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Highly sensitive dendrimer-based nanoplasmonic biosensor for drug allergy diagnosis

Maria Soler [a,1], Pablo Mesa-Antunez [b,c,1], M.-Carmen Estevez [a,*], Antonio Jesus Ruiz-Sanchez [b,c], Marinus A. Otte [a], Borja Sepulveda [a], Daniel Collado [b,c], Cristobalina Mayorga [c,d], Maria Jose Torres [c,e], Ezequiel Perez-Inestrosa [b,c], Laura M. Lechuga [a]

[a] Nanobiosensors and Bioanalytical Applications Group, Institut Català de Nanociència i Nanotecnologia (ICN2), CSIC and CIBER-BBN, 08193 Bellaterra (Barcelona), Spain
[b] University of Malaga, IBIMA, Department of Organic Chemistry, 29071 Malaga, Spain
[c] Andalusian Centre for Nanomedicine and Biotechnology-BIONAND, Parque Tecnologico de Andalucia, 29590 Malaga, Spain
[d] Research Laboratory, IBIMA, Regional University Hospital of Malaga, UMA, 29009 Malaga, Spain
[e] Allergy Unit, IBIMA, Regional University Hospital of Malaga, UMA, 29009 Malaga, Spain

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ABSTRACT

A label-free biosensing strategy for amoxicillin (AX) allergy diagnosis based on the combination of novel dendrimer-based conjugates and a recently developed nanoplasmonic sensor technology is reported. Gold nanodisks were functionalized with a custom-designed thiol-ending-polyamido-based dendron (d-BAPAD) peripherally decorated with amoxicilloyl (AXO) groups (d-BAPAD–AXO) in order to detect specific IgE generated in patient's serum against this antibiotic during an allergy outbreak. This innovative strategy, which follows a simple one-step immobilization procedure, shows exceptional results in terms of sensitivity and robustness, leading to a highly-reproducible and long-term stable surface which allows achieving extremely low limits of detection. Moreover, the viability of this biosensor approach to analyze human biological samples has been demonstrated by directly analyzing and quantifying specific anti-AX antibodies in patient's serum without any sample pretreatment. An excellent limit of detection (LoD) of 0.6 ng/mL (i.e. 0.25 kU/L) has been achieved in the evaluation of clinical samples evidencing the potential of our nanoplasmonic biosensor as an advanced diagnostic tool to quickly identify allergic patients. The results have been compared and validated with a conventional clinical immunofluorescence assay (ImmunoCAP test), confirming an excellent correlation between both techniques. The combination of a novel compact nanoplasmonic platform and a dendrimer-based strategy provides a highly sensitive label free biosensor approach with over two times better detectability than conventional SPR. Both the biosensor device and the carrier structure hold great potential in clinical diagnosis for biomarker analysis in whole serum samples and other human biological samples.

1. Introduction

Health and clinical care increasingly demand new modalities of diagnostic instrumentation which can satisfy strict requirements that conventional methods cannot provide. In this sense, decentralized reliable analysis with diagnostic platforms performed at patient bedside, or in primary care doctor's office, which are fast, sensitive and minimize sample manipulation is still needed. The development of these point-of-care (POC) devices could simplify disease diagnosis and could aid in following disease progression (or regression). Optical biosensors stand out among other configurations as the best candidates to fulfill POC requirements. They show exceptional potential for integration in portable lab-on-a-chip devices. They provide direct quantification of molecules in a real time and label-free manner (Estevez et al., 2012; Ligler, 2008). Plasmonic sensors and Surface Plasmon Resonance (SPR) sensor in particular are currently routine analytical instruments in laboratories due to its simplicity, robustness and versatility (Homola, 2008). Some of the well-known SPR limitations, such as moderate sensitivity, limited throughput capabilities and difficult

* Corresponding author.
E-mail address: mcarmen.estevez@cin2.es (M.-C. Estevez).
[1] These authors contributed equally.

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miniaturization can be surpassed by using nanoplasmonic-based biosensors (Estevez et al., 2014; Petryayeva and Krull, 2011; Sepúlveda et al., 2009), whose configuration is based on the localized surface plasmon resonance (LSPR) rather than conventional SPR. Basically, the LSPR is the localized oscillation of free electron charges of metallic nanostructures with a resonance wavelength (λ_{LSPR}) which is determined by their composition, size, shape, and the refractive index (RI) of the surrounding dielectrics. LSPR has emerged during last decade due to the eruption of a wide variety of plasmonic nanostructures. However despite its demonstrated potential, its applicability as biosensing devices has not been fully exploited and mainly proof-of-concepts are reported in the literature. Appropriate biofunctionalization of the nanostructures is crucial to take full advantage of such sensitive devices and the complexity of this step may lie behind the lack of reported complete bioanalysis in contrast with the high amount of examples based on conventional SPR. New biofunctionalization strategies which ensure stable and reliable assays, which in addition reduce potential nonspecific adsorption, are always sought. In particular, initial coatings or modification of the surface using functional linkers that in turns allows further specific receptor attachment have a direct influence on the final performance. Besides proteins, which are commonly used as carrier structures, dendrimers have profiled themselves as attractive materials because of the inherent capabilities conferred by their three-dimensional architecture (Astruc et al., 2010). Their structural homogeneity, controlled composition and multifunctionality make them unique for biomedical applications (Svenson and Tomalia, 2012), especially for drug delivery (Kesharwani et al., 2014) but also for biosensing (Fu and Li, 2013). Their use in immunochemistry has increased exponentially due to their interesting properties for the immobilization of biomolecules or small haptens. The globular-shaped poly(amido amine) PAMAM dendrimers (carrying amino groups in their structure) is the first and most extensively studied family (Trinchi and Muster, 2007). Conventional peptide carriers such as bovine serum albumin (BSA) or poly-L-lysine (PLL) usually lead to uncontrolled density and randomly distribution of the haptens in their structure, which can eventually induce reproducibility issues. In contrast, the regular geometry, stability, and high surface functionality of the dendrimers provide better control over the density and spacing of the immobilized molecules and also on the thickness of the overall receptor layer.

Herein, we propose an unprecedented approach that combines the advantages of a recently reported novel and highly sensitive label-free nanoplasmonic biosensor based on gold nanodisks with a generation 2 (G_2) BAPAD dendron-based structure in order to directly quantify specific antibodies in human samples. The optical label-free sensor is based on a novel waveguiding mechanism occurring in nanoplasmonic structures composed out of short-range ordered arrays of gold nanodisks (Otte et al., 2011) which results not only in higher sensitivities to RI changes, but also in improved signal to noise ratios, assuring an overall significant improvement of the RI sensor performance. We have designed a compact read-out setup for the real time evaluation of the biomolecular interactions occurring in the functionalized nanostructures.

Based on these grounds we have focused on the development of a highly sensitive and reproducible diagnostic tool for the quantitative detection of specific IgE antibodies in amoxicillin (AX) allergic patients. Current available diagnostic methods employed to assess subjects with an immediate allergic reaction include a complete clinical history plus in vivo skin tests or/and in vitro determination of specific IgEs in blood (Torres et al., 2003), whose production is triggered by the organism during an allergic outburst. Skin testing has been the most generalized approach. However this technique is invasive, painful (especially for children) and is not always appropriate particularly when the patient has suffered a severe allergic reaction. It requires the patient to temporarily stop medication, and results can be affected by skin condition. Solid phase immunoassays, such as the radioallergosorbent test (RAST) and subsequently the ImmunoCAP test have profiled themselves as best alternatives and are nowadays complementary in clinical diagnostics to the skin prick tests. Typically less sensitive than *in-vivo* skin tests (Chinoy et al., 2005), RAST is of a more qualitative nature and the results are usually expressed in a specific numerical scoring system (RAST scale from 0 to 6). On the other hand ImmunoCAP tests, based on fluorescent enzyme-labeled immunoassay (FEIA) (Mayorga et al., 2010), show improved performance resulting from the high-binding capacity of the solid phase used to keep the allergen bound, which results in enhanced sensitivity. This technology has been cleared by the Food and Drug Administration (FDA) to provide quantitative measurements of IgE concentration in blood, and is a validated method with a working range of 0.35–100 kU/L (0.84–242 ng/mL), and a cutoff value of 0.35 kU/L (Alonso et al., 1995; Blanca et al., 2001). In fact, RAST scores have been correlated with IgE concentration determined with ImmunoCAP tests. As alternative to these conventional techniques, PAMAM dendrimers have been used as carrier structure to conjugate β-lactam antibiotics mimicking the behavior of protein–hapten conjugates. They have been able to recognize specific IgEs (Sánchez-Sancho et al., 2002), attached to cellulose disks (Montañez et al., 2008) or to silica nanoparticles (Vida et al., 2013) and used as solid phase in RAST assays of serum patient samples. However none of these studies provided quantitative information of IgE concentration.

In this study we have developed not the whole dendrimer but a novel BAPAD–dendron structure as carrier molecule which can be easily transferred to (nano)plasmonic biosensor devices, allowing orthogonal coupling both to the gold surface and to amoxicillin molecules through the reactive amine groups in the outer shell. Using this strategy, very low concentrations of specific antibodies have been detected (LoD = 0.6 ng/mL), that is better than the one achieved with the ImmunoCAP test. The appropriate surface coating minimizes nonspecific interactions which enables direct whole serum evaluation. A validation of the assay has been done with real serum samples from hospital patients and the excellent correlation with ImmunoCAP allergy test confirms the reliability and viability of our approach as a potential tool for fast and real-time diagnosis of drug allergy.

2. Materials and methods

2.1. Nanodisks preparation and optical setup description

Short-ordered arrays of gold nanodisks (D = 100 nm, H = 20 nm (Ti/Au = 1/19 nm)) were fabricated on glass substrates via hole-mask colloidal lithography (HCL) (Fredriksson et al., 2007). Before use, the substrates were cleaned by consecutive 1 min sonication cycles in acetone, ethanol and MilliQ water, dried with N_2 stream and placed in an UV/O_3 cleaner tor for 20 min, after which they were rinsed with ethanol and water and dried with N_2. Then, the substrates were clamped between a trapezoidal glass prism (n = 1.52) contacting the sample through RI matching oil ($n \approx 1.512$) and a custom-made Delrin flow cell (volume = 4 μL), which is connected to a microfluidic system consisting on a syringe pump (New Era, NE-1000) with adjustable pumping speed that ensured a constant liquid flow and a manually operated injection valve (IDEX Health and Science, V-451). The biosensing surfaces were excited by a collimated halogen light source (HL-2000, Micro-pack) set in TE polarization for gold nanodisks (LSPR mode). The light reaches the substrates through the prism and the