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Vibrational spectroscopy for probing molecular-level interactions in organic films mimicking biointerfaces

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ABSTRACT

Investigation into nanostructured organic films has served many purposes, including the design of functionalized surfaces that may be applied in biomedical devices and tissue engineering and for studying physiological processes depending on the interaction with cell membranes. Of particular relevance are Langmuir monolayers, Langmuir–Blodgett (LB) and layer-by-layer (LbL) films used to simulate biological interfaces. In this review, we shall focus on the use of vibrational spectroscopy methods to probe molecular-level interactions at biomimetic interfaces, with special emphasis on three surface-specific techniques, namely sum frequency generation (SFG), polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) and surface-enhanced Raman scattering (SERS). The two types of systems selected for exemplifying the potential of the methods are the cell membrane models and the functionalized surfaces with biomolecules. Examples will be given on how SFG and PM-IRRAS can be combined to determine the effects from biomolecules on cell membrane models, which include determination of the orientation and preservation of secondary structure. Crucial information for the action of biomolecules on model membranes has also been obtained with PM-IRRAS, as is the case of chitosan removing proteins from the membrane. SERS will be shown as promising for enabling detection limits down to the single-molecule level. The strengths and limitations of these methods will also be discussed, in addition to the prospects for the near future.

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1. Introduction

The importance of biointerfaces has been emphasized in view of the increasing use of biomaterials and for biomedical applications, both in diagnosis as well as in therapy. Adequate interactions at the interface are necessary between biomaterials replacing parts of living systems and living tissues [1,2]. A clear example is the area of tissue engineering [3], for cell growth and differentiation are essential for producing artificial organs [4–8] and implants [9–19]. In drug delivery systems, surface coatings may be required for some types of release [20–24]. For the design of new pharmaceutical drugs, an important ingredient is the

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identification of the mode of action which is normally associated with the cell membranes (i.e. biointerfaces). In clinical diagnosis, the fabrication of novel biosensors relies increasingly on functionalized surfaces that may also be considered as biointerfaces [25–29].

Biointerfaces are now investigated using a myriad of experimental methods and computer simulations [30]. These include techniques to probe surface properties such as wetting and adhesion, methods to determine structure, e.g. X-ray [31–33] and neutron reflectivity [34–37], several types of microscopy and various spectroscopic methods [38–43]. For the purposes of this review paper, we shall concentrate on the vibrational spectroscopy methods, whose use will be exemplified for two types of systems associated with biointerfaces. The first is model cell membranes that are mimicked with nanostructured films, including Langmuir monolayers [44–46], Langmuir–Blodgett (LB) films [47,48] and layer-by-layer (LbL) films [49–51]. The second type of system is functionalized surfaces where biomolecules are employed in coatings for several applications.

The review is organized as follows. Section 2 brings a brief description of the three vibrational spectroscopic methods considered here, namely infrared absorption-based spectroscopy, sum-frequency generation (SFG) spectroscopy and surface-enhanced Raman scattering (SERS) spectroscopy. Examples of their use for cell membrane models and functionalized surfaces are given in Section 3. We emphasize here that our survey of possible uses of these methods is by no means exhaustive; we simply selected a variety of papers to illustrate the strengths of the methods for biointerfaces. Section 4 is dedicated to a comparison of strengths and limitations of the methods considered, which is followed by Conclusion and future prospects in Section 5.

2. Vibrational spectroscopy techniques

In this section, a brief introduction to the spectroscopy techniques most useful for biointerfaces will be provided, with the aim of offering some background for understanding the results and contributions to be discussed throughout the review paper. Experimental details and the theoretical background behind the techniques are either omitted or presented very briefly, and the readers are referred to the literature. For instance, readers interested in the use of vibrational spectroscopy for investigating biological applications may consult ref. [52]. Because it is less frequently used, sum-frequency generation (SFG) is described at a greater length.

2.1. Infrared absorption-based spectroscopic methods

The electromagnetic radiation in the infrared (IR) region of the spectra has oscillation frequencies that match the characteristic frequency of vibrational modes of matter, and therefore IR spectroscopies have been ubiquitously used as characterization techniques. A variation of the traditional transmission Fourier Transform Infrared spectroscopy (FTIR), developed by *Greenler* [53], was based on measuring the reflected light from a film supported on reflective substrates (e.g. metals), and is now referred to as IRRAS (infrared reflection-absorption spectroscopy). With IRRAS one has improved sensitivity and orientation specificity, which is achieved with the interference of the incident and reflected components of the electric field, attained at incidence angles of ca. 80°, and the surface selection rule according to which only *p*-polarized light will be reflected from the surface [53]. Derivations of reflection–absorption IR technique were developed over the years, which include the internal total reflection–absorption FTIR spectroscopy (nowadays known as ATR — attenuated total reflection) [54]. ATR is now among the most useful tools to characterize biological films supported by solid crystals [55,56]. As a method to probe biointerfaces, IRRAS had its applicability largely expanded when it was adapted to Langmuir monolayers [57,58].

An extension to IRRAS was made by Golden and co-workers in the early 1980s [59], where the incident polarized infrared source had its beam polarization alternated between *s* and *p* at a frequency of tens of kHz. They were then able to calculate the differential reflectivity (S), given in Eq. (1), where R_p and R_s are, respectively, the reflectivities for *p* and *s* polarizations. The new variant was named PM-IRRAS, where the letters PM stand for polarization modulated.

$$S = \frac{R_p - R_s}{R_p + R_s}. \quad (1)$$

Blaudez and co-workers [60,61] realized the importance of PM-IRRAS and applied it to the characterization of Langmuir films. Water is not a perfect reflector and therefore both *p* and *s* polarizations can be simultaneously absorbed because the surface selection rule is not applicable for the air/water interface. Since *p*-polarized light is more sensitive to vertically oriented dipoles and *s*-polarized beam is sensitive to horizontally oriented ones, the relative orientation of chemical groups from the film constituents can be estimated from the analysis of the differential reflectivity. Moreover, by subtracting the bare water reference spectrum from the film reflectivity ($\Delta S = S_{film\ +\ water} - S_{water}$), contributions to the signal can be filtered out from isotropically oriented molecules, such as CO_2 and H_2O (the latter from vapor right above the film or from the subphase beneath it), which are the main noise sources to the final spectrum.

In the 1980s another FTIR-related technique was developed based on the enhancement of IR absorption by *plasmons* in metallic nanostructures, which was named Surface-enhanced infrared-absorption spectroscopy (SEIRA). In recent years, SEIRA has been used to probe metal-supporting biological and organic thin films [62,63].

Because of the large number of contributions in the literature associated with IR-related methods, we chose a few examples of the use of ATR, SEIRA, IRRAS and PM-IRRAS to characterize films mimicking biointerfaces to be mentioned in this review, while many others can be found in other pieces in the literature [64,65].

2.2. Sum-Frequency Generation spectroscopy

Sum-Frequency Generation spectroscopy (SFG) is a nonlinear optical spectroscopic technique with which to obtain the vibrational spectrum of interfacial molecules, discriminating them from those in the bulk material. It is therefore surface-specific, with intrinsic selectivity to interfacial contributions. Here we describe only the fundamentals of SFG spectroscopy. A detailed theory can be found elsewhere [66–68], and references [69,70] are tutorial reviews of its applications to many fields of surface science. Recent reviews of applications of SFG spectroscopy to selected fields are also available [71–73].

In SFG spectroscopy, two high-intensity laser beams at frequencies ω_{vis} and ω_{IR} overlap at an interface and generate an output beam at frequency $\omega_{SFG} = \omega_{vis} + \omega_{IR}$ in the reflection direction. The intensity of the SFG signal is proportional to the square of the effective second-order nonlinear susceptibility of the interface, $\chi^{(2)}_{eff}(\omega_{SFG} = \omega_{vis} + \omega_{IR})$. As second-order process, SFG is forbidden in media with inversion symmetry, such as gases, bulk liquids, amorphous solids and most crystals of achiral molecules, but allowed at interfaces where the inversion symmetry is broken. This is why SFG spectroscopy is intrinsically sensitive to interfaces. Since the process relies on broken inversion symmetry, only molecules without inversion symmetry may be detected in SFG. However, if such molecules arrange at an interface with random orientations, the net SFG signal vanishes. Conversely, if there is a substantial SFG signal, it can be concluded that molecules have a net average orientation at the interface. Thus, we can obtain information about the average orientational ordering of the interfacial molecules. For vibrational spectroscopy, ω_{IR} is tunable in the mid-infrared, (in the range of the vibrational modes of the surface molecules), while ω_{vis} is a fixed frequency within the visible spectrum, so that ω_{SFG} is also in the visible–UV and can be detected with high sensitivity. In some cases, ω_{vis} may be tunable as well, yielding the electronic spectrum of interfacial