Microcantilever Biosensors: using a micromechanical resonator to quantify specific biological targets

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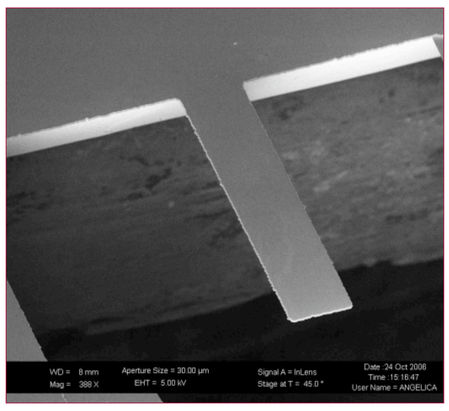
Introduction

Microcantilever (MC) biosensors are label-free platforms that combine a biologically sensitive with a physical transducer in order to selectively and quantitatively detect the presence of specific compounds in a given external environment. Since they can be operated either as micro-mechanical resonator or as surface stress sensor, MCs - activated with antibodies for molecular recognition - enable the measurement of mass with extraordinary sensitivity [1]. This contribution deals with the development of mass detector biosensor based on MC systems that would permit to shift from qualitative data to quantitative measurements of key molecules involved in physiological processes such as angiogenesis.

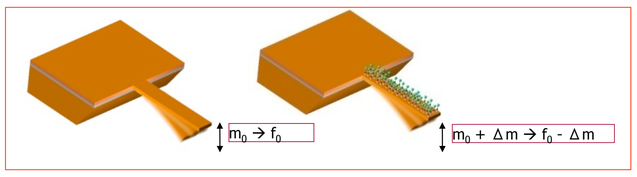
Moreover the aim of our work is the integration of a microfluidic circuit in order to develop a Lab on a Chip (LOC) platform which permit to perform *real time* measurements of the binding processes occurred on the cantilever surface. In vacuum and in liquid experiments are reported to demonstrate the sensitivity and specificity of the MC bio-sensor.

MCs as Mass Detector Biosensors



Dynamic mode

(frequency shift evaluation)

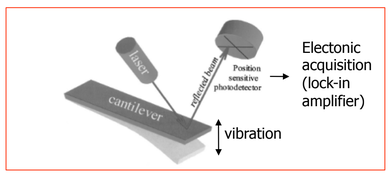


[2]

Detection by **optical lever tecnique**

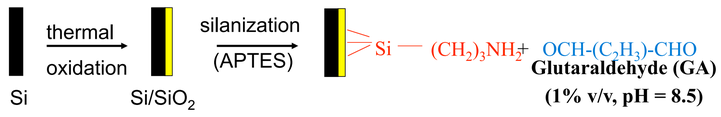
Frequency shift can be related to the additional mass of the analyte adsorbed on the surface

[2] $$\Delta m = -2\frac{\Delta f}{f_0}m_0$$



Experimental Design

i) Surface Activation

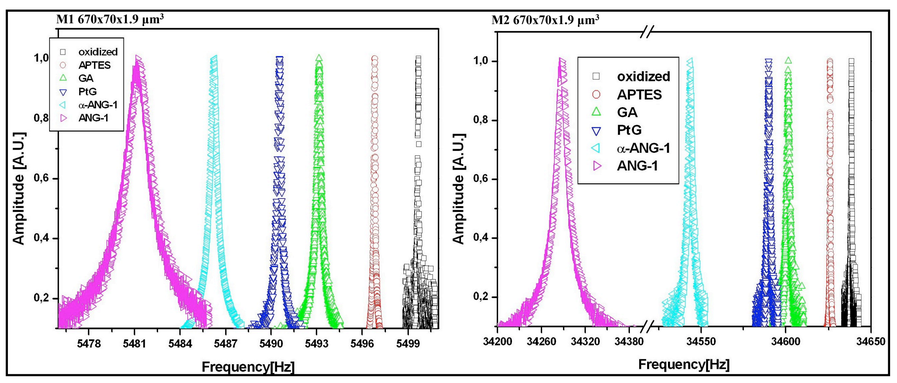


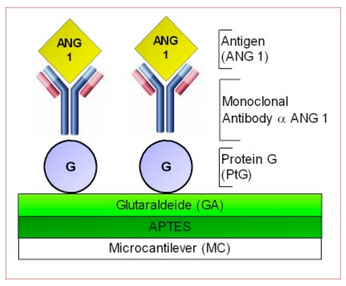
ii) Protein A/G binding (correct orientation of the Fab fragment)

Cancer marker detection: Angiopoietin 1

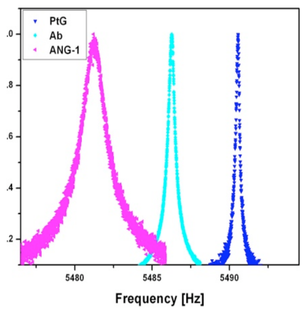
Biological background

Angiogenesis is a complex multistep process regulated by several different growth factors and their associated tyrosine kinase receptor (KDR). Among these, Angiopoietin 1 (ANG-1) and vascular endothelial growth factor-A165 (VEGF-A165) associated with activated KDR has been recognized as the system at the heart of network which governs differentiation, survival, proliferation and migration of Endothelial Cells (EC). Thus ANG-1 is a critical component of several human diseases, including cancer, diabetic microvascular and rheumatic diseases, psoriasis, hemangioblastoma, and ischemia.





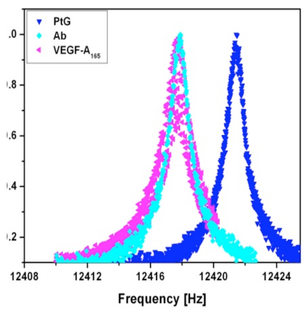
Diagnostic Application



Antibody binding and hybridization with target

Anti-ANG-1 Surf. Density: (2.5 +/- 0.05) x10^{12} molecules/cm^2

ANG-1 Surf. Density: (5.14 +/- 0.02) x10^{12} molecules/cm^2



Selectivity Tests [3]

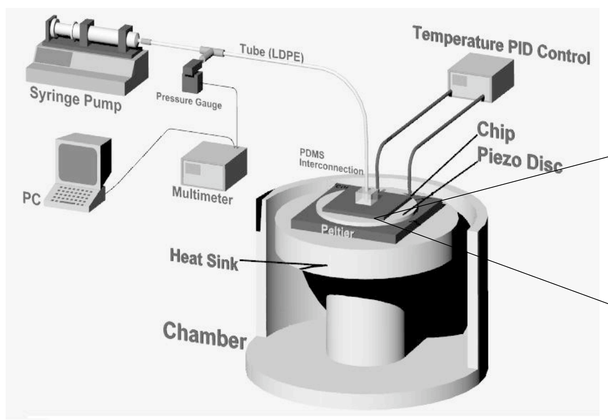
VEGF-A165 is chosen as a false antigen to check the chemical and physical interaction of the cantilever-based platform with different antigens

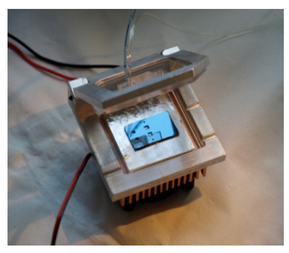
→ **Nearly perfect selectivity**

Integration of microfluidic technology

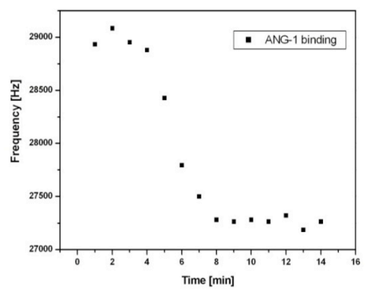
Liquid measurement advantages:
- Physiological structure and function of molecules
- Real-time and kinetics measurement
- Reduction of salt residuals

Integration with LOC instead of fluid cell advantages:
- Reduction of reagents
- Cost efficiency
- Portability
- Restricted dimension tolerance
- Bench production





Real-time biosensing in liquid environment [4]



The capability to monitor the kinetics of molecular binding as well as the interesting opportunity to integrate the sensor device in more complex lab on a chip has moved the attention towards measurements in liquid.

Conclusions

- An experimental protocol was developed for MC-based biosensing of tumor markers.
- Data Analysis demonstrates:
  - high sensitivity
  - measure specificity and reproducibility

→ MC Biosensor approach allows to perform a shift from qualitative data acquisition to accurate quantitative measurements (masses lower than 1 femtomol have been revealed).

→ Microfluidic technology permits the development of Lab on Chip MC bio-sensor which allows to monitor the kinetics of molecular binding

References:
[1] J. Fritz. et al., *Science* (2000) 288-316; [2] N.V. Lavrik et al., *Rev. Sci. Instrum.* (2004) 2229-2253;
[3] C. Ricciardi et al., *Biosens. Bioelect.* (2010) 1193-1198 ; [4] C. Ricciardi et al., *Biosens. Bioelect.* (2010) 1565-1570;