One-step antibody immobilization-based high sensitivity immunoassay procedure for potential in vitro diagnostics

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Summary

A potential one-step antibody (Ab) immobilization strategy was developed for the leach-proof binding of capture Ab to the polystyrene microtiter plate (MTP) [1-3]. It enables the development of high sensitivity, cost-effective and analytically superior immunoassay (IA) for potential *in vitro* diagnostic (IVD) kits. The NIF-based IA detected HFA in the range of 4.9 pg mL^{-1}-20 ng mL^{-1} with limit of detection (LOD) of 7 pg mL^{-1} (Fig. 1B (a)). The analytical sensitivity was 10 pg mL^{-1}, which was 51-fold more sensitive than the convention immobilization format (CIF) based commercial ELISA.

1 Material and Methods

Human fetuin A (HFA) was taken as a model analyte system as all the components of sandwich HFA IA are available commercially in the form of a sandwich enzyme-linked immunosorbent assay (ELISA) kit from RnD Systems, USA. HFA is a specific biomarker for atherosclerosis and hepatocellular carcinoma, and is associated with arthritis, cardiovascular diseases, diabetes, metabolic syndrome and multiple sclerosis. Therefore, the highly sensitive and precise determination of HFA is of immense biomedical importance. The developed one-step Ab immobilization strategy based new immobilization format (NIF) involves simply the dilution of Ab in 3-aminopropyltriethoxysilane (APTES) (Fig. 1A). It leads to the formation of stable complex of Ab with APTES by ionic and hydrophobic interactions and its subsequent adsorption on polystyrene MTP. The Fourier transform infrared (FTIR) spectra shows all the characteristics Si-O-Si, primary amine and Ab peaks (Fig. 1A).

2 Results

The NIF-based IA detected HFA in the range of 4.9 pg mL^{-1}-20 ng mL^{-1} with limit of detection (LOD) of 7 pg mL^{-1} (Fig. 1B (a)). The analytical sensitivity was 10 pg mL^{-1}, which was 51-fold more sensitive than the convention immobilization format (CIF) based commercial ELISA. It enables highly specific HFA detection without any interference with immunological reagents (Fig. 1B (b)). The developed IA can detect HFA with high precision in various sample matrices, as demonstrated by the detection of HFA spiked in diluted human whole blood and plasma (Fig. 1B (c). It can detect the entire pathophysiological range of human CRP in serum (0.08-480 µg/mL) after appropriate sample dilution. The inter-day and intra-day variability of NIF-based IA were 2.1-10.2% and 1.2-8.5%, respectively, while the maximal half-effective concentration (EC_{50}) was 2.6 ng mL^{-1}. NIF-based IA was also 3-times more sensitive than our previously-developed covalent immobilization format (CovIF) (Fig. 1B (a)).

The Ab-bound and BSA-blocked MTPs stored in 0.1 M PBS, pH 7.4 at 4°C were highly stable as there was no significant decrease in their functional activity for 8 weeks (Fig. 1B (d)). Therefore, they can be employed in clinical and industrial labs, where greater number of samples need to be rapidly analysed.

3 Conclusions and Outlook

ELISA-based technologies have a lucrative commercial market and have been extensively investigated, as evident from more than 400,000 peer-reviewed articles to date. The NIF-based IA procedure is generic and have been similarly employed for the development of high sensitivity IVD assays for other biomarkers such as C-reactive protein (CRP), human albumin and human lipocalin-2, where it gave similar results. Therefore, it has large potential for the development of high sensitivity IVD assays and kits.

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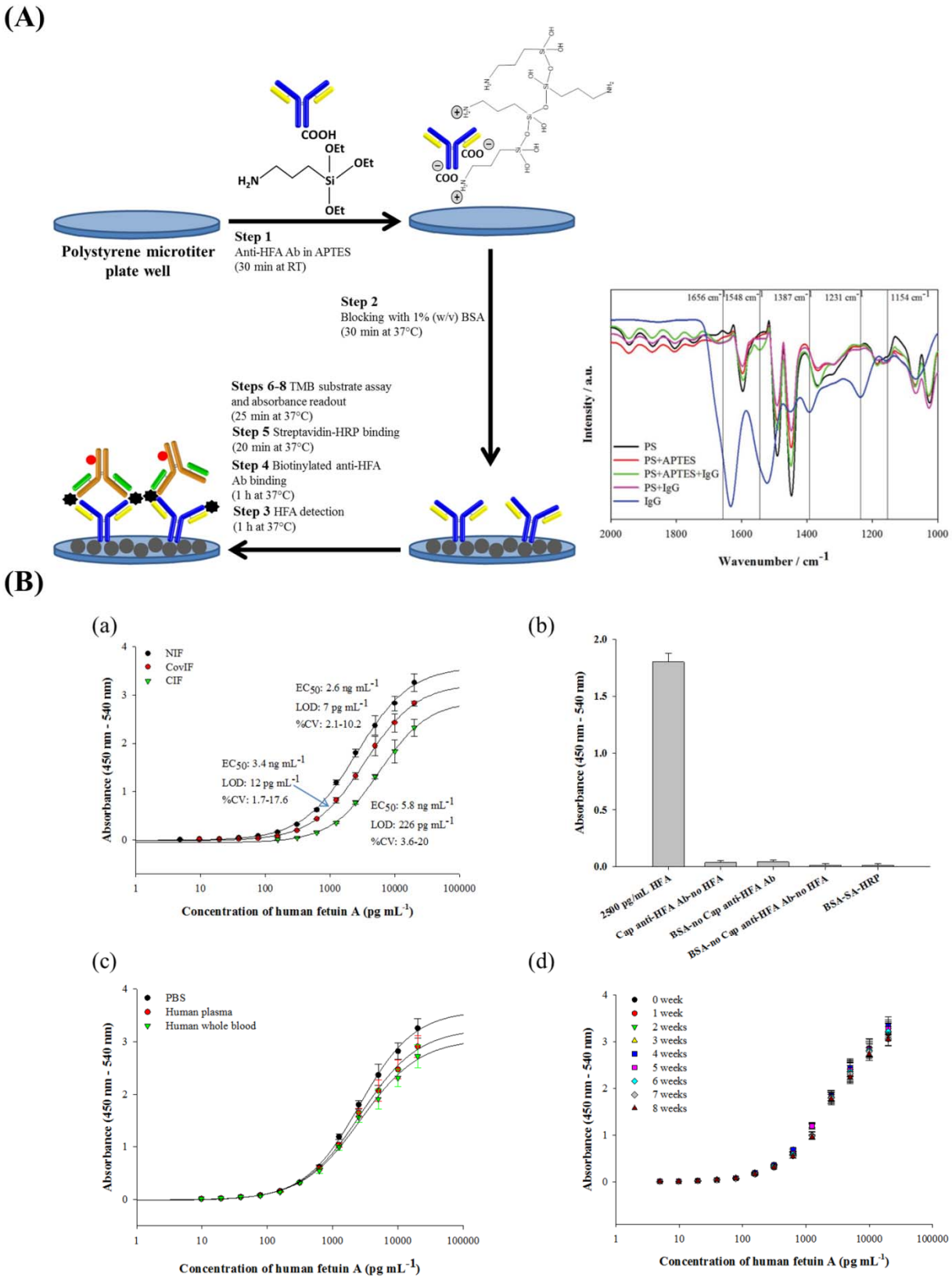

Figure 1. (A) Left: One-step Ab immobilization-based high sensitivity HFA IA. Right: FTIR characterization. (B) (a) Detection of HFA by the developed (NIF), conventional (CIF) and covalent (CovIF) IA formats. (b) Experimental process controls. (c) Detection of HFA spiked in various sample matrices. (d) Stability of anti-HFA Ab-bound MTPs stored in 0.1 M PBS, pH 7.4 at 4°C.

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