

Nanotechnologies for In Vitro IgE Testing

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Abstract

Purpose of Review This review discusses the recent advances in the development of IgE antibody assays based on nanotechnologies. IgE blood testing is an important part of the diagnostic workup of IgE-mediated hypersensitivity. We also address the challenges in moving from an academic proof-of-concept to a product routinely used by allergy experts.

Recent Findings Several nanotechnologies have been applied to the field of IgE testing: nanoparticles are used either as a support to capture analytes or as a detection tool to enhance the measurement signal. Nanofluidics allows to reduce assay time by enhancing molecular interaction.

Summary Nanotechnologies bring forth new methods for in vitro IgE testing. Substantial advantages such as lower sample volume, shorter assay time, simplified procedures, and lower analytic sensitivity, without affecting test precision and accuracy, can be achieved thanks to nanotechnologies.

Keywords IgE blood test · Allergy diagnosis · Nanotechnology · Nanofluidics · Nanoparticles

Introduction

Advances in physical, material, and chemical sciences as well as micro- and nanofabrication technologies have brought

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nanotechnology to play an important role in numerous biomedical applications. In particular, nanotechnology has been a driving parameter to enhance biosensors used for in vitro diagnostic purposes. Performance properties such as time-to-result, sample volume, sensitivity, and multiplexing are strongly dependent on the feature size of the different elements forming the biosensor. The building blocks based on nanotechnology used for the enhancement of biosensor attributes are commonly nanostructured surfaces and nanoparticles of various materials of submicron dimensions [1–3].

Nanostructured surfaces are often used to create integrated on chip biosensors providing the solid substrate where biomolecules are immobilized. Due to the submicron structuration, the binding surface for the capture biomolecule is strongly increased which enhances the sensitivity of the sensor and the biomolecular interaction leading to reduced assay times. The miniaturization allows a multitude of biomolecules to be immobilized in arrays offering multiplexing solutions while keeping sample volumes very low [2]. In combination with optical detection methods such as surface plasmon resonance (SPR) techniques [4•] or fluorescence measurements [5], nanostructured surfaces provide enhanced biosensor properties for highly integrated devices.

Nanoparticles of different shapes, sizes, and composition offer high versatility and allow pushing the performance limits of bio-assays. In general, they as well extend the binding surface and can target and label biomolecules in samples inside a complex biological matrix for the enhancement of electronic, optical, or magnetic signals.

Current Status of Allergy Diagnostic Testing

Diagnosis of allergic diseases is complex and relies on the integration of information provided first by clinical history

and physical examination, and then by results from skin prick tests and serologic IgE tests. Additional assays, such as basophil activation tests and, if necessary, provocative challenges, may also bring valuable information leading eventually to a comprehensive understanding of a patient's IgE antibody-mediated disease, guiding disease management, and therapy. IgE blood tests, which identify patient's sensitization to allergens, are today mostly performed in large automated laboratory platforms. These platforms are today mainly based on fluorescent enzyme immunoassays or enzyme-enhanced chemiluminescent assays to amplify the signal. Assay time is usually within a few hours to reach the needed analytic sensitivity. Industry gold standard methods have low analytic sensitivity (0.35 kU_A/l or less) and have shown excellent reproducibility and precision. However, marked variability in the quantitative estimation of allergen-specific IgE is reported [6]. These platforms offer a robust tool to allergists that are highly complementary to *in vivo* skin testing. With the increasing availability of purified allergen components from recombinant or natural sources and a better understanding of IgE response to allergenic molecules, assays are evolving from crude extracts, which contain a mixture of proteins, toward measurement of IgE levels for specific allergenic components. Molecular allergology identifies potential disease-eliciting allergens. It also gives information on genuine sensitization and cross-reactivities to perform a risk assessment of severe reaction, as sensitization to some allergens are known to be associated with severe reaction [7–9].

In recent years, a number of microtechnology-based assays, such as chips containing microarrayed multiple allergic molecules, have been developed. To date, one microarray is commercially available for IgE antibody testing, the Immuno Solid-phase Allergen Chip (ImmunoCAP ISAC, ThermoFisher Scientific). This array is composed of 112 allergens covering a wide range of allergen sources (allergens from trees, grasses and weed pollens, food and venoms...) and protein groups (PR-10, profilin, nonspecific lipid transfer protein (nsLTP), polcalcine...). With an assay time of approximately 4 h, the ImmunoCAP ISAC is performed in laboratories. It is also more widely used in research activities, in particular, in epidemiology studies as highlighted by the numerous data obtained from a modified version of this chip, the Medall Chip [10]. Several other allergen microarrays have been, or are, currently developed. Comparison studies on analytical performances and diagnostic accuracy are found in several research articles and reviews [11, 12]. On a general basis, multiplexed arrays offer the advantage of providing information on the sensitization pattern of a patient for a large number of molecules from a tiny volume of serum. However, analyzing all the information produced by IgE multiplexed assays is not trivial, especially to differentiate clinically relevant from irrelevant sensitizations. Moreover, the clinical utility of testing large allergen panels is a matter of intense discussion among the

allergist community. As part of this, The European Association for Allergy and Clinical Immunology (EAACI) has recently published a guideline on Molecular Allergology recommending an approach that combines both usual cares followed or completed by multiple testing of allergen components to further investigate potential clinical consequences of sensitization to an allergen component associated with high risk of severe reaction and/or cross-reactive sensitization [13].

As an intermediate approach between singleplex and multiplex testing, assays measuring a moderate number of allergen components from a unique sample are often judged as an interesting alternative. As an example, a microbead technology is based on the use of fluorescent polystyrene microspheres coated with allergens in a suspension array (Map technology, Luminex Corporation, Austin, TX) [14]. Using spectrally distinct fluorophores allows to simultaneously detect multiple analytes, in the present case, a panel of six aeroallergens plus the total IgE from 20 µl of serum. This assay is quantitative (an IgE standard curve is prepared using beads coupled to nDer p 2 for allergen-specific IgE measurements or anti-IgE for total IgE measurements). A good agreement was found with microtiter plate ELISA and ImmunoCAP. With an assay time of 6 h and the necessity to process whole blood to serum, this technology is however not intended to leave the laboratory. Other color-coded fluorescent microbeads (Becton Dickinson, New Jersey, USA) were also used in a flow-cytometer setting in which different allergen-specific IgE were detected in a multiplexed array [15]. Performances of this assay were compared to ImmunoCAP ISAC and showed mixed results: while allergen-specific IgE levels showed a good agreement between the two methods for some allergens, substantial differences were however observed for other. These discrepancies may not directly be related to the technology but rather to the allergen source, as it is often observed in IgE antibody assays.

ImmunoCAP ISAC and microbead-based IgE assays are recent examples of the evolution of IgE assay from singleplex to multi- and oligoplexing. They are relying on microtechnologies and therefore remain tests that will inevitably be performed in the laboratory with blood samples processed into serum or plasma. As discussed below, nanotechnologies offer promising opportunities to further develop such assays.

The holy grail of IgE assay providing valuable information on a patient's IgE sensitization pattern must fulfill key requirements such as low sample volume, no (or fully integrated and automated) sample processing to facilitate test handling, short turnaround time in order to have a near-patient testing platform, and tests with analytical performances and diagnostic accuracy similar to current laboratory reference methods. Nanotechnology not only can respond to the sensitivity and multiplexing requirements of IgE testing but can bring molecular IgE diagnostics to a new level by providing rapid, sensitive, specific, and easy to use solutions at the point-of-care.

Most emerging assays based on nanotechnologies use nanoparticles. Gold nanoparticles are well-known for their size-dependent optical properties. The absorbance and scattering properties can be tuned in accordance with their shape and size. In combination with optical detection methods, such as spectroscopy or refractive index sensitive techniques, gold nanoparticles offer an inexpensive and highly sensitive sensor solution [16]. Magnetic nanoparticles can isolate captured biomarkers within a biological sample by means of an external magnetic field. Due to this controlled separation, nonspecific background signal is reduced and sensitivity of the assay enhanced [17]. Other nanoparticles with specific properties are available and provide solutions for rapid and inexpensive measurements of biomolecules [18]. These particles are coupled either with the capture molecules, typically an allergen or a fragment thereof, or with the detecting molecules. Table 1 summarizes the key characteristics and development stage of the assays discussed in this article.

IgE Assay with Nanoparticles Used as a Support for Capture of Analyte

Nanoparticles are chemically modified on their surface to allow the grafting of biomolecules. As peanut allergies may cause severe reactions, including life-threatening anaphylactic shock, several groups are using peanut allergens to develop a new assay. Electrochemical immunosensors have been prepared with gold nanoparticles covalently coated with an Ara h 2 peptide containing a well-characterized IgE binding site [19]. These beads were immobilized on a poly-diallyldimethylammonium chloride-pyrolytic graphic electrode, and human serum spiked with chicken IgY antibody specific for this Ara h 2 epitope were captured and detected by horseradish peroxidase-coupled anti-chicken IgY antibody. Among the different evaluated detection methods, the one based on nonfaradaic impedance gave the most promising analytical performances. However, in view of a commercial application, data with anti-Ara h 2 human IgE are lacking in this study to clearly endorse the performances and potential of this technical approach. In addition, the polyclonal nature of the sensitization to an allergen raises also the question of the pertinence of relying on a unique peptide.

An assay was developed with iron-oxide magnetic nanoparticles coated with peanut extract [20]. These beads are incubated with a human serum sample. An external magnetic field is then applied to isolate the beads and let them react with alkaline phosphatase-coupled anti-human IgE. Thanks to the magnetic property of the nanoparticles, the isolation of the beads makes it possible to achieve an analytic sensitivity at 0.1 kU_A/l. This analytic sensitivity is however dependent on the incubation time and is reached only after a 2-h incubation. Shorter incubation times resulted in a higher analytic sensitivity value. Therefore, despite a clever approach to overcome

the limitation caused by diffusion, this assay is today not applicable to near-patient IgE testing but may be interesting in applications where higher analytic sensitivity is acceptable.

With the aim of reducing assay time, Teste and colleagues tried to tackle the limitation of molecule diffusion in a microfluidic assay [17]. They used magnetic core-shell nanoparticles on which alpha-lactalbumin, a major allergen of cow milk allergy, was coated. Confinement of this colloidal immunosupport allows to detect low-concentrated allergen-specific IgE in serum in only 5 min. This proof-of-concept is promising, and it would be very interesting to run this assay with more samples to better evaluate the test performances at sub-kU_A/l concentrations.

IgE Assay with Nanoparticles Used as a Detection Agent

Nanoparticles are commonly chemically modified to allow coupling of detecting molecules. These molecules can be antibodies, aptamer (oligonucleotides or peptides specifically binding a target molecule), or an enzyme. The objective of coupling these molecules onto a nanoparticle generally consists in amplifying the signal to reach lower analytic sensitivity values.

Gold nanoparticles coated with oligonucleotide aptamers with high specificity for human IgE have been used in several systems, in particular, in a system relying on SPR [21]. Aptamers offer several advantages over antibodies: ease and reproducibility of manufacturing processes, site-specific labeling, product stability, cost-effectiveness, and so forth. However, it is challenging to select aptamers with subnanomolar avidity for its ligand. In addition, aptamers may have a short half-life in blood, even if this problem has today mostly been circumvented thanks to chemical modifications. In this SPR assay in which an anti-human IgE antibody was immobilized to detect human IgE, initial analytic sensitivity was in the microgram per milliliter range, not being sufficient for IgE testing. Signal amplification was then achieved by adding IgE aptamer-coated gold nanoparticles. This resulted in an improved analytic sensitivity in the nanogram per milliliter range. This example clearly highlights how nanoparticles can improve analytical performances of an assay. The same group reported the following year the development of an electrochemical immunoassay in which IgE was captured and detected by means of IgE antibody-specific aptamer-coated nanoparticles. These beads incorporated methylene blue to produce an electrical current by oxidation of methylene blue on the electrode that was proportional to the concentration of human IgE [22]. These two aptamer-based assays need further evaluations with human blood specimen, as matrix effects may influence test performances, in particular, the analytic sensitivity. Moreover, these assays have been developed for the measurement of the total IgE level. It would be interesting to see if this technology could be extended to allergen-specific IgE testing.

Table 1 Summary of key parameters of the assays discussed in this review.

Technology	Example/references	Development status	Matrix	Sample volume	Assay time	Analytic sensitivity
Microarray	ImmunoCAP ISAC, (ThermoFisher Scientific)	CE-marked	Serum or plasma from venous or capillary whole blood	20 μ l	5 h	0.3 ISU
Microparticles	Luminex (Indoor Biotechnologies)	Proof-of-concept	Serum	50 μ l	6 h	0.3 kU _A /l (equivalent to 0.7 ng/ml IgE)
	ABA (flow cytometry-based assay)	Proof-of-concept	Serum and plasma	50 μ l	~3 h	N.D.
Nanofluidic	abioSCOPE, Abionic SA	CE-marked	Capillary whole blood, serum and plasma	50 μ l	5 min	0.7 kU _A /l (equivalent to 1.4 ng/ml IgE)
Nanoparticles as capture support	[19]	Proof-of-concept	Serum	10 μ l	~3 h	5 pg/ml IgY
	[20]	Proof-of-concept	Plasma	Unknown	2–4 h (depending on sample incubation time)	0.1 kU _A /l (with the longest incubation time; equivalent to 0.24 mg/ml IgE)
Nanoparticles as detection support	[17]	Proof-of-concept	Serum	20 μ l	~30 min	~1 ng/ml
	[21]	Proof-of-concept	Phosphate-buffered saline (PBS)	Unknown	Unknown	~10 ng/ml
	[22]	Proof-of-concept	Phosphate-buffered saline (PBS)	10 μ l	2 h	0.52 ng/ml
	[23]	Proof-of-concept	Serum	10 μ l	Unknown	~1 ISU
Nanocrystal	[24]	Proof-of-concept	Serum	Unknown	Unknown	N.D.
	[25]	Proof-of-concept	Serum	<100 μ l	>1 h	1 ng/ml
Nanodisk	[26]	Proof-of-concept	Serum	Unknown	15 min	0.6 ng/ml

ISU immunoCAP ISAC standard unit, N.D. not determined

A paper-based vertical flow array has been developed in which ten allergens were immobilized in the paper fibers. Allergen-specific IgE in serum that bound to the immobilized allergens are then detected by colorimetry using gold nanoparticles coated with anti-human IgE. Such an assay offers results in 10 min and has a claimed analytic sensitivity of approximately 1 ng/ml (1 ISAC standard unit). A comparison with ImmunoCAP ISAC performed with a small panel of polysensitized sera showed a good agreement between the two methods [23]. This technical approach seems promising and such immunochromatographic assays are already commonly used as home tests (pregnancy test) or point-of-care test (typically in the field of the diagnosis of infectious disease). It would however be interesting to see how the test could be compatible with capillary whole blood instead of serum, as processing of the blood requires time and equipment not readily available outside laboratories.

An alternative to gold nanoparticles are quantum dots (QD). QD are nanometer-sized semiconductor particles with optical properties different from larger particles. In life-sciences, their higher brightness and photostability than traditional fluorescent reporters offer substantial advantages in applications where such properties are valuable. Coated with streptavidin, they have been used to amplify signals in an assay designed with solid phase-bound milk allergens that capture allergen-specific IgE from serum. As detection by dual polarization interferometry (DPI) with anti-IgE resulted in sub-optimal analytic sensitivity, a biotinylated anti-IgE was used to detect captured allergen-specific IgE. Streptavidin-coupled QD then bound to anti-IgE to amplify the signal, what successfully resulted in a lower limit of detection [24].

Nanocrystals are biocompatible particles that encapsulate cations and are labeled with the detecting antibody. Zinc-selenium (ZnSe) nanocrystals coupled to anti-IgE release zinc cations that associate to the poorly fluorescent FluoZin-3 to form strongly fluorescent Zn-FluoZin-3 complexes [25]. A substantial signal amplification is reached with such an approach, as thousands of cations are encapsulated in a single nanocrystal. This allows quantifying IgE in the nanogram per milliliter range.

Not Particles: Nanodisk

Plasmonic structures at the nanoscale are today manufactured to develop nanoplasmonic biosensors. SPR assays are often limited by the analytic sensitivity. Soler and colleagues reported on an assay in which dendron-based structures are coupled to amoxicillin to detect amoxicillin-specific IgE, as a proof-of-concept assay for the diagnosis of IgE hypersensitivity to antibiotics [26]. Arrays of nanodisks of 100 nm in diameter have high sensitivity to changes of refractive index and low signal-to-noise ratio compared to classical SPR sensors. While

interesting, the authors claim that this technology could be developed as a user-friendly point-of-care diagnostic devices, a promise that has not become a reality yet.

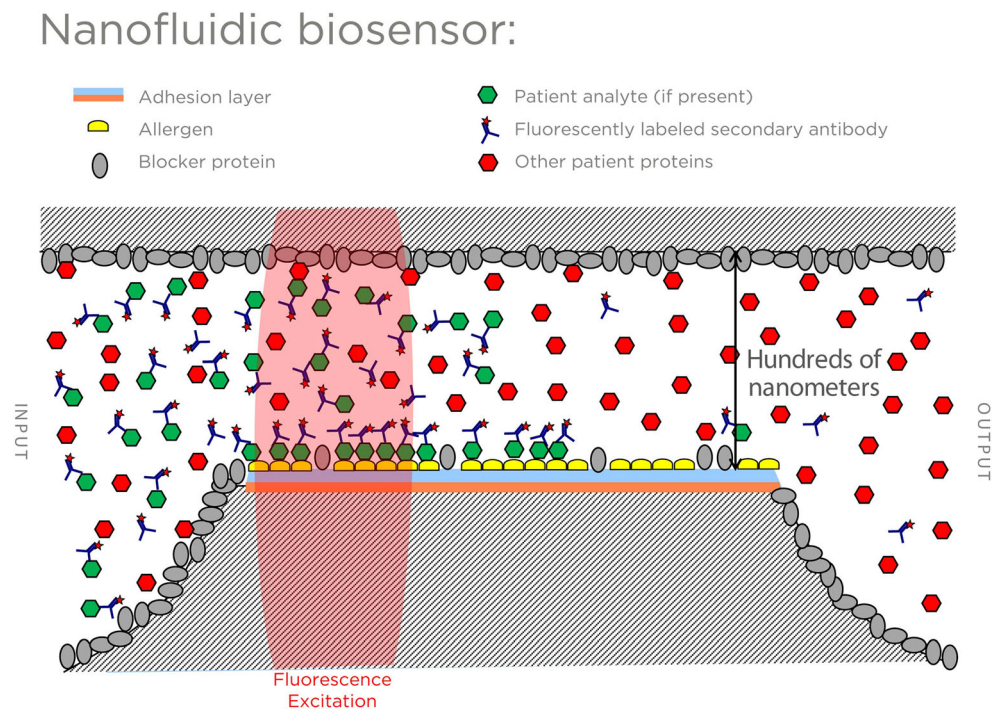
Not Particles: Nanofluidics

Nanofluidic channels strongly influence the diffusional behavior of biomolecules and increase molecular interactions [27••]. In addition, due to the high surface to volume ratio, the interaction of biomolecules with the surfaces is strongly enhanced, and therefore nanofluidic systems provide excellent ways to improve performances for biomedical applications. Abionic Ltd., introduced nanofluidic biosensors for the detection of IgE. Figure 1 illustrates a cross-section through a nanofluidic biosensor including the nanochannel and lateral openings. The surfaces inside the nanochannel are biochemically functionalized and coated with allergen components. The lateral openings with dimensions in the micrometer range allow a patient sample to enter and to exit the nanofluidic channel by simple capillary forces. A patient sample is prepared with fluorescently labeled biomolecules and deposited into a capsule that contains multiple biosensors including the nanofluidic channels, each prepared with a different allergen component. The biosensors are filled with the solution within a few seconds. The patient's fluorescently labeled allergen-specific IgE molecules interact immediately with the coated surfaces, i.e., the specific allergens, forming molecular immobilized complexes. The fluorescent complexes are then optically measured by a reader system. As the system is calibrated to the international IgE standard from the World Health Organization, the detected fluorescent signal is proportional to the IgE concentration in the sample and therefore quantifies the patient IgE level specific for a given allergen. The nanofluidic biosensor configuration not only provides analytic sensitivity levels of 1 ng/ml but also accelerates the biomolecular interaction eliminating incubation times and therefore reduces assay times to a few minutes. The sub-micrometric dimension of the nanofluidic channel requires very little patient sample volume and produces very little background signal thus making washing steps unnecessary. Furthermore, the multiple miniaturized biosensors contained in one test capsule enable multiplexed assays that are especially interesting in the field of allergy diagnosis. The here presented nanofluidic technology provides a solution with all the necessary attributes for a rapid whole blood near-patient test.

Conclusion and Expert's View

Nanotechnologies may bring a new technical revolution by allowing testing large panels of allergen components

Fig. 1 Vertical cut through a nanofluidic biosensor including lateral openings for the fluidic capillary action. The patient sample containing the analyte and the fluorescently labeled secondary antibody immediately interacts with the immobilized allergens inside the nanochannel. An optical system reads the specific fluorescence signal being proportional to the concentration of the patient's specific IgE



from a very low sample volume at the point-of-care, thereby reducing the long turnaround time of today's laboratory-based singleplex assays and multiple allergen microarrays. Real-time measurements of IgE antibodies allow allergists to make a comprehensive diagnosis of IgE hypersensitivities within a single patient consultation. We believe that the current trends seen in other medical fields should also affect allergy diagnosis, taking into consideration the specificities of the allergy field. A rational use of blood tests and in vivo tests, together with patient history and physical examination should guide allergy experts toward a comprehensive understanding of a patient's phenotype and its underlying molecular mechanisms.

How can novel technologies support this? Nanotechnologies offer the opportunity to reach an extremely low analytic sensitivity with assays having very short turnaround times. The low sample volume allows testing many parameters from as little as a few microliters. These test parameters offer unprecedented opportunities to develop IgE antibody assays widely available to both allergists, pediatricians, thanks to the low sample volume and primary care centers, thanks to short turnaround time.

The fact that very few technologies evolve from an academic proof-of-concept to a commercially available product highlights the difficulties that must be taken into consideration early in the product development. Cost, ease-of-use, miniaturization, robustness, and manufacturability are parameters that must also be considered when developing a new product extending the list of analytical and performance requirements. In general, a couple of months are necessary to produce first results for a proof-of-concept

with a new technology from an academic environment. However, bringing a proof-of-concept to a product takes a lot of testing and optimization in order to achieve the needed performance characteristics and reproducibility. In a next phase, the designed product needs to be industrialized, transferred to production, and go through production volume scale-up steps. This might be very challenging due to new processes and manufacturing methods that must be developed as well. The different development stages must be accompanied by clinical tests with patient samples and by putting the product into the hands of the allergists. Eventually, regulatory requirements must also be respected before the product can be commercialized. In conclusion, a new in vitro diagnostic assay based on nanotechnology that offers the highest simplification for the different development phases has the greatest potential to be successful on the path to market.

Compliance with Ethical Standards

Conflict of Interest The first author has a patented nanofluidic biosensor and its use for rapid measurement of biomolecular interactions in solution and methods issued; a patented apparatus and method for detecting and measuring biomolecular interactions; and is one of the inventors of the nanofluidic technology described in the text.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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