Solid surface development for an ultrafast immunoassay concept

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Introduction

Diagnostic devices are important tools in resolving the right diagnosis. Futuristic diagnostic tools may prevent from having to re-visit the doctor as results may be provided within minutes.

In heterogeneous immunoassays, the reaction of interest happens on solid surface. Reaction consumes reagents near the surface, thus reducing reaction rate. Efficient mixing balances the lack of reagent near surface.

The aim of this study was to develop a solid surface treatment to work with a novel stirring technique. The baseline method was coating the solid surface with streptavidin by adsorption, which had issues of low streptavidin density and high background signal from nonspecific binding of plasma samples.

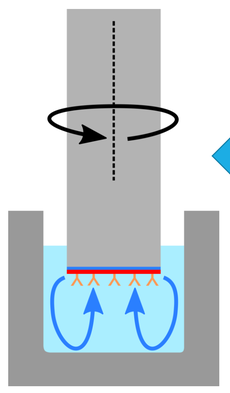


Fig 1. *Rapid spinning of cylindrical solid surface forms a strong current towards the surface of the test cylinder. With proper equipment, the solid surface may be automated to incubate, wash and dry the surface by moving it between different pools.*

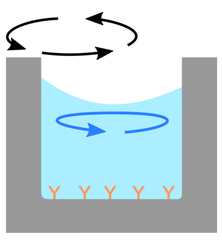


Fig 2. *Eccentric rotation of microplates causes fluid to spin around the edges of wells, side to side rather than up and down.*

Methods

The silane treatment (Fig 3) was meant to fill cracks in handcrafted test cylinders and block nonspecific components of blood plasma from sticking into the solid surface. Side chains of silane were carbonylated for covalent binding of streptavidin.

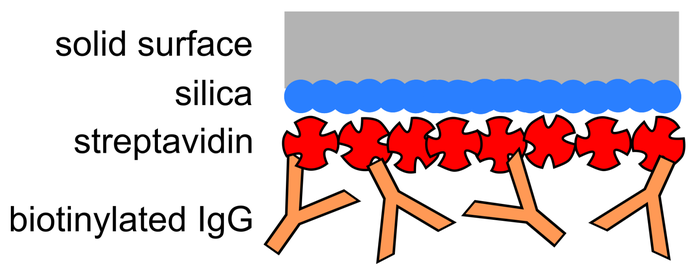


Fig 3. *Silane treatment*

Functionality of the treatment was analyzed with two immunoassays (Fig 4, Fig 5) and SEM-based element analysis.

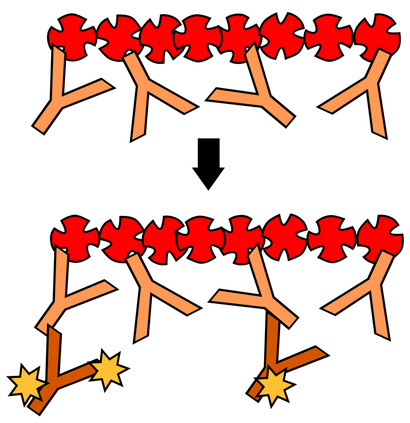
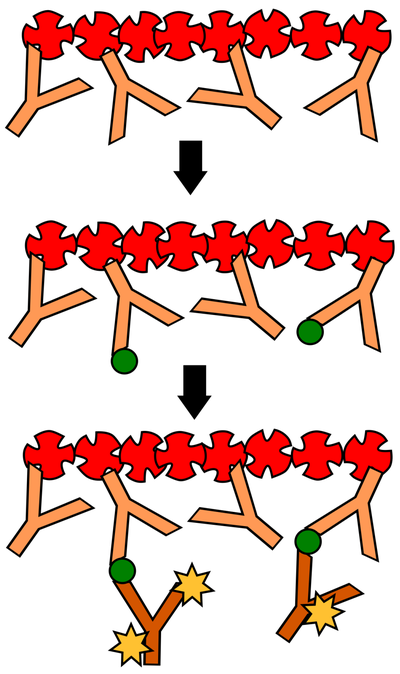


Fig 4. *Immunoassay for determining the streptavidin density of the developed surface. Biotinylated mouse antibody was bound to streptavidin. Labelled rabbit anti-mouse antibody was bound to mouse antibody.*

Fig 5*. Sandwich immunoassay for analysing efficiency of the silane method with a realistic immunoassay and studying the effects of blood plasma on surface. Biotinylated, analyte-specific antibody was bound to streptavidin. Analyte was bound to the antibody and a labelled, analyte-specific antibody was bound to the analyte.*



Results

Streptavidin density of the solid surface has multiplied due to the new surface treatment (Fig 6).

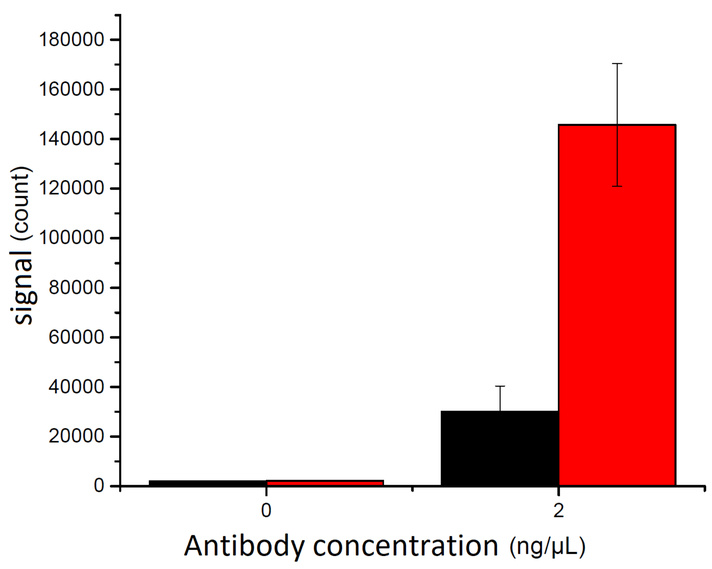


Fig 6. *Comparison of surface streptavidin density in the developed surface treatment (red) and passive adsorption of streptavidin (black).*

The novel spinning method reached reaction rates of similar assay with traditional microplate shaking (Fig 7). Although, deviation within the new solid surface is still larger than that of commercial microplates and the surface can't yet be used with blood plasma.

SEM-analysis of silica surfaces displayed no signs of silicon.

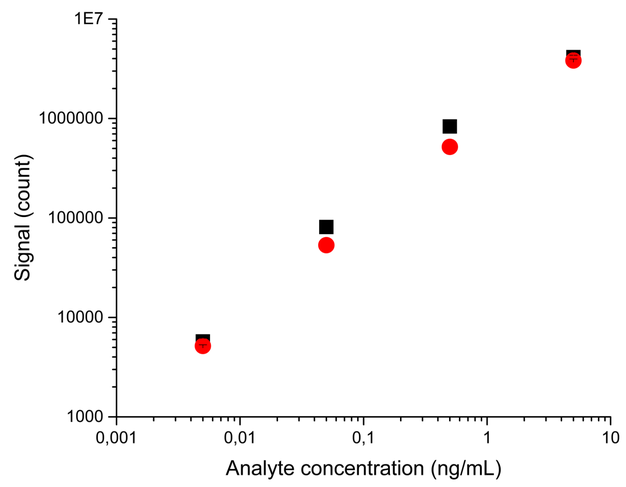


Fig 7. *Comparison of the developed surface treatment (black) and commercial microplates (red) in a time-restricted sandwich-assay.*

Conclusions

Silane does not seem to bind to the side groups of polystyrene. Earlier studies have proven synthesis of silanol-coated polystyrene microparticles possible. In this study, the surface was 10^6 times larger, which likely makes it impossible to synthesize a silane grid that traps the surface inside itself without bonding of any sort.

Despite the failure in silica coating, the chemically induced binding of streptavidin has likely happened onto polystyrene surface.