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Design of a surface plasmon resonance immunoassay for therapeutic drug monitoring of amikacin

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ABSTRACT

The therapeutic drug monitoring (TDM) of pharmaceutical drugs with narrow therapeutic ranges is of great importance in the clinical setting. It provides useful information towards the enhancement of drug therapies, aiding in dosage control and toxicity risk management. Amikacin is an aminoglycoside antibiotic commonly used in neonatal therapies that is indicated for TDM due to the toxicity risks inherent in its use. Current techniques for TDM such as high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) are costly, time consuming, and cannot be performed at the site of action. Over the last decades, surface plasmon resonance (SPR) biosensors have become increasingly popular in clinical diagnostics due to their ability to detect biomolecular interactions in real-time.

We present an SPR-based competitive immunoassay for the detection of the antibiotic amikacin, suitable for TDM in both adults and neonates. We have obtained high specificity and sensitivity levels with an IC_{50} value of 1.4 ng/mL and a limit of detection of 0.13 ng/mL, which comfortably comply with the drug's therapeutic range. Simple dilution of serum can therefore be sufficient to analyze low-volume real samples from neonates, increasing the potential of the methodology for TDM. Compared to current TDM conventional methods, this SPR-based immunoassay can provide advantages such as simplicity, potential portability, and label-free measurements with the possibility of high throughput. This work is the foundation towards the development of an integrated, simple use, highly sensitive, fast, and point-of-care sensing platform for the opportune TDM of antibiotics and other drugs in a clinical setting.

1. Introduction

In the last decade research related to immunosensors has been focused towards the development of point-of-care devices that can allow management of a particular issue whether it is food safety, security, environmental, or medical related [1,2]. Therapeutic drug monitoring (TDM) is a branch of clinical chemistry and pharmacology that specializes in the measurement of therapeutic drug concentrations in blood or other body fluids. TDM is indicated for drugs that have narrow therapeutic ranges, i.e. drugs that present a high risk of toxicity when overdosed or ineffectiveness when underdosed [3]. Moreover, neonate (newborn) patients have unique drug dispositions that indicate the need of TDM in order to ensure a safe drug therapy [4]. This monitoring aims to enhance drug efficacy through toxicity risk management, dosage control, and assistance in clinical diagnosis [5,6].

Antibiotics are drugs that are suitable for TDM and they are the most widely used drugs in neonatal therapy due to newborns' high susceptibility to infections [7]. Amikacin (AK) is an aminoglycoside antibiotic commonly used in neonatal therapies [8], and is indicated for TDM due to the toxicity risks inherent to its use as well as its narrow therapeutic range (1–30 μg/mL) [9,10]. TDM of amikacin and other pharmaceutical drugs is mostly performed in blood serum or plasma [6,11] and given the low blood volume in neonates of approximately 80 mL/kg, small sample sizes are most desirable in order to avoid detrimental impact to the patient due to blood loss [4]. Conventional methods for TDM include high performance liquid chromatography (HPLC) [12,13], gas chromatography–mass spectrometry (GC–MS) [14,15], and other commercial immunoassays (IA) [16,17]. Specifically, TDM of AK (and other aminoglycoside antibiotics) is mostly done by immunoassays in different configurations (i.e. Fluorescence Polarization IA, FPIA, turbidimetric IA or enzyme

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multiplied IA, EMIT) [10,18–20] although other methods such as liquid chromatography tandem mass spectrometry LC–MS/MS have been applied [21]. All these methods are costly and time consuming and commonly they cannot be performed at the site of action [6].

Surface plasmon resonance (SPR) is a widely known optical technique thoroughly studied in the last few decades, which uses evanescent waves to investigate surface phenomena and generate a signal related to a change in refractive index at an interface. It has become popular in a variety of fields including environmental protection, fundamental biological studies, food safety and clinical diagnosis applications [22]. Advantages of SPR biosensing include real-time and label-free monitoring, simplicity, high levels of sensitivity and fast-response measurements, making it a potential alternative to current clinical and laboratory measurement techniques such as enzyme immunoassays (ELISA, EMIT) and chromatographic techniques (LC–MS, HPLC) [22,23]. While SPR biosensing has been used for the detection of antibiotics in milk samples for food safety applications [24,25], their application for TDM purposes, especially in neonates, has not been approached. The antibiotic kanamycin has been monitored using a fluorometric technique based on plasmon resonance light-scattering (PRLS) of gold nanoparticles (AuNPs) [26], albeit in urine samples for monitoring of nephrotoxicity and ototoxicity.

In this work we present a SPR-based competitive immunoassay for the detection of AK, suitable for TDM in both adults and neonates. We have obtained high specificity levels and a high sensitivity which complies with the drug's therapeutic range, demonstrating the effectiveness of this approach to further point-of-care on-site TDM monitoring.

2. Materials and methods

2.1. Chemicals and reagents

Reagents for the self-assembled monolayer (SAM) formation (16-mercaptohexadecanoic acid (MHDA) and 11-mercaptoundecanol (MUOH)), common reagents (N-hydroxysulfosuccinimide sodium salt (NHS), 1-ethyl-3(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC), ethanolamine), inorganic salts for buffer preparation (phosphate buffer saline (PBS), 2-(N-morpholino)ethanesulfonic acid (MES), Tween 20), bovine serum albumin (BSA) and AK were purchased from Sigma Aldrich.

Polyclonal IgG anti-AK antibody was purchased from Abcam (England) and AK–BSA conjugate was synthesized as described in Section 2.4.

2.2. Instrumentation

SPR measurements were performed using a homemade SPR biosensor based on the Kretschmann configuration under a prism-coupling scheme. A polarized 3 mW laser beam with an operational wavelength of 670 nm is directed at a fixed angle towards the gold-coated sensing surface. The sensing chips are 10 mm × 10 mm × 0.3 mm glass coated with 2 nm of chromium and 45 nm of gold, from Ssens (Netherlands). SPR measurements are performed at a fixed angle of incidence. The optimum measurement angle is selected during calibration to ensure maximum sensitivity to changes in the refractive index, which in turn are caused by mass variations at the sensing surface. These variations are detected as changes in the reflected light intensity at a photodiode, and are subsequently amplified and converted to a digital signal, which we refer to as SPR signal (intensity variation of the reflected light at a fixed angle).

Sample sizes of 250 μL are injected by a diaphragm pump to the flow delivery system, which incorporates a flow cell with two independent channels, which are both used for measurements. The system incorporates all the optics, electronics and fluidic components necessary to function autonomously.

2.3. Synthesis of the AK–BSA conjugate

AK–BSA conjugate was prepared based on the conjugation of the amino groups of AK to the carboxylic groups present in BSA. Briefly, a solution of AK (11.11 mg/mL in MES buffer pH 5.0) and the carrier protein BSA (10 mg/mL in MilliQ water) was incubated for 3 h with 10 mg of EDC as a linker. Next, purification of the conjugate was achieved by using PBS 10 mM (pH 7.4) as a purifying buffer and a 30 kDa centrifugal filter. The solution was mixed with 250 μL of purifying buffer in the filter and was centrifuged at 12,000 RPM during 120 s, after which the residues from the collection tube were discarded. This process was repeated three times, yielding the final conjugate solution. Conjugate concentration was determined by UV resulting in 14.3 mg/mL.

2.4. Immobilization procedure

Gold chips were prepared for immobilization by a wash with ethanol, followed by 20 min of surface cleansing with UV-ozone (Bioforce Nanosciences, Inc, model UV-TC.220) to eliminate organic contamination. A second rinse with ethanol was performed and finally the chips were dried with N_2.

A covalent strategy was used for biofunctionalization of the surface. After chip cleaning, the formation of an alkanethiol self-assembled monolayer (SAM) was carried out ex-situ by coating the gold chip overnight with a mixed solution of MHDA:MUOH in ethanol, with a molar ratio of 1:1 and at a total concentration of 250 μM. The chip was rinsed with ethanol and dried with N_2, and mounted in the SPR platform. Next, an AK–BSA conjugate was covalently coupled to the carboxylic groups of the SAM through activation of the acid groups with a solution of 0.2 M EDC and 0.05 M NHS in MES buffer (pH 5.0). Subsequently, after the concentration of AK–BSA conjugate in PBS buffer was injected, a 1 M ethanolamine solution (pH 8.5) was used for the deactivation of unreacted carboxylic groups. This procedure was done at a flow-rate of 14 μL/min.

2.5. SPR immunoassay format

An indirect competitive immunoassay format was used for the detection of AK levels given the low molecular weight of the molecule (585.60 Da). Therefore, the AK–BSA conjugate was immobilized and used as the sensing bioactive surface. After assay optimization, samples consisting of mixtures (1:1 v/v) of different analyte concentrations (ranging from 1 pg/mL to 5 μg/mL in PBS or PBST (0.05% Tween 20)) and a fixed antibody concentration were flowed over the sensing layer at a flow rate of 22 μL/min. Samples were incubated for 10 min at room temperature before injection and monitoring was performed in real time. Since antibody binding to the immobilized conjugate is inhibited by the presence of analyte, lower analyte concentrations resulted in high SPR signals and vice versa. Standard calibration curves were constructed with at least 7 points (7 different AK concentrations) with three replicate measurements each. Reutilization of the sensor surface was achieved using a solution of NaOH 45 mM as a regeneration solution, during 120 s at a flow rate of 22 μL/min after each sample injection.

2.6. Data analysis

Standard calibration curves were obtained by plotting the average SPR signal and standard deviation of three samples for