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Short Communication

Introduction to nanoMIPS Prepared by Solid-Phase approach as an Alternative for Antibodies in the pseudo-ELISA Diagnostic Assay

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Abstract

The presented article describes the possibilities of replacing antibodies with artificial molecules in the pseudo-ELISA diagnostic assay. The enzyme-linked immunosorbent assay ELISA is based on specific antibodies, linked to an enzyme, to detect the determined molecule. However, the production of antibodies is complicated and therefore its price is high. Also, the stability of the antibodies is inadequate for their price. For this reason, research into artificial molecules that could replace natural immunoglobulins is becoming extremely important. The article presents studies comparing the use of antibodies and molecularly imprinted nano molecules, nanoMIPs, as artificial antibodies in the ELISA assay.

Keywords: nanoMIPS; pseudo-ELISA; Solid phases imprinted method

Introduction

The principle of antibody activity is based on the recognition and binding of antibodies to an antigen, or more precisely an epitope. This means that each antibody molecule has a specific binding site. Multi specific antibodies are able to recognize and bind to more than one antigen. In contrast, monoclonal antibodies bind to only one specific antigen and thus are characterized by their high affinity and selectivity [1,2]. Considering the complexity and sophistication of these molecules, creating their artificial counterparts is not easy. The difficulty of this task is confirmed by the fact that researchers have been working on this type of material for over 25 years. The first demonstration that the recognition material in diagnostic assays can be replaced by artificial antibodies based on molecularly imprinted polymers (MIPs) was published in 1993 [3]. MIPs are synthetic materials, polymerized in the presence of a specific target molecule called the 'template'. The template molecule can be considered as an antigen or in some cases as a fragment of the antigen molecule. They are based mainly on acrylic or methacrylic monomers which functionality enables to form a monomer-template complex in a very similar manner as the interactions between amino acids (from antibody) and antigen are formed [4]. Superior of MIPs compared with natural antibodies is also reflected in the types of the analytes that MIPs can be produced for such as toxins and some pesticides which prove to be difficult to produce antibodies for. In effect, the plastic antibodies/imprinted polymers in various formats (nanoparticle or film/microparticles) have successfully been produced against a wide range of targets such as: ions (phosphate[5], carbonate[6], copper ions [7], small molecules (caffeine[8], cocaine[9], melamine[10], ascorbic acid[11], sugars[12], fentanyl[13], doxorubicin[14,15], phosphorganic pesticides[16], explosives[17], etc), peptides (vancomycin[18-20], peptides/protein epitopes [21,22]), proteins (trypsin[21,23,24], acetylcholinesterase [25]), *E. coli* [26].

The further development of solid-phase imprinted method made it possible to obtain particles with surface imprinting in nano sizes with the similarity to monoclonal antibodies [4]. This method is based on interactions between the monomer and the functional groups present in the template, which is previously immobilized on the solid phase. After the polymerization process, the low-affinity particles and non-reacted monomers are removed by the low-temperature washes. Subsequently, the temperature is raised and the high affinity nano MIPs are collected while the template molecule is still attached to the solid phase. The template-derived sites created within a polymeric matrix allow MIPs to selectively recognize and bind to the target molecule (antigen) [27-31]. Produced nanoMIPs

are more resistant to chemical and biological damage and inactivation than antibodies [26]. For this reason, the imprinted nanoparticles have high potential to be generic alternatives to antibodies in sensors, diagnostics, separations and in enzyme-linked immunosorbent diagnostic assays [19,31,32]. One of the first results, showing the advantage of the technology, were published in 2013 and presents the use of nano MIPs in the new, highly specific, sensitive, and clinically relevant enzyme-linked assay for vancomycin detection [19].

The developed assay was based on nanoMIPs-coated microplates and allowed the accurate determination of vancomycin over the concentration range of 0.001–70 nM, with a limit of detection of 0.0025 nM (Figure 1). It also allows determining vancomycin in plasma at clinically relevant concentrations with a mean accuracy of 98% and very low cross-reactivity with three other antibiotics (amoxicillin, gentamicin, and bleomycin) comparable to high-quality monoclonal antibodies [19]. Another interesting approach, based on nanoMIPs with the catalytic core, provides a new route towards replacement of unstable biomolecules in immunoassays and fully abiotic assays where the nanoparticles possess both recognition and signaling properties [33]. The nanoMIPs were also successfully synthesized for another antibiotic - gentamicin and used in pseudo-ELISA assay. This antibiotic was determined in milk at clinically relevant concentrations with a mean accuracy of 94%. The cross-reactivity of such nanoparticles was investigated with streptomycin and ampicillin as control antibiotics, demonstrating excellent specificity [34].

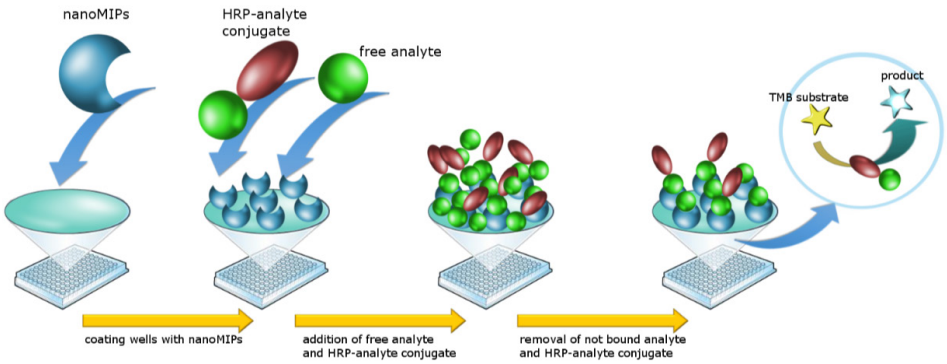

Figure 1: Pseudo-Elisa assay using nanoMIPs-coated microplates.

The nano MIPs can be created with relatively short development times for various types of molecules and still demonstrate comparable or better performance to commercially produced antibodies in enzyme-linked competitive assays (Table 1) [35]. It has been also shown that the polymerization type has influence on the nano MIPs performance in the pseudo-Elisa assays depending on the size of imprinted molecule [10]. The studies concluded that the best performances were obtained for particles synthesized in aqueous media for the larger analytes such as horseradish peroxidase (HRP. 44 kDa), cytochrome C (Cyt C. 12 kDa) and biotin (244.31 g mol−1) but completely failed for the smallest template melamine (126.12 g mol−1). The high-performance Elisa for melamine was therefore achieved using nanoMIPs prepared by UV polymerization in an organic media with a shell created by poly (glycol ethanol).

Table 1: Limit of detection, LoD for nanoMIPs and antibodies-based assay.

Template	LoD, nano MIP based assay, nM	LoD, Antibodies based assay, nM	Ref.
Biotin	1.20x10-3	2.54x10-3	[35]
L-Thyroxine	8.07x10-3	17.5	[35]
Glucosamine	4.01x10-4	3.38x10-4	[35]
Fumonisine B2	6.12x10-3	2.5x10-2	[35]
Fumonisine B1	1.90x10-3	4.18x10-2	[36]
Hemoglobine	8.70x10-3	1.54x10-4	[37]
Glycated hemoglobin	2.46x10-3	2.38x10-4	[37]
Vancomycin	2.50x10-3	-	[19]
Gentamycin	1x10-4	-	[34]
Horseradish peroxidase	0.5	-	[10]
Cytochrome C	45	-	[10]
Melamine	25	-	[10]
LC-mycocistin	2.64x10-4	-	[38]
Cocaine	4.24x10-3	3.3	[38]

In conclusion, the nanoMIPs prepared by solid-phase imprinted methods have high potential to replace antibodies in pseudo-ELISA assays with high specificity and no cross-reactivity. Moreover, the nanoMIP-coated microplates can withstand exposure to high temperature for prolonged periods without affecting the sensitivity of the assay. Conducted research suggested also that microplates coated with nanoMIPs do not require refrigeration during transportation and were also stable during the storage at room temperature for at least 1 month, which brings additional social and economic benefits [19,33].

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