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Using Microcantilevers with Biofunctionalized Surfaces for Malaria Vaccine Diagnosis

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Abstract

The purpose of this project was to test candidate vaccines for their immunogenic response using a technology consisting of functionalized microcantilevers and an optical readout system. Serum samples from a clinical trial were analyzed and quantitative diagnostic results were obtained. This was compared to the immunoassay testing system ELISA using a calibration curve. It was shown that the microcantilever system was as sensitive as ELISA, while also producing an in-situ, controlled, specific response.

Introduction

Malaria is one of the major causes of death in tropical and sub-tropical countries and in 2018 an estimated 228 million cases occurred worldwide[1].

Reliable diagnostics and point-of-care testing are essential to control the spread of the disease in these areas.

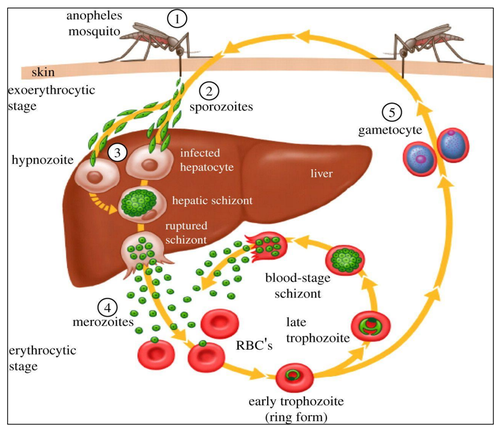

Figure 1: The life cycle of the malaria parasite[4].

The subject of this project is a new generation of biological sensors that can detect the presence of several specific antibodies at once in just 6 µL serum sample.

Volunteer samples from a malaria vaccine trial were tested for immunogenic response, producing some interesting results.

Biofunctionalization

Figure 2: A typical cantilever array[3].

The sensor array used for measurements (fig 2) consists of a silicon chip with 8 individual cantilevers. Each of these are 500 µm in length and 1 µm thick.

The surface of the cantilevers were chemically cleaned and treated with DSU. This forms a self-assembled monolayer (SAM), which binds to the chip's gold surface on one side and the amino groups of the vaccine on the other.

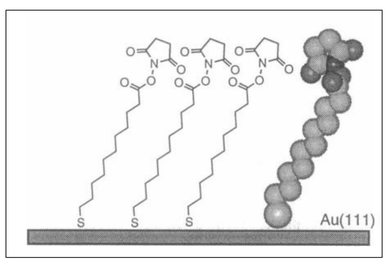

Figure 3: A self assembled monolayer of DSU on a gold surface[2].

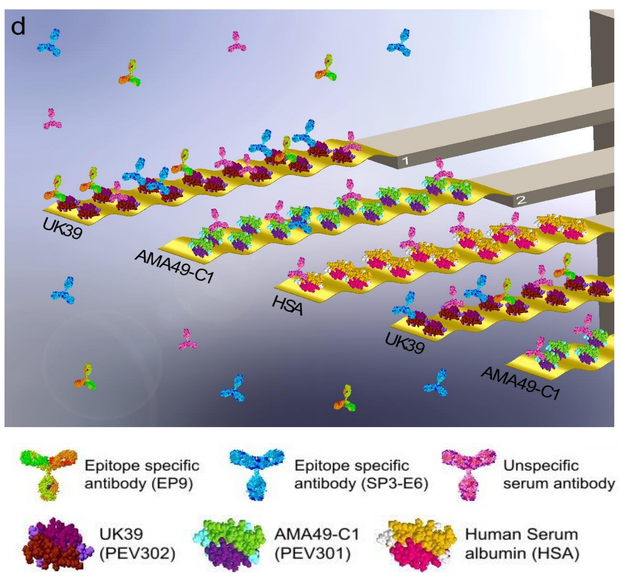

Figure 4 : Antibodies in human serum binding to the functionalized surface during the experiment. Reproduced with permission from author.

In this case, chips were functionalized with two malaria vaccine candidates, amical membrane antigen 1 (AMA49-C1) and circumsporozite protein (UK39). These are virosomal formulations designed to produce an antigenic response In humans.

UK39 is designed to protect against the first stage of infection, the pre-erythrcytic stage, while AMA49-C1 protects the body against the blood stage, when symptoms first appear.

Frequency Measurements and Device

The functionalized chip is placed inside a chamber where an electrical piezo element causes it to resonate at frequencies of between 10 kHz and 2.8 MHz. The human serum samples are then injected into the chamber for analysis.

If the target biomarker (specific antibodies) is present in the sample injected, they will bind to the receptor molecules on the sensors (AMA49-C1 or UK39). The increased mass will make the system resonate at a lower frequency, and show a definite change, Δf.

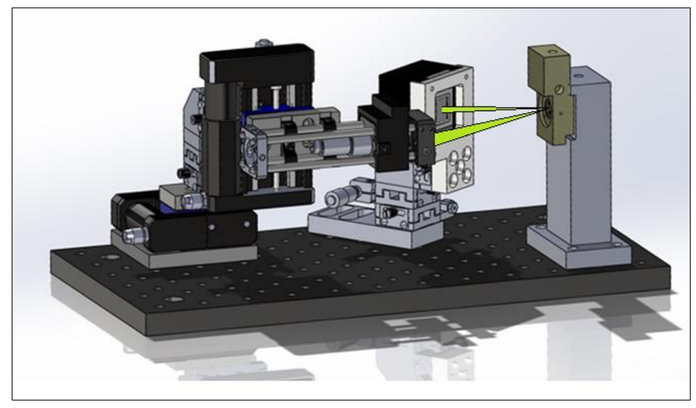

Figure 5: The measurement device. A laser is reflected off the cantilever surface onto a position sensitive diode (PSD). This tells us the resonant frequencies of each cantilever[4].

The recorded Δf is then converted to Δm through data processing. The mass addition is calculated differentially by subtracting the non-specific response (AMA49-C1) from the measurement response (UK39). This shows the epitope specific UK39 biomarker binding.

Results and Discussion

Figures 6 and 7 below show a typical mass differential measurement performed by the Nanobio group[4]. The frequency change directly correlates to the uptake of a specific biomarker. In this case, the immunisation against UK39 is established by the presence of UK39-specific antibodies in the volunteer serum.

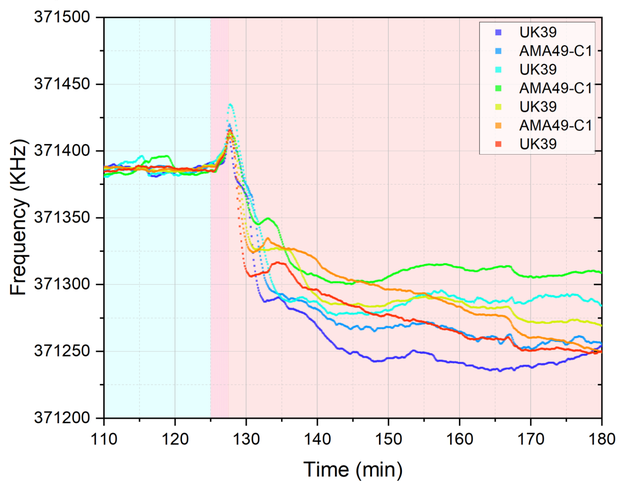

Figure 6: The frequency shift for all cantilevers upon injection of patient serum is shown. The change in frequency is directly proportional to the mass of molecules (both specific and unspecific) that have attached to the cantilever surface.

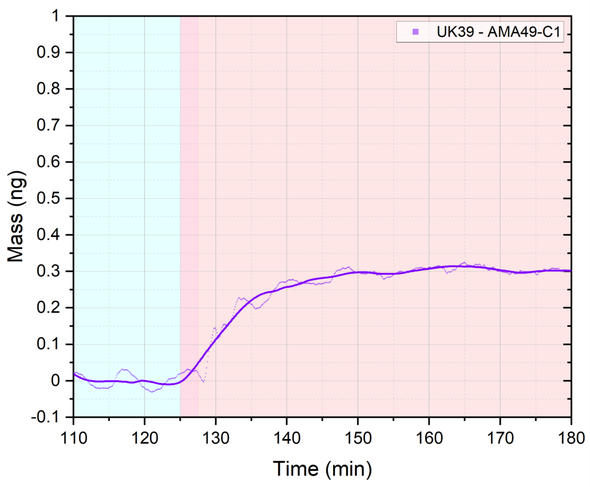

Figure 7: The mass uptake for the same injection of serum as figure 6 was plotted. By looking at the differential between AMA and UK39, we can see the antibodies produced by the volunteer were for the pre-erythrcytic stage of the malaria life cycle.

The figures above confirm that this testing method is reliable and matches the ELISA test in sensitivity. This, along with a differential read-out and the ability to evaluate several biomarkers simultaneously, make it a powerful technology not only for vaccine testing but also for virus detection, blood coagulation analysis and cancer diagnostics.

References

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