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Antibody recognition of tumor cells on the cantilever surface

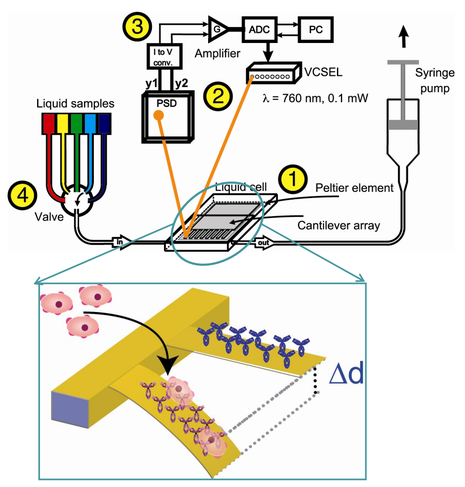
J. Zhang[1], F. Huber[1], D. Rimoldi[2], H.P. Lang[1], Ch. Gerber[1]

1 Swiss Nanoscience Institute, University of Basel, CH-4056 Basel
2 Ludwig Institute for Cancer Research, University of Lausanne, CH-1066 Epalinges)

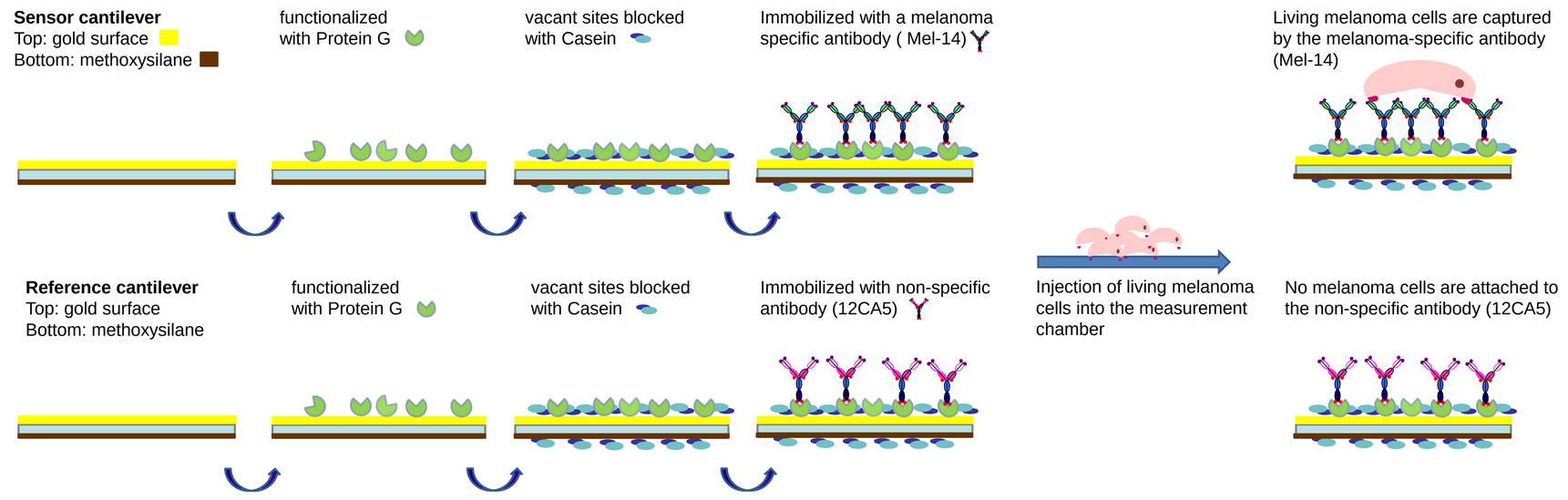
SNI SWISS NANOSCIENCE INSTITUTE
EINE INITIATIVE DER UNIVERSITÄT BASEL UND DES KANTONS AARGAU

Introduction

We propose the combination of nanomechanical cantilever with melanoma-specific antibody (Mel-14) as a biosensing method to detect living melanoma cells *in situ*. Our detection scheme is based on the interaction between antibody and melanoma associated antigen (HMW-MAA) on the surface of melanoma cells, which should lead to a change in cantilever deflection. After injection of viable melanoma cells, we monitored cantilever surface bending signals originating from a gold surface coated with a melanoma-specific antibody. Specifically, we evaluated differential deflection data calculated by subtracting the response of a reference cantilever from the melanoma-specific antibody response. The comparison with a flow experiment under a microscope reveals that changes in nano-mechanical responses are associated with attachment of living cells on the sensor cantilever surface.



Melanoma cell detection measurement setup



Schematics of the functionalization and detection process on both sensor and reference cantilever surfaces

Experiment results

1. Real-time capturing of live melanoma cells 4.2*10^5 cells/ml in RPMI/Hepes/Pen-strep

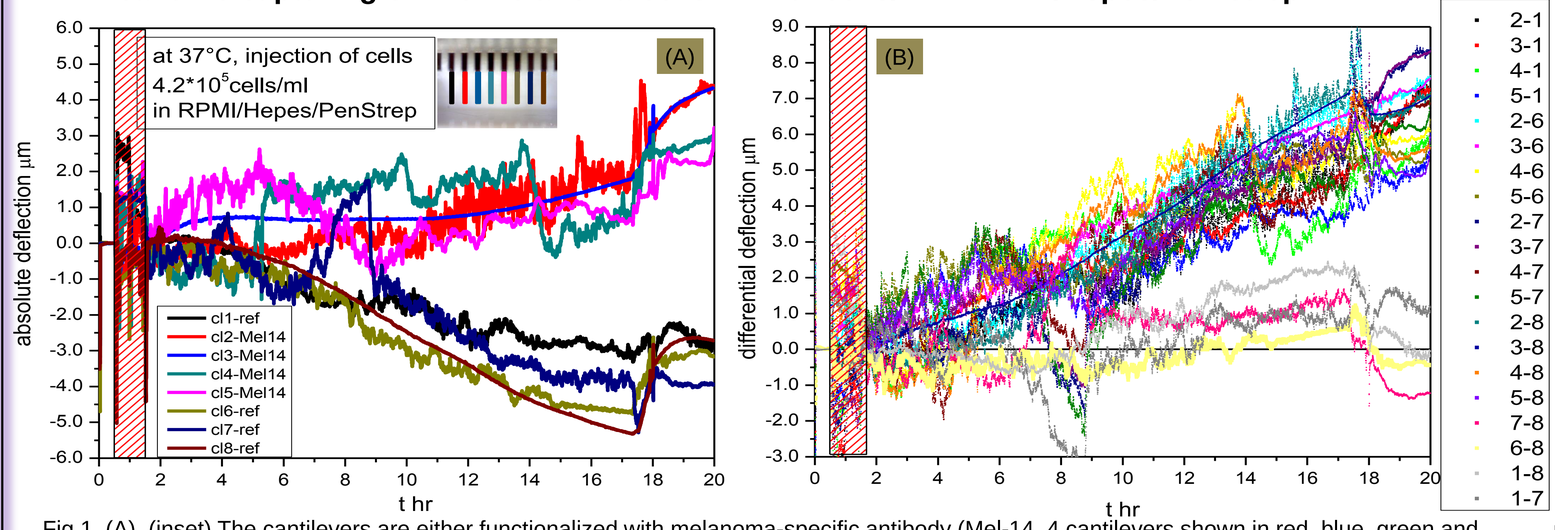


Fig.1. (A) (inset) The cantilevers are either functionalized with melanoma-specific antibody (Mel-14, 4 cantilevers shown in red, blue, green and pink) or with a non-specific antibody (12CA5, 4 cantilevers in black, olive, dark blue and brown). The absolute bending signal after interaction of the melanoma-specific antibody and its antigen, as well as the response of the non-specific antibody shows different reactivity (two groups) for both sensors and reference cantilevers. (B) Differential bending signal showing a very reproducible positive signal that corresponds to tensile stress building up on the sensor cantilever. The four curves at the bottom show the difference in the responses of four pairs of reference cantilevers, yielding smaller signals than the sensor cantilevers.

2. Melanoma cell concentration-dependent measurements

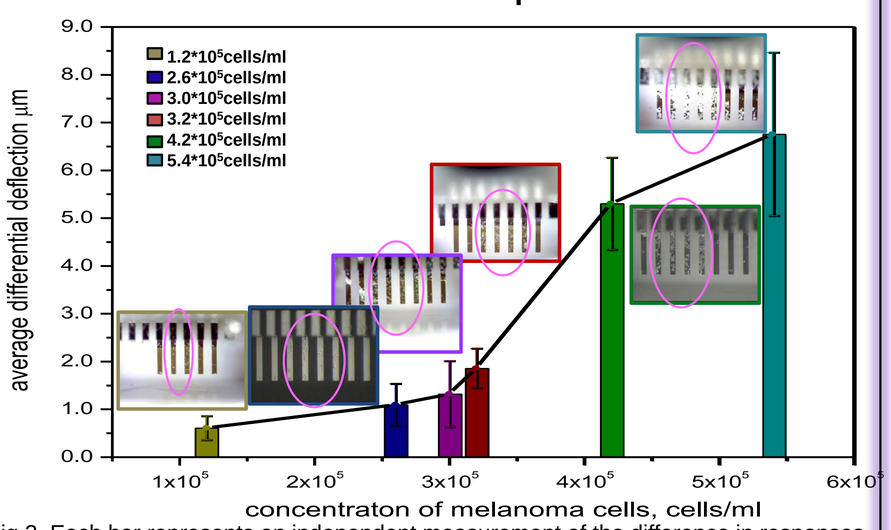
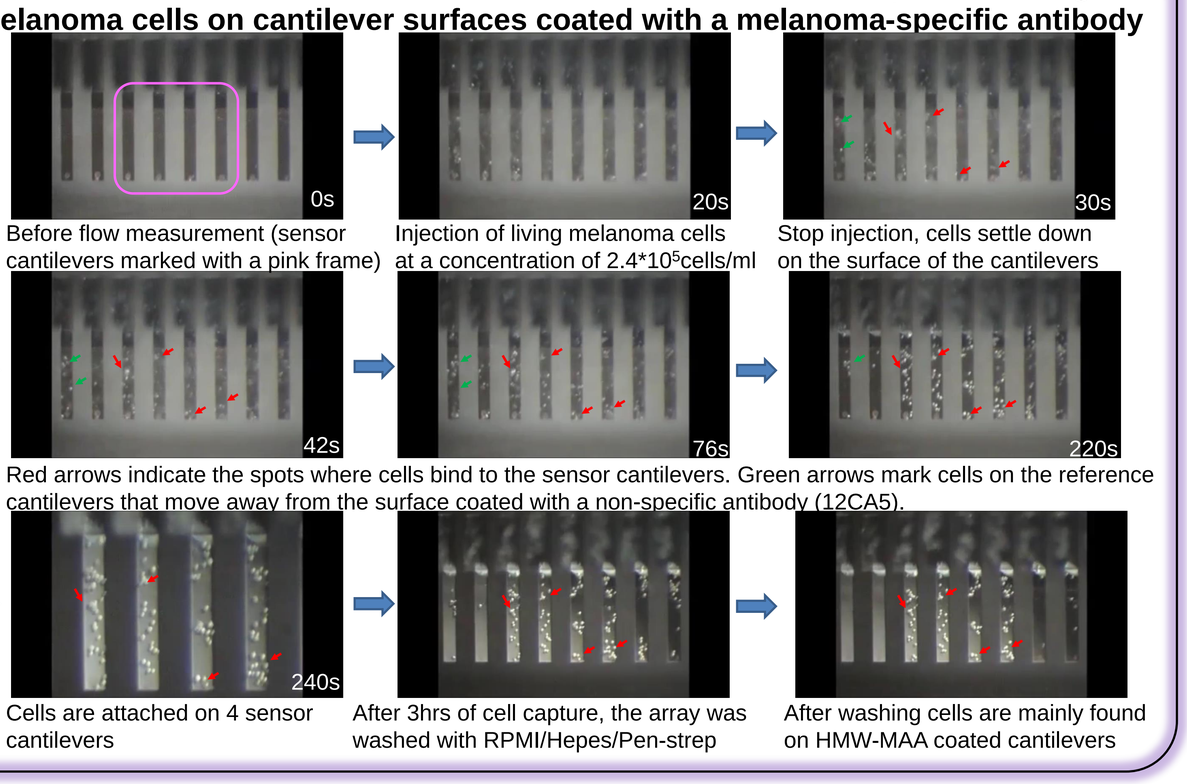


Fig.2. Each bar represents an independent measurement of the difference in responses of a sensor and a reference cantilever. The average differential responses at six various concentrations of melanoma cell are marked by different colors and corresponding standard deviation error bars. The micrographs show the cantilever array at the end of experiment. The color of the frame matches the color of the bar. Sensor cantilevers are marked with a pink oval.

3. Flow measurements in the chamber under a microscope – Capture of living melanoma cells on cantilever surfaces coated with a melanoma-specific antibody



Before flow measurement (sensor cantilevers marked with a pink frame)

Injection of living melanoma cells at a concentration of 2.4*10^{5}cells/ml

Stop injection, cells settle down on the surface of the cantilevers

Red arrows indicate the spots where cells bind to the sensor cantilevers. Green arrows mark cells on the reference cantilevers that move away from the surface coated with a non-specific antibody (12CA5).

Cells are attached on 4 sensor cantilevers

After 3hrs of cell capture, the array was washed with RPMI/Hepes/Pen-strep

After washing cells are mainly found on HMW-MAA coated cantilevers

Conclusions

1. Using cantilever arrays, we demonstrated that living melanoma cells can be captured by cantilevers coated with the melanoma-specific antibody Mel-14. The concentration dependence of the binding process was studied.
2. In a living cell assay, the nanomechanical responses due to capture of cells were detected on sensor cantilevers; binding was also verified using optical microscopy after measurement in the cantilever setup. Only few cells were observed on the surface of reference cantilevers.
3. The antibody orientation is crucial for successful capture of melanoma cells.
4. Binding of melanoma cells on the cantilever surface functionalized with HMW-MAA-specific antibody Mel-14 generated tensile stress.
5. A comparison with the flow experiment under the microscope reveals that the nanomechanical response is associated with the attachment of living cells on the sensor cantilever surface.