

DETECTION OF ANGIOGENIC GROWTH FACTOR BY MICROCANTILEVER BIOSENSORS

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Introduction. Angiogenesis - the process of new blood vessel growth - plays an essential role in the development of tissues in the vertebrate embryo, and is also involved in a wide variety of physiological and pathological conditions in adults, including wound repair, metabolic diseases, inflammation, cardiovascular disorders, and tumor progression. In order to achieve new insight in the cellular mechanisms that affects angiogenesis, as well as to enable the early detection of low abundant tumor biomarkers, specific and quantitative measurements of target proteins appear to be necessary. In this work, we present the development of a microcantilever-based biosensor suitable to perform quantitative and high sensitive detection of specific proteins. We chose Ang-1 as our target molecule representative of the wide range of angiogenic factors, whose expression level is largely investigated in different tumors.

Experimental. Arrays of Si microcantilevers with dimensions of 250-700 μm in length, 50-80 μm in width and 2-7 μm in thickness were fabricated using a combination of surface and bulk micromachining process [1]. The samples were oxidized, silanized with 3-aminopropyltriethoxysilane (APTES) and derivatized with glutaraldehyde (GA) to covalently bind protein A or G and thus optimize the antigen/antibody recognition. Enzyme Linked ImmunoSorbent Assay (ELISA) experiments were specifically designed to verify silicon surface-protein binding specificity and immobilized protein activity; Pull-down Assay and Immunoprecipitation (IP) experiments were used to establish the optimal experimental conditions. After each binding step, the first and second flexural modes of the MCs were measured by means of an optical lever based set-up [2].

Results and Discussion. In this study, we used the Ang-1-detection system as a demonstrator of the capability of a proper functionalized microcantilever-based biosensor to give quantitative measurements not only in terms of high sensitivity but also in terms of specificity and reproducibility. To this purpose Ang-1 binding experiments were performed using two hybridization reactions: 1) Tie2/Fc–Ang-1, that is characterized by a high receptor-ligand binding affinity, thus representing a suitable reference model to validate the experimental procedure and to demonstrate the feasibility of employing MCs for this application; 2) monoclonal antibody–Ang-1, that allowed us to deal with a biosensor that could be considered as a prototype for further development of highly sensitive detection of biomarkers such as angiogenic factors on the basis of the antigen-antibody reaction.

Interesting results were achieved both with the “proof-of-concept” and the “real” experimental design: Ang-1 masses as low as some picograms (corresponding to an amount lower than a femtomol) were detected in vacuum (see Figure 1).

Furthermore, specificity measurements were performed using the PBS buffer solution without any antigen and with a non-complementary growth factor (VEGF): no appreciable frequency shifts were detected, indicating a very good selectivity of the whole system.

Very Recently we integrated a microfluidic platform with the MC array and developed a Q-enhancement feed-back in order to perform online measurements directly in plasma/serum samples. The work is still in progress, but first measurements showed very interesting features.

References:

- [1] G. Canavese et al., J. Micromech. Microeng. **17** (2007) 1387
- [2] S. Bianco et al., J. Vacuum Sci. Tech. B (2006)

Figure:

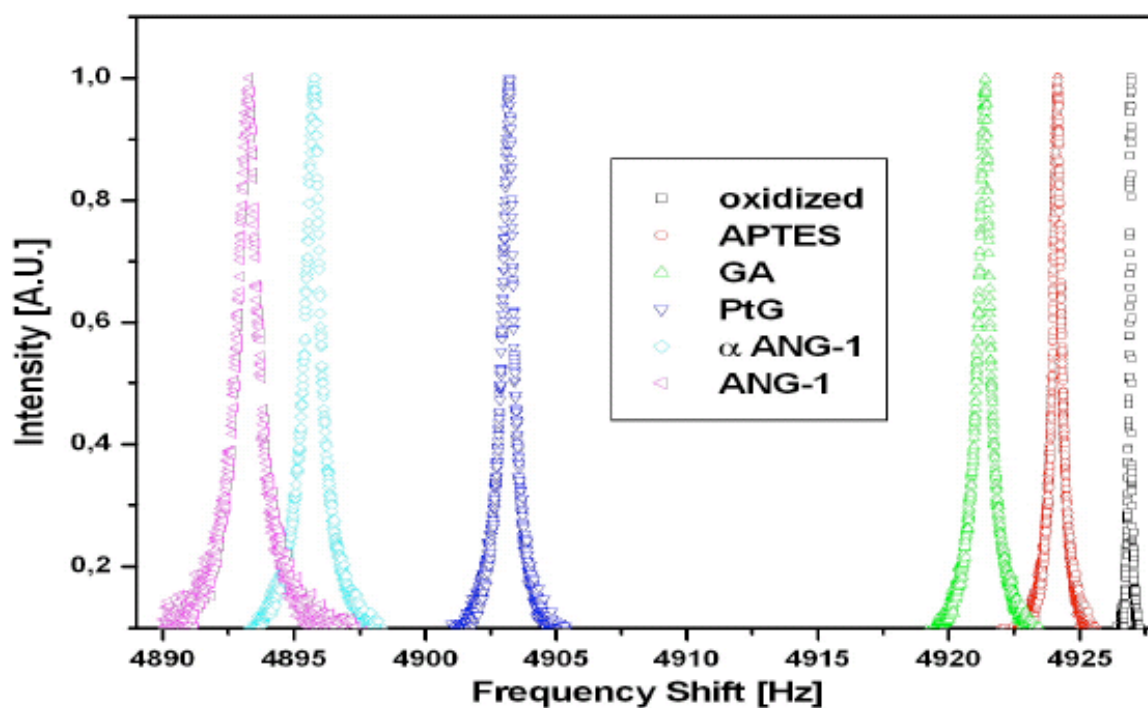


Fig. 1 First flexural mode recorded in vacuum for a MC after each modification step