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CHEMICAL SCIENCES

Liposome Adsorption Dynamics Induced by Gold Surface Functionalization

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Abstract: Early disease detection is vital. Biosensors using phospholipid films or core-shell lipid-based nanostructures can aid this process. This study examined the influence of gold surface functionalization with self-assembled monolayers (SAMs) on liposome adsorption using Atomic Force Microscopy and Quartz Crystal Microbalance. Comparing hydrophobic octanethiol and hydrophilic 3-mercaptopropionic acid (3-MPA) SAMs, we found that liposome adsorption on the hydrophilic surface occurred significantly faster, requiring only 20 seconds, compared to hydrophobic surfaces which took over 1,000 seconds. Furthermore, the hydrophilic surface exhibited a higher adsorbed mass of approximately 200 ng, suggesting multilayer formation, while hydrophobic surfaces showed around 150 ng of deposited liposomes. These results highlight the crucial role of surface wettability, dictated by SAM polarity, in controlling the kinetics and extent of lipid bilayer formation for applications in biosensors and drug delivery nanotechnology.

Key words: Liposome, dynamics, gold, functionalization, thiol SAM, sensor.

INTRODUCTION

Early disease detection is of vital importance, and biosensors utilizing phospholipid films or core-shell lipid-based nanostructures can significantly aid in this process. In the development of reliable biosensors for diagnosis and effective therapeutics, including conditions such as COVID-19, the use of phospholipid films has emerged as a successful strategy to mimic biological conditions (Behera et al. 2020, Nele et al. 2024). Despite the widespread acceptance and employment of phospholipid membrane models in studies concerning the specificity of binding targets, there is still a limited understanding of the overall interaction between immersed planar surfaces and the adjacent liquid, although this aqueous environment near the interface is fundamental for living organisms and related technologies, such as the development of drug delivery systems and biosensors (Mazur et al. 2017, Seo & Park 2021, Philipp et al. 2023). Functionalization of metal electrodes with thiol self-assembled monolayers (SAMs) is widely used to alter the wettability of the surfaces and is an essential component for the formation of supported lipid bilayers used in biosensors, influencing the balance between solid-liquid intermolecular adhesive forces. This work evaluates how the functionalization process affects the water interfacial region microscopically and macroscopically. The macroscopic effects were visualized by contact angle measurements, and the microscopic effects were investigated by monitoring the interaction of the DMPC (1,2-Dimyristoyl-sn-glycero-3-phosphocholine) liposome solution/surfaces using Atomic Force Microscopy (AFM) and Quartz Crystal Microbalance (QCM).

MATERIALS AND METHODS

All chemicals used in the study were of analytical grade and used as purchased without further purification: DMPC, Octanethiol (P.A., Allkimia products) and 3-Mercaptopropionic Acid (3MPA) were purchased from SIGMA. Milli-Q water was obtained fresh before each experiment. DMPC liposome solution was prepared by solubilizing DMPC in an Eppendorf containing chloroform, the solvent was dried, forming lipid bilayers on the surface of the preparation Eppendorf. After drying, deionized water was added to a concentration of $1.0x10^{4}$M and then placed in ultrasound to form liposomes.

Functionalization of gold electrode surfaces: sputtered gold surfaces are hydrophobic, but additional hydrophobic functionalization was performed by immersing the electrode in 5 mL of ethanol containing octanethiol monomers at a concentration of 0.1 mM for 24 h. The electrodes were then rinsed with Milli-Q water and dried using nitrogen flux. Similarly, formation of hydrophilic films on gold was performed by immersing the electrode in 5 mL of ethanol containing 3-MPA monomers at a concentration of 0.1 mM for 24 h. The electrodes were then rinsed with Milli-Q water and dried using nitrogen flux. Atomic Force Microscopy (AFM) was used to characterize the surfaces and structures of adsorbed liposomes. Contact angle images of 5 µL water droplets onto surfaces were observed and subsequently measured using ImageJ software. AFM images of dried samples were obtained using a Thermo AutoProbe CP-Research Microscope. Silicon nitride (Si_3N_4) cantilevers with an ultra-low spring constant (ca. 0.03 N m^{-1}) and a small tip radius of curvature (ca. 5 nm radius) were used. The scan rate of 1 to 4 Hz allowed us to scan the samples without causing any physical damage. Particle topographies were obtained using Gwiddion AFM software. Then, ~10 µL of the liposome solution was placed on the gold electrode surfaces and dried for 24 h. The percentage of surface coverage was measured.

Quartz Crystal Microbalance measurements: A thin disk of mirror-polished piezoelectric quartz crystal sandwiched between two metal electrodes forms the QCM sensor. The QCM sensor used is a Stanford Research QCM200, a 5 MHz quartz crystal with parallel capacitance compensation (Cp). The resonant frequency (f) and resonant resistance (R) values are measured (Systems 2018). These values characterize mass deposition or viscoelastic coupling. One of the crystal surfaces is in contact with air, the one in contact with the liquid generates a shear wave that extends into the fluid, in the direction normal to the electrode, disappearing at a maximum penetration depth λ (for a 5 MHz crystal immersed in water, λ ≈ 238 nm). The frequency shifts Δf are given by the Sauerbrey Equation, $\Delta f = -C_f \Delta m$, where the observed frequency shift is given in Hz, Δm is the change in mass per unit area (g/cm^2), and C_f is the sensitivity factor for the crystal (-5.3 ng/Hz for a 5 MHz AT-cut quartz crystal at room temperature, gravimetric area of ≈0.3cm^2). Tests to characterize the surface adsorption dynamics used nanometer-sized DMPC liposomes in aqueous solution. The quartz crystal microbalance was initially transferred from a beaker with Milli-Q water at 25 °C to a beaker containing 100 mL of 10^{-4} M DMPC solution. Assuming a full coverage of the electrode area by DMPC polar heads, which corresponds to 0.6 mol/nm^2, then a monolayer of closely packed phospholipids would cause a frequency shift of -3.82 Hz, or 20.26 ng. The coverage of the phospholipid bilayer then corresponds to a frequency shift of -7.64 Hz. Thin film deposition results in a rigid mass adsorption on the electrode. All measurements were performed in triplicate.

After AFM imaging of the electrode surface, each functionalized electrode was mounted on the QCM. Initially in air, then net mass loading was obtained by immersing the sensor in a 100mL beaker with Milli-Q water. Tests were performed with this surface immersed in water and in a liposome solution with DMPC concentrations of $1.0x10^{-4}$ M. After 10 minutes, the QCM sensor was placed back in the water and the measurements were performed with mechanical agitation.

RESULTS AND DISCUSSION

Characterization of Functionalized Surfaces by AFM, Figure 1: Octanethiol functionalization rendered the gold surface more hydrophobic, while 3-MPA thin film rendered the surface hydrophilic. The wettability changes cannot be explained by roughness effects alone, since hydrophobic and hydrophilic surfaces exhibit approximately the same R_a value (~2.0±0.5 nm). The pronounced effect on wettability is mainly due to the polar heads of the SAMs.

AFM imaging of liposomes adsorbed on gold surfaces (Figure 2) showed variation in adsorption of the liposome structure with functionalization. Hydrophobic surfaces showed non-uniform coverage compared to the pure gold surface. The hydrophilic surface had the most uniform coverage. Liposome clusters were

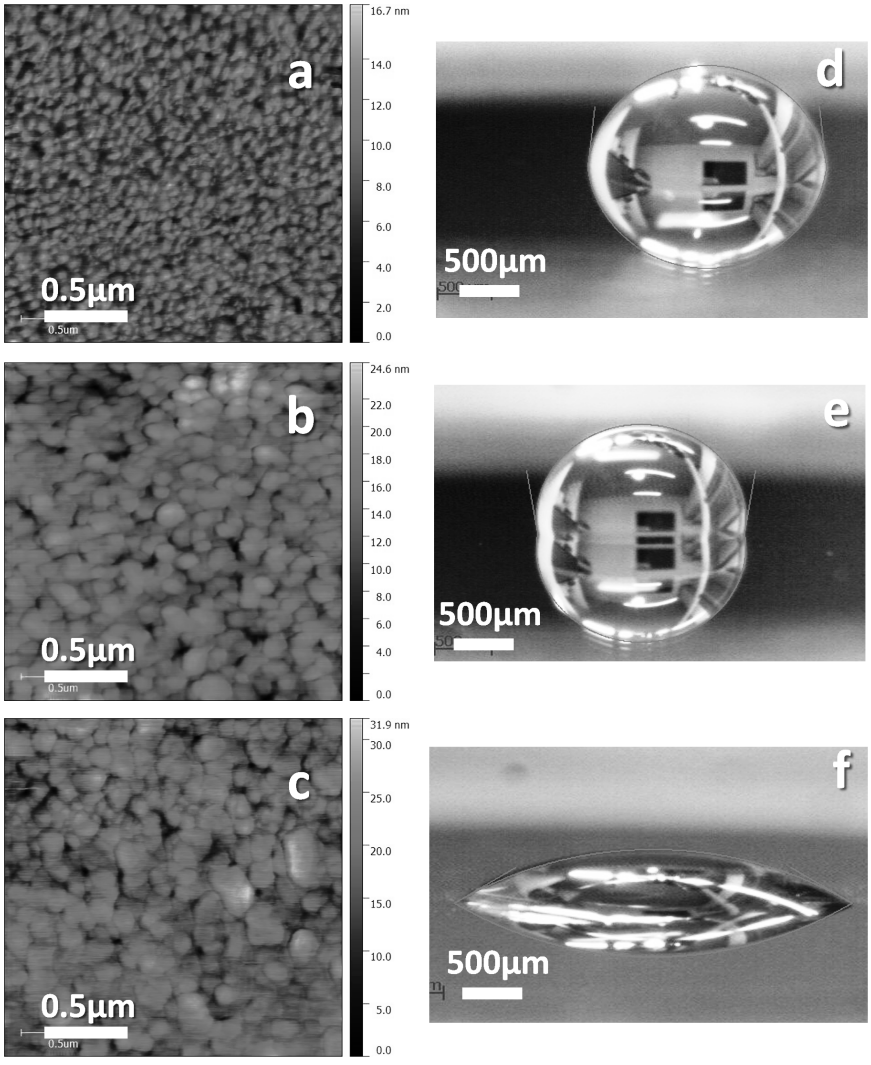

Figure 1. This figure demonstrates the characterization of the functionalized surfaces by means of Atomic Force Microscopy (AFM) and contact angle measurements. a, b and c) presents AFM topographic images of the surfaces of the gold electrodes: pure gold, gold functionalized with octanethiol (hydrophobic) and gold functionalized with 3-MPA (hydrophilic). d, e and f) illustrates the respective contact angle measured for each surface (83°, 99° and 32°).

observed on all surfaces. The DMPC structures covered almost 60% of the three surfaces studied, with some liposome structures remaining intact, partially corroborating the results of Goda & Miyahara (2017, 2018) and Morita et al. (2006). The formation of liposome clusters on all surfaces and the difference in the coverage and uniformity of the lipid layer is a function of the functionalization, as the diameter of the liposome structures adsorbed on the different surfaces. This demonstrates that most of the adsorbed structures have a diameter close to 200 nm on all surfaces. However, the maximum diameter of the structures varies significantly between surfaces, being larger on the hydrophilic surface (3-MPA) and smaller on the hydrophobic surface (octanethiol).

The dynamic test consisted of immersing the QCM electrodes in a beaker with water, then changing it to nanoscale DMPC liposomes in aqueous solution. For all surfaces, there is a deposition of intact liposome structures, observed with AFM and QCM techniques. The QCM technique revealed significant differences in liposome adsorption dynamics depending on the functionalization of the gold surface. Adsorption on hydrophilic surfaces (gold functionalized with 3-MPA) was considerably faster, occurring in only 20 seconds, compared to adsorption on hydrophobic surfaces (pure gold

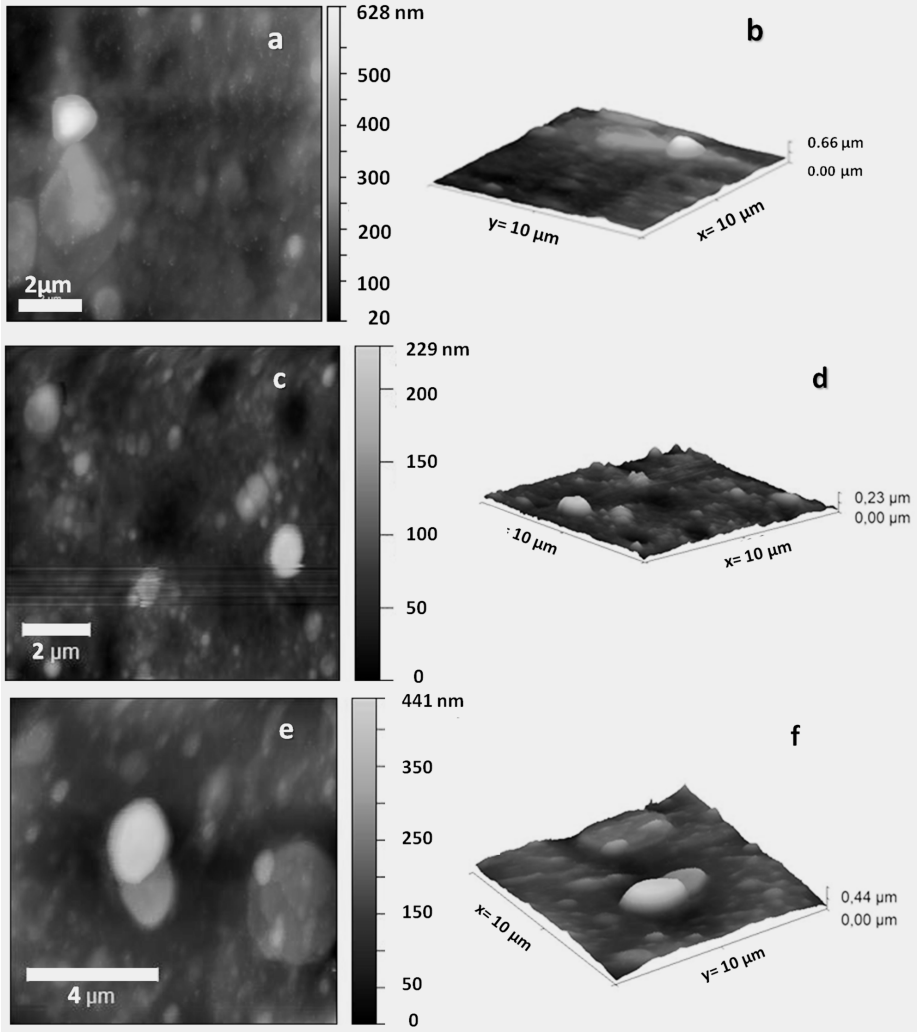

Figure 2. This figure compares the AFM images of DMPC liposomes adsorbed on gold surfaces with different functionalization after air drying for 24h. The 2D and 3D images (a-f) show the variation in the adsorption of the liposome structure on pure gold (a-b), hydrophobic gold (c-d) and hydrophilic gold (e-f). The coverage area of the liposome structures is (47±5)% onto pure gold (a-b), (48±5)% onto hydrophobic gold (c-d) and (57±5)% onto hydrophilic gold (e-f).

and gold functionalized with octanethiol), which required much longer times (>1,000 seconds) and even mechanical agitation to stabilize the resonance frequency. This difference in adsorption kinetics can be attributed to the greater affinity of zwitterionic liposomes for the hydrophilic surface, which has a more organized water layer that is favorable for interaction with the polar heads of phospholipids (Zafiu et al. 2011, Soares et al. 2010). Hydrophobic surfaces, on the other hand, have a less structured water layer, which makes it difficult for liposomes to approach and adsorb (depositing ~150ng). Additionally, adsorption on hydrophilic surfaces resulted in a higher amount of deposited mass (~200 ng), compatible with the formation of multiple lipid bilayers onto gold electrode. These results suggest that surface functionalization, and consequently wettability, plays a crucial role in the dynamics of supported lipid bilayer formation, influencing the rate and extent of liposome adsorption (Figure 3). Hydrophilic surfaces, obtained with 3-MPA functionalization, promote faster and more uniform adsorption of liposomes, forming more robust supported lipid bilayers, indicated by the higher adsorbed mass measured by QCM. In contrast, hydrophobic surfaces, both pure gold and octanethiol-functionalized gold, exhibit slower and less homogeneous adsorption.

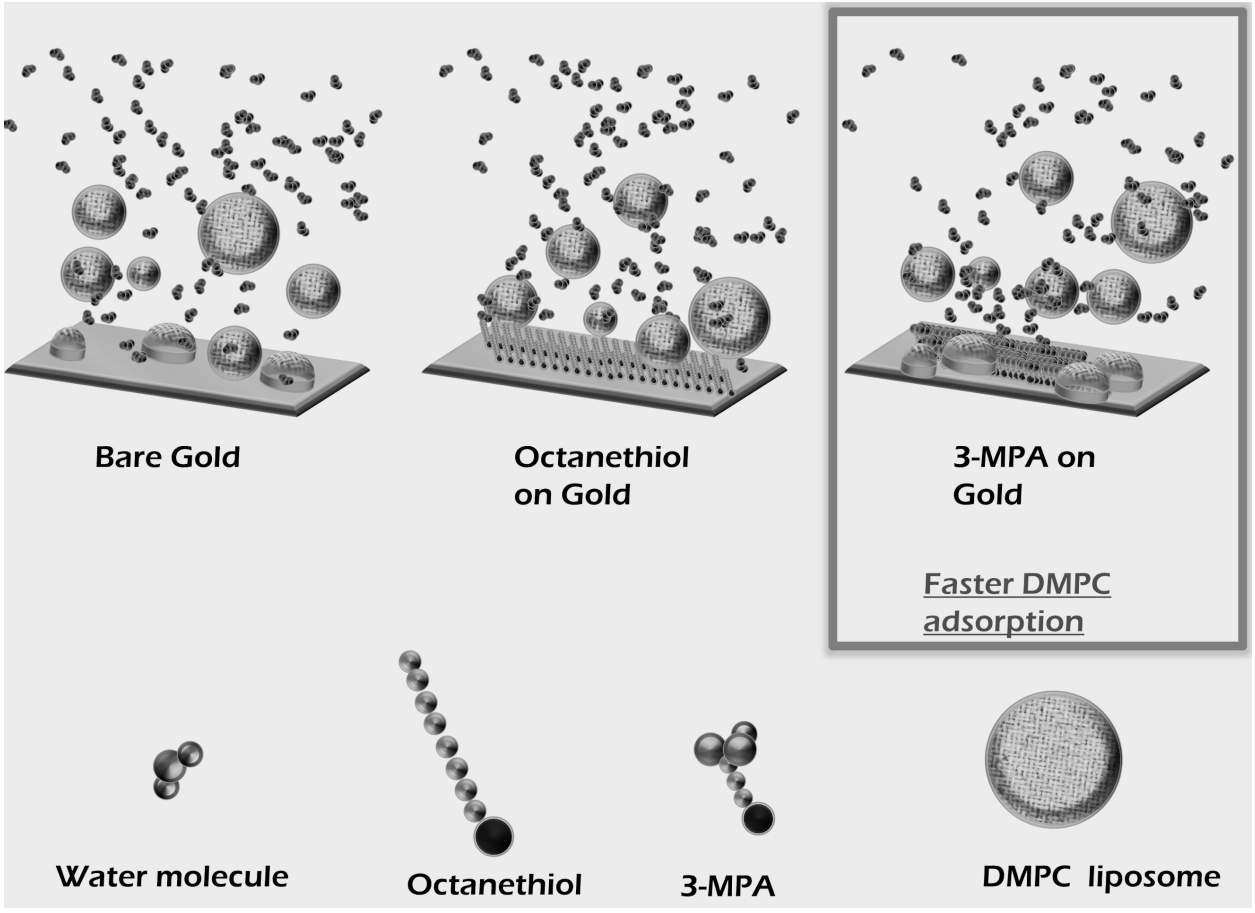

Figure 3. Functionalization of gold surfaces with thiol self-assembled monolayers (SAMs) significantly influences surface wettability and adsorption of DMPC liposomes, especially hydrophilic surfaces of 3MPA SAM´s (highlight).

CONCLUSIONS

A thin thiol monolayer drastically alters the wettability of the gold surface due to its different molecular polarities. The wettability is not due to increased roughness, but rather to the polarity of the film. Depending on the designed functionalization, it drastically alters the solid-liquid interface, both macroscopically, with respect to contact angle values, and microscopically, as observed by the adsorption dynamics of zwitterionic liposome species, resulting in faster adsorption on the hydrophilic surface to form solid-supported lipid bilayers.

The QCM study shows great potential for other technological applications related to solids and liquids in relation to functionalization processes, such as the use of liposome structures as vectors for drug delivery systems. The results of AFM surface topographic imaging show that the hydrophilic coating allows for a more uniform solid-supported lipid bilayer, compared to hydrophobic surfaces, DMPC structures larger than 200 nm in diameter are extended over the surface. The QCM study provides information on the speed of DMPC liposome coating, showing that liposome adsorption on hydrophobic surfaces is much slower.

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