Optical grating coupler biosensors

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Abstract

By incorporating a grating in a planar optical waveguide one creates a device with which the spectrum of guided lightmodes can be measured. When the surface of the waveguide is exposed to different solutions, the peaks in the spectrum shift due to molecular interactions with the surface. Optical waveguide lightmode spectroscopy (OWLS) is a highly sensitive technique that is capable of real-time monitoring of these interactions. Since this integrated optical method is based on the measurement of the polarizability density (i.e., refractive index) in the vicinity of the waveguide surface, radioactive, fluorescent or other kinds of labeling are not required. In addition, measurement of at least two guided modes enables the absolute mass of adsorbed molecules to be determined. In this article, the technique will be described in some detail, and applications from different areas will be discussed. Selected examples will be presented to demonstrate how monitoring the modification of different metal oxides with polymers and the response of the coated oxides to biofluids help in the design of novel biomaterials; how OWLS is useful for accurate bioaffinity sensing, which is a key issue in the development of new drugs; and how the quantitative study of protein–DNA/RNA and cell–surface interactions can enhance the understanding of processes in molecular and cellular biology.

Keywords: Optical grating coupler biosensors; Adsorption kinetics; Cell–surface interactions; Protein–DNA interactions; Lipid bilayers; Surface modification

1. Introduction

The optical grating coupler biosensor (OGCB) is a relative newcomer in the field of integrated optics, i.e., the science of light guided in structures smaller than the wavelength of the light. As a technique for investigating processes at the solid/liquid interface, it pushes the sensitivity (as well as the convenience and wealth of obtainable information) to levels even higher than the already impressive achievements of the older techniques of ellipsometry [1,2], scanning angle reflectometry (SAR) [3] and surface plasmon resonance (SPR) [4]. Ellipsometry and SAR are closely related to optical waveguide lightmode spectroscopy (OWLS) [5], and SPR can be cast in the same form [6]. Each has certain advantages: ellipsometry can be used with both transparent and non-transparent substrates; SAR can draw on a large collection of associated theoretical work, especially regarding the properties of thin films; and SPR can, and indeed must, be used with a noble metal substrate. However, OWLS not only has superior intrinsic sensitivity but also is convenient and versatile, especially for biological applications.

Theory, methods and application examples from the very beginning of the history of grating coupler waveguide sensors up to 1995 have been reviewed by Lukosz [7]. The purpose of this review is to offer a recent summary of the most commonly used OGCB, OWLS, as it is currently applied in practice to solve biological and biomedical problems. Theory, methods and applications are covered. The review is aimed at the molecular biologist, biochemist, biomaterials specialist or biomedical researcher who is searching for an advantageous method for investigating biomolecular...
2. Theory of OWLS

OWLS is a powerful method for monitoring the adsorption of macromolecules on a sensor surface. Its sensing principle is based on the evanescent electromagnetic field of guided light, which extends a few hundred nanometers above the waveguide and is similar to the evanescent field of surface plasmons used in the SPR technique [4,6,8]. A grating serves to incouple light into a planar optical waveguide in which the light then propagates, generating an evanescent field. This evanescent field is used to probe the optical properties of the solution in the vicinity of the surface. This is the basis for the sensitivity of the method to changes in the refractive index when deposition of macromolecules occurs.

2.1. Measuring principle

The OWLS technique uses an optical grating to excite the guided modes of a planar waveguide. The incident plane-polarized laser is diffracted from the grating and starts to propagate via internal reflections inside the waveguide. The thousands of internal reflections under the illuminated area interfere and excite a guided mode if the phase shift during one total internal reflection equals zero (mono-mode waveguide) (Fig. 1). This only occurs at two distinct incident angles for mono-mode waveguides (represented by the two effective refractive indexes for the transverse electric (N_{TE}) and the transverse magnetic (N_{TM}) polarization modes) according to the mode equations [9] (see Eq. (1) and inset of Fig. 2).

\[ 0 = \frac{2\pi}{\lambda} \sqrt{n_{TE}^2 - N^2} \]

\[ \times \left( d + \frac{n_{A}^2 - n_{C}^2}{n_{F}^2 - n_{C}^2} \left[ \frac{(N/n_{C})^2 + (N/n_{A})^2 - 1}{(N/n_{C})^2 + (N/n_{F})^2 - 1} \right]^p \right) d_{A} \]

\[ - \arctan \left( \frac{n_{F}^2}{n_{S}^2} \sqrt{n_{F}^2 - N^2} \right) \]

\[ - \arctan \left( \frac{n_{F}^2}{n_{S}^2} \sqrt{n_{F}^2 - N^2} \right). \]  

(See Nomenclature.)
These two equations ($\rho = 1$ for the TM mode and $\rho = 0$ for the TE mode) depend on the two effective refractive indexes ($N$), $N_{\text{TE}}$ and $N_{\text{TM}}$, which can be related to the incoupling angles, $\alpha_{\text{TE}}$ and $\alpha_{\text{TM}}$, respectively. These angles correspond to peaks in the light-mode spectrum according to

$$N = n \sin \alpha + l \lambda / A,$$

(2)

where $n$ is the refractive index of air, $\lambda$ is the wavelength of the laser, $A$ is the grating constant and $l$ is the diffraction order. Continuously measuring the shift of these incoupling angles allows the direct on-line monitoring of the adsorption of macromolecules above the grating without the need for any labeling procedure (see Fig. 2). The method is highly sensitive (i.e., detection limits < 1 ng/cm$^2$) up to a distance of a few hundred nanometers above the surface of the waveguide. Furthermore, a measurement time resolution of seconds permits an in situ, real-time study of adsorption kinetics.
The advantage of this technique over the SPR sensor is that two parameters: the thickness \( (d_A) \) and the refractive index \( (n_A) \) of the adsorbed layer can be simultaneously determined from the two measured parameters using the mode equations [10]. Since two equations are solved for two parameters, no assumption (e.g., for the refractive index as commonly used in SPR and ellipsometry calculations) has to be made other than the usually reasonable assumption of a homogeneous adsorbed layer. The absolute values for the surface adsorbed mass density \( (M) \) then can be directly calculated from the thickness and refractive index values according to de Feijter’s formula (see Eq. (3)) [11]. This is very useful especially when the density (thus the refractive index as well) of the adsorbed layer changes during adsorption (i.e., due to hydration, denaturation, etc.)

\[
M = d_A \frac{n_A - n_C}{dn/dc} \tag{3}
\]

A refractive index increment \( (dn/dc) \) value of 0.18 cm\(^3\)/g can be used for most of the protein adsorption calculations [10,12]. However, by using a Rayleigh interferometer, \( dn/dc \) values can be explicitly measured for the chosen analyte and buffer.

OWLS instruments are equipped with flow-through cuvettes to introduce the liquid sample solution to the grating part of the sensor surface in a reproducible manner (see Fig. 1) [13,14]. A standard measurement consists of the following steps (see Fig. 2):

- **Baseline-run** in pure (non-film forming) buffer: This allows the determination of the refractive index, \( n_F \), and the thickness, \( d_F \), of the waveguiding film and eliminates the influence of small differences in optical properties from one sensor chip to another.
- **Adsorption experiment**: This monitors the evolution of two characteristic parameters, \( n_A \) and \( d_A \), as the adsorbed layer forms, allowing in situ determination of the adsorbed protein mass and the kinetics of adlayer formation.
- **Desorption phase**: This is a washing step in pure buffer, which provides information about the stability of the formed adlayer and possible desorption kinetics.

These last two steps are used for the kinetic analysis, which is discussed in the next section. They can be followed by subsequent adsorption and desorption experiments if the interaction among several analytes is of interest (e.g., in bioaffinity sensing).

### 2.2. Analysis of binding kinetics

An important advantage of optical grating coupler biosensors is that their intrinsically high sensitivity allows the kinetics of binding to a smooth planar surface to be measured. A further advantage of OGCB is that the absolute mass of bound ligands can readily be calculated. Not only can the stoichiometry of ligand–receptor binding then be unambiguously determined, but saturation binding levels can be used to determine the area occupied per bound molecule and, by comparison with the area deduced from knowledge of the native biomolecule, used to infer the extent of conformational changes upon binding.

Given that the prerequisite of a smooth sensor surface of known geometry is satisfied, ideally the solution of protein or other molecules is introduced by smooth laminar flow. Knowledge of the geometry of the flow-through cuvette permits the application of the equation for convective diffusion to the transport step. Hence transport can be effectively separated from the binding. The two are characterized, respectively, by the diffusion coefficient, \( D \), and the adsorption rate coefficient, \( k_a \), and either or both can be determined from a series of OWLS measurements.

The rate of adsorption can be written as the product of three terms: the adsorption rate coefficient, \( k_a \); the vicinal biomolecule concentration, \( c_v \); and the available area function, \( \phi \) (i.e., the fraction of the surface available for adsorption or binding):

\[
\frac{dM}{dt} = k_a c_v (c_b, D, k_a) \phi(M, a), \tag{4}
\]

where \( M \) is the adsorbed mass per unit area, \( c_b \) is the bulk concentration and \( a \) is the area occupied by a single molecule on the surface. This equation can be either fitted directly to numerically differentiated kinetic \( (dM/dt, M) \) data or numerically integrated and fitted directly to the \( (M, t) \) data.

The hydrodynamic conditions prevailing in a flow-through cuvette are discussed in more detail in Ref. [15]. Approximate expressions for \( c_v \) (which is always \( \leq c_b \)) rely on the fact that it relaxes more slowly than \( M \). Thus in the absence of desorption,

\[
c_v = c_b \frac{(1 + k_a \phi \delta / D)}{D}, \tag{5}
\]

where \( \delta \) is the thickness of the diffusion boundary layer.

The function \( \phi \) can be expanded as a polynomial in \( M \):

\[
\phi = 1 - b_1 M + b_2 M^2 + b_3 M^3 + \cdots, \tag{6}
\]

where the coefficients \( b_1, b_2 \) and \( b_3 \) depend on the mode of adsorption and on the particle size \( (a) \) and shape [16,17]. Experience has shown that it is generally unnecessary to take powers of \( M \) higher than the third into account. Often visual inspection of the \( (M, t) \) dataset or its derivative provides sufficient information to identify the adsorption mode. In most cases, Eq. (4) or its integral can be fitted to the data with just two free parameters, \( k_a \) and \( a \).
Dissociation, investigated experimentally by replacing the ligand solution flowing over the waveguide by the corresponding ligand-free solvent, is incorporated into Eq. (4) by subtraction of the term $k_d(t)M$ from the right-hand side. Time-invariant dissociation rate constants, $k_d$, leading to pure exponential dissociation, are the exception rather than the rule. In general, multiple binding interactions between receptor and ligand produce a strongly non-exponential behavior [18]. Sometimes desorption can be approximated by a double exponential [19], and sometimes the time constant of the second exponential may be taken as infinite (irreversible adsorption) [20].

3. Instrumentation and sensor manufacturing

Various types of optical biosensors and instrumentation have been reviewed by Robinson [21] and more recently by Ramsden [5]. Instruments based on the optical grating coupler principle are commercially available.

3.1. The OWLS instrument

The optical setup of an OWLS instrument is illustrated in Fig. 1. A computer-controlled stepping motor rotates the sensor holder with high accuracy and reproducibility. When the incoupling conditions, described in the previous section, are fulfilled, the waveguide guides the incoupled light of the TE$_0$ or TM$_0$ mode to the photodetectors. In order to determine precisely the direction normal to the waveguide, which is needed to calculate the absolute values of the incoupling angles, the OWLS instrument scans from $-7$ to $+7$ degrees and measures the incoupling angles in both directions. The absolute values correspond to the means of the angles measured in the two directions.

The positive half of a typical lightmode spectrum measured by an OWLS instrument is shown in the inset of Fig. 2. Typical angular resolution is better than $10^{-4}$ degree, and in the continuous effective refractive index measurement mode, when only one incoupling angle change is monitored, the temporal resolution is a few seconds. The instrumental resolution in effective refractive index change is $\Delta N \approx 10^{-6}$.

The main drawback of the OWLS setup is that the rotating sensor configuration is not suitable for miniaturization. Several alternative sensing schemes based on the grating coupler waveguide sensor principle and excluding moving parts have been proposed (two or more gratings, tunable laser light source, reflection mode, bidiffracting or chirped grating, etc.) and it seems likely that one or more of this kind of instrument will appear in the market in the near future.

3.2. Sensor manufacturing

The fabrication of a cheap, reliable, reproducible grating coupler waveguide sensor requires efficient manufacturing of two components: a low-loss optical waveguiding layer and a low-modulation, high-frequency grating.

Good optical waveguiding films are usually made from metal oxides that have a high-optical refractive index in the visible range. The required thin (in the range 100–200 nm), and low-loss ($\leq 1$ dB/cm) waveguiding layers need to be deposited on glass or plastic substrates with high reproducibility. Commercially available vacuum evaporators and sputtering equipment can be used for this purpose but are too expensive for medium-volume sensor production. An economical and highly effective method for waveguide fabrication is the sol–gel technique [22], in which solid, glassy layers are produced from metal alkoxides or colloidal solutions after heat treatment at a high temperature. The thickness uniformity, optical loss and smoothness of these films are excellent.

To obtain high sensitivity, a low-modulation (typically 10 nm), high-periodicity (typically $A \leq 500$ nm) optical grating is needed. Conventional photolithography, dry etching, holographic exposure of photosensitive layers, and direct laser ablation of the solid surface are all suitable methods, but are quite capital intensive.

An elegant and economical method to produce high-frequency gratings is to emboss a sol–gel-coated substrate against a master grating [23]. Although embossing itself is straightforward, high precision is needed to produce reproducible gratings. The method is based on the fact that a sol–gel layer that is applied as a thin film onto the surface of the substrate will harden spontaneously. If the semi-hardened sol–gel layer is embossed by a master grating the sol–gel layer can be deformed in the right way without sticking to the master grating. An atomic force microscope (AFM) image of an embossed grating is shown in Fig. 3.

After heating the sol–gel layer, a solid material with a grating depth of 5–20 nm is obtained. High-refractive index, metal-oxide-based sol–gel materials that can be used for grating embossing are available so the same material can be used for both the waveguide and grating. Commercially available grating-coupler waveguide sensors have been made from SiO$_2$, Ta$_2$O$_5$ and SiO$_2$/TiO$_2$.

The above-mentioned methods are also suitable for producing a variety of grating-coupler waveguide structures, including waveguides with more than one grating, with chirped or bidiffracting gratings, and with stacked gratings, as well as stacked waveguides with gratings in each waveguide layer [24].
3.3. Surface modifications

An important aspect with regard to both basic studies and applications is the possibility of adapting the sensor chips’ surface chemistry to the particular needs of the experiment. For OWLS applications, coating the sensors with thin films of an appropriate material is a straightforward way to provide the necessary flexibility. The requirements for such surface-modifying coatings are that they should be optically transparent and should have low optical loss.

3.3.1. Inorganic coatings

Among the possible inorganic coatings fulfilling the optical requirements, metal oxide and nitride layers are the most commonly used. Vacuum evaporation, sputtering, LPD, CVD and sol–gel processes are used to deposit reproducible thin films. The coating layer should be thin enough not to alter the optical parameters (incoupling angle, sensitivity, etc.) of the grating-coupler sensor significantly; on the other hand it should be thick enough to cover the underlying waveguide completely. Several kinds of metal oxide coatings have been used (SiO$_2$, TiO$_2$, Ta$_2$O$_5$, ZrO$_2$, Nb$_2$O$_5$) for different purposes [13,25]. Even electrically conductive, transparent oxide layers (such as indium tin oxide) can be applied as coatings on the grating coupler sensor chip. With such materials, the grating-coupler sensor can be combined with an electrochemical sensor, opening up new fields of application [26].

3.3.2. Organic coatings

Several organic surface-modification techniques for waveguides are commercially available for different analytical purposes. These permit the immobilization of a variety of saccharides (such as dextran, gelatin, heparin, etc.) or biomolecules (biotin, streptavidin, etc.) via covalent coupling to silanized waveguides, for example. Different terminal functionalities, such as amine, hydroxyl or carboxyl are also available and allow the further modification of the sensor surface if required by the application.

Various types of surface modifications are possible by spin-coating a thin polymer layer onto the sensor surface. Teflon-AF, phosphorylcholine containing polyurethanes (PCPUR) [27] and polyethylene (PE) [28] are typical examples of surface coatings that can be produced by spin coating.

Using copolymers such as poly(1-lysine)-g-poly(ethylene glycol) provides a fast and convenient platform for modifying the surface of a biosensor [29,30]. Recently, it has also been shown that octadecylphosphoric acid esters self-assemble on a variety of amorphous/nanocrystalline metal-oxide surfaces. These self-assembled monolayers (SAMs) represent a powerful and highly flexible approach for the creation of concentrated planes of surface functionality [31]. These two surface modifications are further discussed in Section 4.4.2.

Pelelectrolyte multilayers also have a great potential in the areas of surface modifications of biomaterials and stabilization of colloids [32].
Another convenient method for organic surface modification is the Langmuir–Blodgett technique [33]. It can be applied for the deposition of lipid layers onto the sensor surface as described in more detail in Section 4.2.1.

4. Biological applications

4.1. Protein–DNA interactions

Clearly the immobilization of a receptor on the waveguide surface allows the binding and dissociation kinetics of a ligand to be measured. The optical waveguide is a very attractive substrate for receptor immobilization because an enormous variety of biomolecule immobilization schemes can be applied (see, for example, Ref. [34]). As a rule, it is preferable to immobilize the corresponding DNA (or RNA) rather than the protein, unless short oligonucleotides are being investigated because the transport of long DNA molecules through a solution is slow. A convenient and robust nucleic acid immobilization protocol, making use of the polyanionic quality of DNA, is described in Ref. [35]. This procedure is especially suitable for molecules that are thousands of base pairs long. Short oligomers are probably better immobilized in a directed fashion, e.g., by biotinylating and anchoring to avidin [36].

A DNA-coated sensor can be used for the determination of complementary nucleic acid strands, i.e., as a genosensor. It complements high-density oligonucleotide arrays (‘gene chips’) by (a) not requiring fluorescent labeling of the target sequence and (b) by enabling the kinetics of association and dissociation to be precisely monitored. Since the binding of two complementary or quasicomplementary oligonucleotides is prima facie a complex process, the response of a gene chip is likely to depend sensitively on the experimental conditions, but unless the binding events are analyzed in detail, distortion of response may not be detected.

The binding of proteins (e.g., restriction enzymes or transcription modulators) to DNA typically involves three steps [35]: (1) non-base-specific, i.e., relatively weak, association of the protein to anywhere on the DNA molecule; followed by (2) random diffusion along the DNA molecule; until (3) the specific recognition site (typically involving 10–20 bases) is reached, whereupon the protein binds tightly. Processes (2) and (3) compete with a fourth process, dissociation of the protein. Observations of the overall protein association and dissociation kinetics using OWLS readily enable the parameters of all four processes to be determined [35].

4.2. Lipid bilayers

Many interactions in biology take place at surfaces. In most cases these involve a biomolecule (protein) coupled to a lipid bilayer or monolayer. OWLS, as a surface-sensitive, in situ technique, offers a natural way to investigate the properties of lipid layers, their interactions with proteins or other biologically interesting molecules, and the interaction of lipid-layer-coupled receptors with their ligands.

4.2.1. Lipid-layer deposition

Over the years a number of different methods have been introduced to coat the sensor surfaces with phospholipid bilayers (occasionally monolayers) of different compositions. The most common one uses a Langmuir trough and the so-called Langmuir–Blodgett and Langmuir–Schaefer deposition processes to form a bilayer on the sensor surface [37–39]. Another possibility is to use phospholipid vesicles and create a bilayer or a supported vesicle layer through adsorbing them [40]. Highly stabilized layers can be made by covalent attachment of modified (thiol) phospholipids onto specially functionalized waveguide surfaces [41].

4.2.2. Biomembranes

OWLS has been used for biophysical investigations in which the optical anisotropy of phospholipid bilayers with different alkyl chain lengths was determined [38] and the adsorption of lipid vesicles onto smooth metal oxide surfaces was characterized [40].

Characterization of lipid–(bio)molecule interactions is also an important field of interest. The investigated (bio)molecules include detergents involved in membrane-protein solubilization and incorporation, such as Triton X-100 [42], where the solubilization characteristics have been investigated; different drugs such as aspirin and caffeine, where partition coefficients of these molecules have been determined [43]; oligopeptide hormones, where a dosimetric application has been developed [44]; and a variety of proteins including promastigote surface protease (PSP), cytochrome-reductase, laminin, MARCKS-related protein, etc., where the association/dissociation rate of the molecules with respect to the underlying lipid bilayers has been determined [37]. The effects of different modifying/environmental parameters such as the presence of lipid anchors [39], and the influence of ion concentration [45] have also been analyzed. In addition the contributions of different forces to the binding of proteins to lipid layers have also been investigated [46,47].

A promising application of OWLS is to use the biomolecule-associated, lipid-layer-covered sensor chips as artificial model cell membranes and to investigate receptor–ligand interactions in their natural environment. The receptor molecules (proteins or polypeptides) can be incorporated into the membranes either via direct adsorption [37,39,48,49] or via fusion of receptor-associated vesicles or micelles into the already deposited lipid layer [41]. These functionalized lipid layers can be
4.3. Monitoring environmental pollution

Biosensors are ideal tools for monitoring the effect of environmental pollution on biological systems. A general platform for the evanescent wave biosensor consists of a sensor whose surface is coated with a biologically sensitive layer whose optical parameters are sensitive to the effect to be monitored.

A grating-coupler sensor was used for affinity-based pesticide sensing [50]. An immunoassay was developed based on a binding inhibition test format with an anti-simazine Fab concentration of 1 μg/ml, and simazine standard solutions were mixed with polyclonal anti-simazine antibody solution (10 μg/ml) to permit binding and regeneration tests to be carried out. A detection limit of 0.25 μg/l was achieved for pesticide simazine detection.

In a recent publication, OWLS was used to characterize changes in the opto-geometrical parameters of a polycrystalline uracil layer upon irradiation by an artificial UV source [51]. It has been shown that UV irradiation causes a decrease in the refractive index and an increase in the optical anisotropy of the uracil thin layer. The determined kinetic parameters of the UV dose-sensor response curves correlate well with results of optical density measurements, but the sensitivity of OWLS is about 10 times higher. The results show that OWLS is capable of analyzing the response of the uracil layer to UV irradiation and open the way for applications in dosimetry.

4.4. Biomaterials

Reduction or elimination of non-specific protein adsorption is an important aspect with the development of biomedical devices, such as catheters, heart valves and stents, as well as blood-, serum- or plasma-contacting sensors [52]. Protein adsorption onto the implant surface is the first stage in a series of events that, depending on the type and nature of the adsorbed proteins, may lead to a deleterious response. In the case of blood-contacting devices such as stents and catheters, in particular, protein adsorption is the first step in a cascade of surface processes inducing platelet deposition and thrombus formation. Since metals, such as titanium, that are covered by their related surface oxides, are often applied in blood-contacting devices, the OWLS technique, which quantifies protein adsorption on oxides, can be used to study important issues in biomaterials technology.

4.4.1. Interaction of surfaces with blood plasma and serum

OWLS combined with antibody techniques has been used to study the adsorption of proteins and the deposition of a complement factor from human plasma and serum onto titanium oxide as a function of incubation time and wall-shear rate [53].

Exposure to human serum for 30 min resulted in an increased amount of deposition from serum as well as an increase in the subsequent anti-C3c binding compared to 15 s of serum exposure but did not show increased amounts of antibodies to complement factor 1q (C1q) or IgG. Also the wall-shear rate appears to have a significant effect on the amount and the composition of the deposited layer. When the serum exposure was done at very low-wall-shear rates (1.5 s⁻¹), increased binding of antibodies to complement factor 3 was observed.

After incubation in heparinised blood plasma for 3 min, a large subsequent binding of anti-high-molecular-weight kininogen was observed, indicating the potential intrinsic coagulation activity. Traces of anti-fibrinogen and anti-factor XII were also detected, but neither anti-albumin nor anti-fibronectin adsorption was observed.

Ultra-high-molecular-weight polyethylene (UHMWPE) is commonly used in artificial hip joints. OWLS was used to study the effect of oxygen-plasma treatment of spincoated polyethylene (PE) on the adsorption kinetics of proteins onto PE [28]. Wear processes in hip joints are believed to occur chiefly under boundary lubrication conditions. Experiments have shown that an oxygen-plasma treatment of PE, which produces significantly greater hydrophilicity due to the modified surface chemistry, leads to faster and modified protein adsorption. A denser layer of human serum albumin (HSA) on the PE surface appears to enhance boundary lubrication and can be correlated with a 50% reduction of dynamic friction as well as a reduction in stiction, which is believed to be a key factor in wear mechanisms occurring in artificial hip joints [28].

4.4.2. Non-fouling surfaces

Polyethylene glycol (PEG) has been extensively investigated for use in a wide array of biomedical applications, and immobilization of PEG on surfaces has long been known to decrease protein adsorption [54–56]. A class of graft copolymers based on poly (l-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) has been found to spontaneously adsorb from aqueous solutions onto several metal oxide surfaces, such as TiO₂, Si₀.₄Ti₀.₆O₂₅, and Nb₂O₅. The resulting layers are highly effective in reducing the adsorption of blood serum as well as adsorption of individual proteins such as fibrinogen, which is known to play a major role in the cascade of events that leads to
biomaterial-surface-induced blood coagulation and thrombosis [29]. Both the adsorption process and the resistance to protein adsorption were measured by the OWLS technique. The adsorption of the polymer was found to be pH dependent, which can be explained by the ionic nature of the interaction between the metal oxide surface and the PLL (see inset of Fig. 4). Fig. 4 also shows the adsorption of serum proteins on a PLL-PEG modified and on an unmodified TiO₂ surface. Adsorbed protein levels lower than 1 ng/cm² could be achieved for the optimized polymer architectures.

Polyurethanes also have traditionally proved to be relatively bio- and haemocompatible materials and therefore, are widely used for biomedical applications such as artificial organs, blood-contacting devices, peripheral nerve repair, vascular prostheses, or other prosthetic devices [57–59]. PCPUR have been spin-coated onto optical grating coupler waveguide surfaces in order to investigate the adsorption of proteins and lipids on these polymers as a function of the phospholipid content [27]. The measurements showed that the irreversible adsorption of proteins and lipids is drastically reduced as a result of the simultaneous contributions of the PC groups, the molecular mobility and the strong hydrophilicity of the polymers (see Fig. 5). Both the proteins and the lipids which were tested showed markedly reduced irreversible adsorption onto PCPUR surfaces compared to the uncoated waveguide. No significant difference in the masses of adsorbed albumin and fibrinogen was found between the two polymers studied, which is probably due to their small PC concentration difference. However, γ-globulin adsorbed less on the PCPUR with the higher PC content, suggesting that IgG may be more sensitive to a small increase in PC-group concentration. An analogous measurement done with fetal bovine serum (FBS) shows the same trend, with a lower amount of adsorbed species on PCPUR than on the uncoated surface.

4.5. Interactions with cells

4.5.1. Toxicological sensor based on living cells

Recently, it has been shown that living animal cell adhesion and spreading can be monitored on-line and quantitatively via the interaction of the cells with the evanescent electromagnetic field present at the surface of an optical grating coupler waveguide [60]. OWLS data and confocal laser scanning microscopy (CLSM) were compared under identical experimental conditions. This allowed the cell-shape information from CLSM to be correlated with the cell–surface interaction measurements from OWLS. Comparing the OWLS signal (which is restricted to the 100–200 nm vicinity of the surface and thus gives information only about the footprints of the cells) to the morphological response measured by CLSM revealed that OWLS is effective in monitoring not only cell attachment and spreading but also cellular response to toxic compounds. Thus, the potential of an on-line sensor based on OWLS for applications in toxicology, pharmacy and biocompatibility was demonstrated [61]. Fig. 6 shows a comparison of the response of MC 3T3-E1 cell line cells to the presence of sodium hypochlorite measured with a conventional MTT-test and with the OWLS technique. OWLS was found to be sensitive to a 50-fold-lower concentration of sodium hypochlorite than the
MTT-test and produced the results within a much shorter time.

4.5.2. Cell–surface interactions

Protein adsorption plays an important role in the interaction of cells with biomaterial surfaces. The capability of the OWLS technique to quantitatively monitor both the protein adsorption and the cell attachment and spreading processes allows for the study of cell–surface interactions. The spreading kinetics of different fibroblast cell lines and the effect of the presence of growth factors were studied by Hug et al. [62].

To explore the interaction of osteoblasts with titanium, TiO$_2$-coated waveguides were exposed to different protein solutions for 30 min in the OWLS instrument, and the formation of the protein layer was monitored. Subsequently, MC 3T3-E1 cell line cells (400,000/ml, in a cell culture medium containing 5% FBS) were injected into the flow-through cuvette. The kinetics of spreading of the cells on TiO$_2$ was found to depend on the layer of proteins present on the surface (see Fig. 7). Pretreatment of the surface with human fibronectin, an RGD-containing protein, resulted in the fastest cell spreading; however, the cells could also spread on a HSA treated surface or even on a surface with no protein pretreatment. This can be probably attributed to exchange between the surface-bound proteins and the proteins produced by the cells and subsequently deposited onto the surface.

5. Conclusions and outlook

Although OWLS can be used simply as a means of detecting the presence or absence of binding to an immobilized receptor, a lipid membrane, or the surface of a surgical implant, and hence simply as a highly sophisticated analogue of an enzyme immunoassay (ELISA), its capabilities far outweigh such applications. Thanks to its high sensitivity and versatility and the

![](image1)

Fig. 6. Comparison of the response of MC 3T3-E1 cell line cells to the presence of sodium hypochlorite measured with a conventional MTT-test and with the OWLS technique. OWLS is found to be sensitive to NaOCl concentrations 50 times lower than those measurable by the MTT-test.

![](image2)

Fig. 7. The dependence of the kinetics of spreading of MC 3T3-E1 cell line cells on the protein pretreatment of the TiO$_2$ surface as measured by OWLS. Faster cell spreading is observed when the surface is treated with human fibronectin or human serum prior to the introduction of the cells, which is expected due to the presence of integrin binding proteins. Interestingly, within 3 h the cells spread also on the surfaces with a preadsorbed albumin layer, indicating a possible exchange of proteins on the surface (Vroman effect).
transparency of the underlying theory, its uses in the biological and biomedical realms are multifarious.

OWLS is particularly valuable for the detailed analysis of a binding process, with a view to elucidate the underlying physico-chemical mechanisms. Its high sensitivity, coupled with the ease with which experiments can be run under controlled hydrodynamic conditions, enables many crucial details to be revealed, which remain hidden when investigated using less-advanced methods. Given that it is becoming more and more apparent that nature fully exploits the subtle and sophisticated possibilities inherent in most biological macromolecules for modulating association and dissociation, the demand for techniques capable of unraveling them is continually increasing. In parallel, ever-increasing demands for sensitivity, selectivity, reproducibility and speed for applications such as biosensors and gene chips make it essential that each step in a molecular binding process is understood in quantitative detail, which OWLS is poised to offer. Thus, for fundamental biological research, both at the level of individual molecules and of clusters operating on the surfaces of cells, and for research aimed at developing diagnostic biosensors and biomaterials, OWLS is becoming an extremely valuable tool.

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