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Formation and characterization of Langmuir and Langmuir-Blodgett films of Newkome-type dendrons in presence and absence of a therapeutic compound, for the development of surface mediated drug delivery systems

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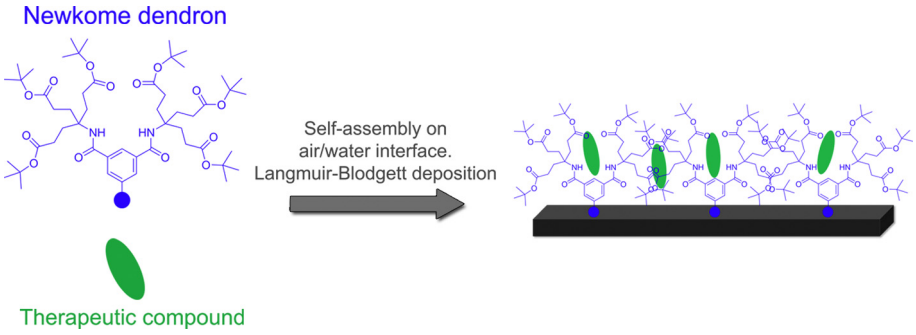
GRAPHICAL ABSTRACT



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ABSTRACT

Organic macromolecules with dendrimeric architectures are polymeric materials potentially useful as nanocarriers for therapeutic drugs. In this work, we evaluate a series of Newkome-type dendrons in Langmuir and Langmuir-Blodgett films as platforms capable of interacting with a potential antitumoral agent. The nanocomposite is proposed as model for the development of surface mediated drug delivery systems. We were successful in the formation and characterization of pure (dendrons) and composite (drug-dendron) stable and reproducible monolayers, and their transfer to solid substrates. A detailed study of topographic characteristics of the generated surfaces by atomic force microscopy was conducted. Furthermore, we probed dendron monolayer films as anchorage surfaces for mammalian cells. Normal cell attachment and proliferation on the surfaces were observed. No evident cytotoxic effects were detected, demonstrating the adequate biocompatibility of the surfaces.

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1. Introduction

Dendrimeric structure is a general category of macromolecules that constitutes the fourth class of polymeric architectures [1]. Dendrimers belong to this family, which are characterized by a well-defined molecular weight and a precise three-dimensional structure [2,3]. Dendrimers are formed by dendrons, which possess a focal point, branches and numerous end functional groups [4]. The structural properties and the monodisperse nature of dendrons and dendrimers make them interesting materials for applications in optoelectronic [5], sensing, catalysis and nanomedicine [6–8]. In particular, numerous studies demonstrate the applicability of dendrons and dendrimers as drug nanocarriers in solution [9–12]. The development of this application has been carried out using two main drug-dendrimer association strategies: covalent attachment of the pharmacological active compounds to dendrimer surface, and the incorporation in its interior or surface, sustained by specific and non-specific interactions. When the drug-dendrimer composite reaches the action site, the drug is released due to physical or chemical changes suffered by the complex in response to some stimulus present in the medium. The drug release trigger mechanism can be originated in exogenous and endogenous stimuli. External induced exogenous stimuli can be generated by temperature changes, illumination, electric and magnetic fields application, etc. On the other hand, endogenous stimuli involve local physiological aberrations originated by the pathological conditions, such as pH variations, proteins and/or enzyme concentrations, and temperature [13,14].

Over the past decade, a novel opportunity has been widely investigated in the field of drug delivery; the utilization of surface polymer films and coatings [15]. In these applications, the deposited polymer films can act as a reservoir for the drug, allowing the controlled release of therapeutic molecules [15–18]. Dendrons are presented as very promising candidates for surface mediated drug delivery systems, because their structure holds a focal point that can be modified in order to optimize the junction to the solid substrate, and functionalizable branches that are able to interact with the active drug [19]. In this sense, it is very important to study how structural properties influence the dendrimeric films conformation and stability. Langmuir and Langmuir-Blodgett techniques provide versatile tools for the study of these characteristics [20–22]. These methods allow the generation and characterization of monomolecular films at the air-liquid interface and their transfer to solid substrates [23–25]. Various authors have described Langmuir and Langmuir-Blodgett formation of dendrimer monolayers. The reports include PAMAM structures [26], poly(propyleneimine) dendrimers [27], carbazole [19], poly(aryl ether) dendrimers [28], poly(benzyl ether) dendrons [29–31], linear-dendritic block copolymers [32], among others. The studies are focused on the influence of various factors (such as generation number, focal point, and terminal groups) on the formation and stability of Langmuir monolayers and transferred films [33–36]. However, there are very few studies where Langmuir monolayers of dendrimeric systems are proposed as potential active compound reservoirs, for the development of surface mediated drug delivery systems [21,37].

In previous works [38–41] we have investigated the association of poorly soluble active drugs with dendrimers in water solution and in air-water interface. Also the phase transfer (water-1,2-dichloroethane) characteristics of a series of dendrons was analyzed [42]. We found that the dendrimer-drug interactions increase the drug solubility in water, and that the composites are able to retain these small molecules at the air-water interface in Langmuir monolayers [41]. However, we did not succeed in their controlled and reproducible transfer to a solid substrate, possibly due to the dendrimer-drug mixture dissolution in the aqueous sub-phase during the transfer process. Thus, it was not possible to construct a mixed film as a model of a drug delivery system. In this paper we describe the formation of Langmuir monolayers by Newkome-type dendrons with different structural characteristics (Fig. 1). The systems hold different focal points, branches and terminal groups, which are highlighted by circles of different colors in Fig. 1. Aniline (in **BBA** and **BTA**) and nitrobenzene (in **BBN**) residues were selected as focal points in order to analyze their influence in the monolayer formation and transfer. On the other hand, two kinds of terminal groups were evaluated: tert-butyl (in **BBA** and **BBN**) and methyl ester (in **BTA**), with the aim of analyzing their possible effect in the monolayer characteristics, and in the formation of composite dendron-therapeutic drug Langmuir and Langmuir-Blodgett films. Methyl thio-5-propyl-1H-benzimidazole 2-yl carbamate (Albendazole, ABZ, Fig. 1) was used as model therapeutic agent. ABZ is a drug commonly utilized as anthelmintic [43] that also holds a good potential as antitumor agent [44,45]. However, ABZ is poorly soluble in water [46], and unable to form ordered Langmuir films. Thus, we evaluated the Newkome-type dendrons as platforms capable of interacting with ABZ, and including the drug in thin films at solid surfaces. We present here the interfacial behavior of pure dendrons and ABZ-dendron mixed systems. We were successful in the formation and characterization of pure and composite stable and reproducible monolayers, and their transfer to solid substrates. Also, we conducted a detailed study of structural characteristics of generated surfaces by atomic force microscopy (AFM). Dendron modified surfaces were used as substratum to evaluate mammalian cell adhesion and proliferation, as evidence of their biocompatibility.

2. Materials and methods

2.1. Materials

Newkome-type dendrons were synthesized, purified and characterized using the methodology already described [4,47,48]. Methyl (5-[propylthio]-1H-benzimidazol-2-yl) carbamate was purchased from Sigma-Aldrich. All solvents were obtained from Sintorgan in HPLC quality. Deionized water was obtained from Elga Classic equipment (resistivity of 18 MΩ cm).

2.2. Langmuir and Langmuir-Blodgett films

Monomolecular monolayers formation and their transference to solid substrates were carried out using a Nima Technology Model 611 Langmuir-Blodgett (L-B) trough. The surface pressure was measured using the Wilhelmy plate method. Deionized water was used as subphase. Blank control isotherms were determined prior dendrons spreading in order to confirm the purity of the surface. Dendron solutions in chloroform (50 μL, 6×10^{-4} M), were carefully spread on the water surface and 10 min were allowed to pass before measurements, in order to permit solvent evaporation. Mixed drug-dendron monolayers were formed from chloroform solutions of both molecules in a molar ratio [ABZ]/[dendron] of 4:1. In all the experiments the subphase temperature was kept constant by thermostatic water circulation in the trough. For monolayers compression and expansion barrier speed was 50 cm^2/min. As solid substrates for monolayer deposition we used freshly cleaved hydrophilic mica sheets, which were previously immersed in the subphase. Then the monolayers were formed at the air-water interface as already described and compressed at 50 cm^2/min. The monolayers were transferred to the slides by the vertical transfer method at 5 mm/min at constant surface pres-