) deposited on quartz by: drop cast (a); dipping (b); Langmuir-Blodgett (c). AFM images of Aβ(1-42) deposited on Si by: drop cast (d); dipping (e); Langmuir-Blodgett (f).

The AFM image of Aβ(1-42) film deposited by drop cast on Si (Figure 7d) show clusters and thick fibrils as was already evidenced in SEM image (Figure 2c). The AFM images of the same amyloid layer deposited by dipping (Figure 7e) and Langmuir-Blodgett (Figure 7f) show predominantly clusters as in SEM image (Figure 3c and Figure 4c) with the tendency to orient preferentially in Langmuir-Blodgett film preserving the potential to generate fibrils (Figure 7f).

Both Aβ(1-40) and Aβ(1-42) show certain tendency to generate fibrils when deposited by Langmuir-Blodgett on solid substrate.

The effect of cholesterol on the amyloid layer formation was also investigated (Figure 8). The presence of cholesterol has increased the roughness of Aβ(1-40) deposited by drop cast both on quartz and Si (Figure 8a,c) compared to Aβ(1-40) deposited by dipping (Figure 8b,d). The highest roughness is presented by Aβ(1-40)+Ch deposited on Si (Figure 8c). The layer is not uniform like pure Aβ(1-40) layer (Figure 6a) and shows bubbles and clusters randomly distributed in the layer.

A tendency of preferential orientation is observed in Aβ(1-40)+Ch layer deposited on quartz (Figure 8b).

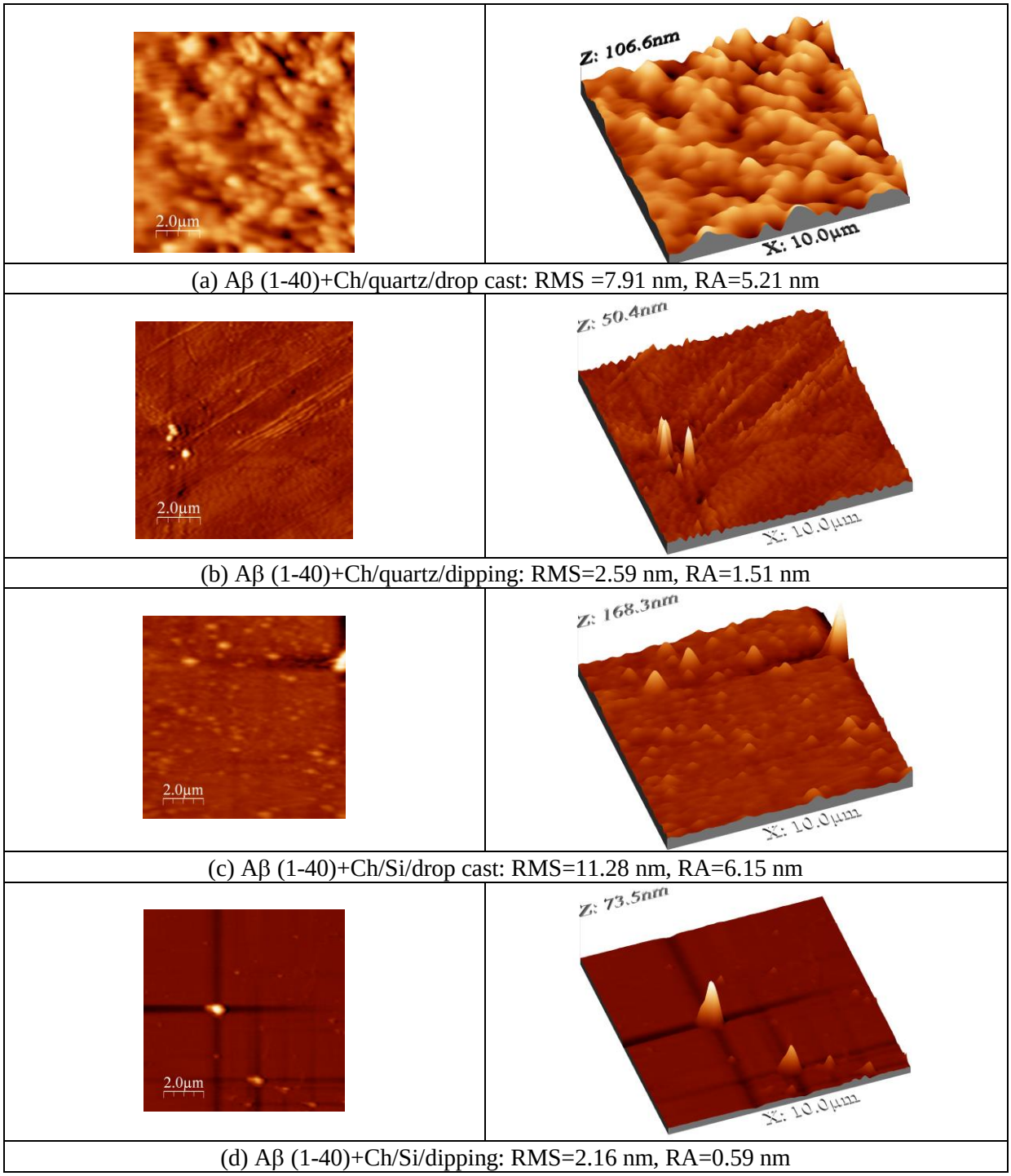
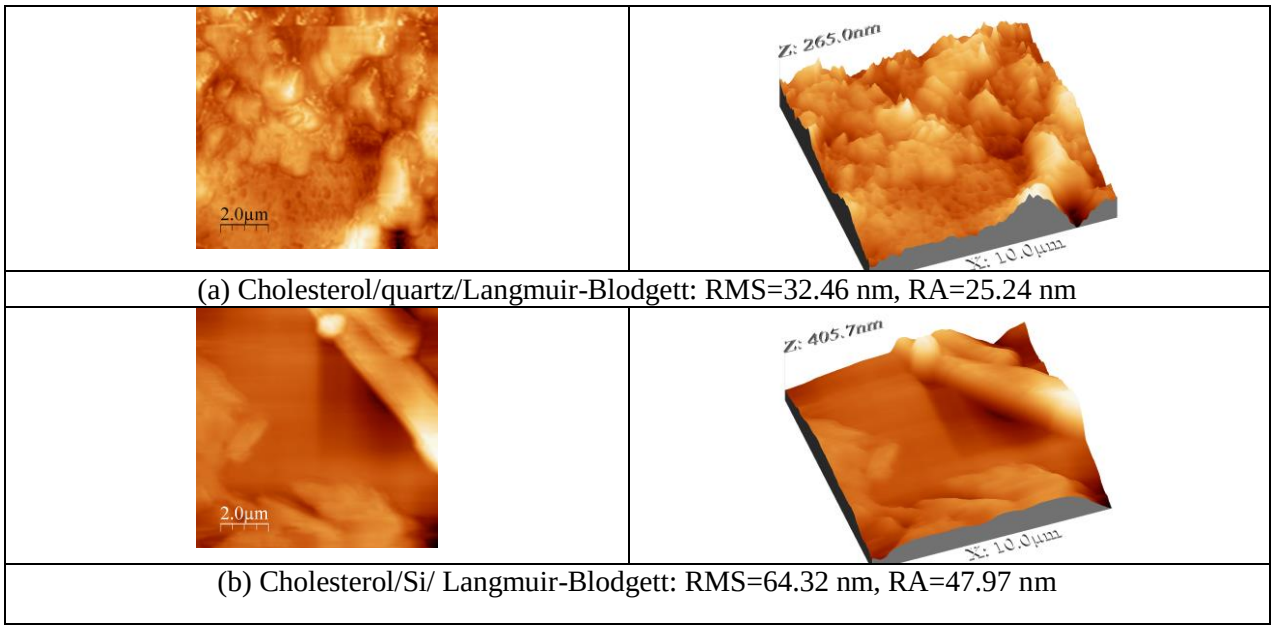


Fig. 8: AFM images of Aβ(1-40)+Ch deposited on quartz by: drop cast (a); dipping (b);. AFM images of Aβ(1-40)+Ch deposited on Si by: drop cast (c); dipping (d).

The morphology of Aβ(1-40)+Ch layer deposited on Si by drop cast and dipping, characterised by the presence of clusters, evidenced by SEM images (Figure 2b and Figure 3b) was also shown by AFM images (Figure 8c,d).

The effect of the Ch considered as dopant on the morphology of amyloid deposits simulates the effect of cholesterol from the human blood on the amyloid aggregation process.

Different surface topography have been shown by the cholesterol film deposited by Langmuir-Blodgett on quartz and Si (Figure 9a,b).



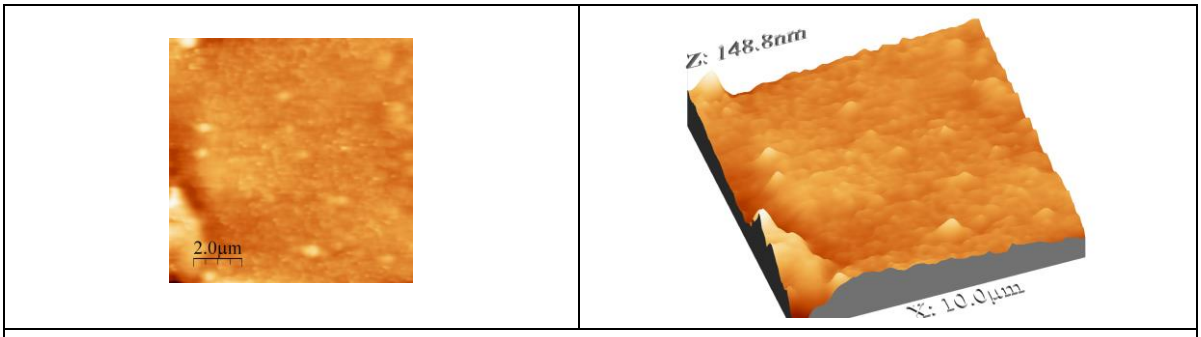
(a) Cholesterol/quartz/Langmuir-Blodgett: RMS=32.46 nm, RA=25.24 nm

(b) Cholesterol/Si/ Langmuir-Blodgett: RMS=64.32 nm, RA=47.97 nm

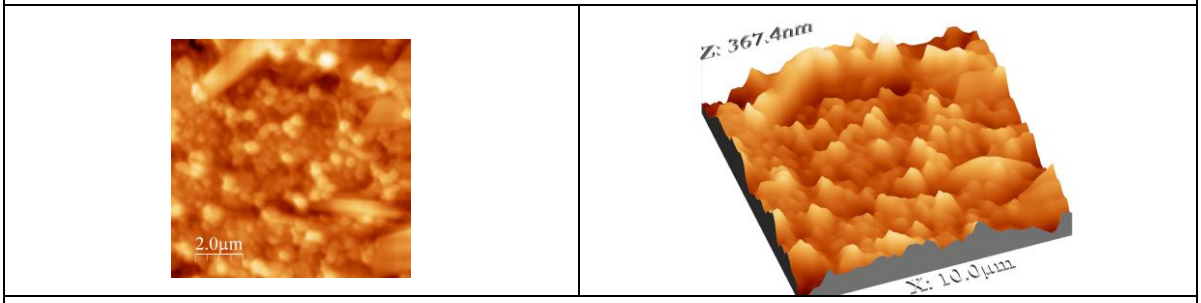
Fig. 9: Cholesterol layers deposited by Langmuir-Blodgett from a solution of 2 mg cholesterol in 1 ml of chloroform on different substrates: quartz (a); Si (b).

As discussed above, it is difficult to obtain the cholesterol multilayer deposition because the molecule of cholesterol is hydrophobic and the Si substrate is hydrophilic, the interaction between cholesterol and substrate is weak and it is difficult to support the successive deposition of different layers. Therefore the assembly of cholesterol on these hydrophilic surfaces show no characteristic, well-defined, morphological features [24] as confirmed by AFM images (Figure 9a,b).

The surface of quartz is covered with a continuous layer of cholesterol showing a typical shape of balls, while the cholesterol deposited on Si shows a less uniform morphology with highly elongated domains suggesting short, thick fibrils (Figure 10b).



(a) Aβ(1-42):drop cast/Ch:Langmuir-Blodgett/quartz: RMS=31.83 nm, RA=23.51 nm



(b) Aβ(1-42):drop cast/Ch:Langmuir-Blodgett/Si: RMS=47.10 nm, RA=37.00 nm

Fig. 10: Aβ(1-42) layers deposited by drop cast on cholesterol layer deposited by Langmuir-Blodgett on different substrates: quartz (a); Si (b).

Two types of interactions could be involved in the process of interaction between Aβ and lipid membrane: electrostatic and hydrophobic interactions [4]. Taking into account that cholesterol has an electrostatic effect it can attract the Aβ peptide and therefore it can be considered as the driving force for amyloid binding sustaining the hypothesis of cholesterol involvement in the toxicity of amyloid [14].

When amyloid peptide is in the proximity of the lipid monolayer supported membrane the peptides can interact forming fibrils or the peptides monomers or oligomers can interact with the lipid head-groups forming aggregates on the lipid surface generating oligomers and fibrils bound to the lipid membrane [4]. Previous work have emphasised a higher propensity of oligomers to lipid surface than fibrils which linked to the higher toxicity of oligomers compared to fibrils [26].

The deposits of amyloid on cholesterol covering Si substrate are spherical oligomers or clusters of spherical oligomers and short fibrils (Figure 10b). The deposits of amyloid on cholesterol covering quartz substrate show no fibrils (Figure 10a) and it is smoother than the layer of amyloid deposited on cholesterol covering Si substrate. Rare, big clusters randomly distributed have been observed on quartz covered by cholesterol. The same number of droplets, 5-6, from the same solution of amyloid, Aβ(1-42), have been deposited on the both substrates covered by Ch and let to evaporate in the same conditions of temperature, humidity and duration. We can suppose that the amyloid surface morphology is determined only by the morphology of the cholesterol layer influenced by the surface of the substrate, quartz or Si.

The tendency to generate short, thick fibrils which are potentially neurotoxic and could be involved in neurodegenerative diseases has characterised the higher hydrophobic Aβ(1-42) interaction with hydrophobic cholesterol deposited on a hydrophilic surface. The different behaviour of the supported Ch membrane is determined by the wettability of the fluid containing Ch on the hydrophilic quartz and Si substrates.

These experimental conditions simulate the interaction between amyloid and cholesterol emphasising that the properties of the cellular membrane (represented by Ch film) affects the aggregation process of amyloids [Aβ(1-42)] deposits.

Conclusions

We have studied the stability of the Aβ(1-40) and Aβ(1-42) monolayer at the water-air interface and the transfer process of these layers on a solid support of quartz or polished Si (100) considering the surface pressure vs mean molecular area isotherms for each peptide. In most cases the transfer rate was >1 confirming the unstable multilayer obtained during the multi cycles deposition process. Because of the high hydrophilicity of both quartz and SiO_2 covered Si it is difficult to obtain a compact, uniform layer covering the substrate. The effect of the solution deposition method, drop cast, dipping and Langmuir-Blodgett, on the morphology has been investigated emphasising the effect of cholesterol used as dopant on the aggregation process of amyloids and generation of fibrils.

The roughness of the Aβ (1-40) layer deposited on quartz is low and the surface is smooth independently of the used deposition method. The layers deposited on Si by dipping and Langmuir-Blodgett have shown lower roughness than the layers prepared by the same method on quartz while the layer deposited by drop cast on quartz showed a slightly lower roughness than the same layer deposited by drop cast on Si.

The behaviour of Aβ(1-42) layers deposited by the mentioned methods on quartz is similar to that of Aβ(1-40) deposited on quartz by the same methods. The surface of the Aβ(1-40) and Aβ(1-42) layers show a low roughness, but the layer of Aβ(1-40) is more compact and homogeneous than the layer of Aβ(1-42).

The layer of Aβ(1-42) deposited on Si by drop cast show the highest roughness and large aggregates determined probably by the low wettability of the more hydrophobic Aβ(1-42) on the hydrophilic Si surface. The Aβ(1-42) layers deposited by dipping and Langmuir-Blodgett show a morphology characterised by droplets showing a preferential orientation in line with the tendency to form fibrils more evident in Langmuir-Blodgett layers.

A certain tendency to generate fibrils when deposited by Langmuir-Blodgett on solid substrate has been evidenced for both Aβ(1-40) and Aβ(1-42). Some similarity has been evidenced between the morphological particularities dominated by clusters or fibrils of amyloids films deposited on Si evidenced by SEM and AFM images.

The effect of cholesterol on the amyloid layer formation was investigated, the cholesterol increasing the roughness of Aβ(1-40) deposited by drop cast, the highest roughness being shown by Aβ(1-40)+Ch deposited on Si and having a morphology characterised by bubbles and clusters randomly distributed.

The deposition of amyloids on a cholesterol layer mimicking the cellular membrane was also studied to evidence the interactions between these peptides and the lipid layer.

The layer of cholesterol deposited by Langmuir-Blodgett on quartz is continuous showing a typical shape characterised by ball, while the cholesterol deposited on Si shows a less uniform morphology with highly elongated domains to evolve in fibrils. The deposits of amyloid on these layers of cholesterol covering Si substrate show clusters and short fibrils. The deposits of amyloid on cholesterol covering quartz substrate show no fibrils and the layer is smoother than the layer of amyloid deposited on Si covered by cholesterol.

This study confirms the importance of cholesterol both present in the fluid (blood) and as lipidic layer component of the cellular membrane in the process of amyloids aggregation and generation of morphological feature such as fibrils and/or clusters of oligomers favouring neurotoxicity.

Selection of the deposition method is very important therefore dipping and Langmuir-Blodgett seem to mimic better the process taking place at the interface cellular membrane – intra/extra cellular space.

Acknowledgments

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