ELISA-like nano-immuno assay for the proteomic analysis of malignant gliomas.

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Abstract

The quantitative analysis of protein markers for malignant glioma is becoming relevant in the diagnosis and prognosis for this kind of tumors. Despite the efforts in cancer therapy, the prognosis of this common brain tumor remains dismal. In order to face this problem we implemented a promising strategy for the high throughput analysis of few glial cells with potential capability of real-time pathological screening and sub typing of brain tumors. We developed an ELISA like nano-immuno array [1] for proteomic/secretomic analysis suitable for low sample volumes.
In particular, we first fabricate DNA nanoarrays exploiting nanografting [2,3], a tip assisted AFM deposition technique used in order to produce spatially confined monolayers of thiolated oligonucleotides on gold surfaces. By exploiting DNA-directed-immobilization (DDI) of DNA-protein conjugates, we are able to immobilize antibodies for the detection of a specific protein of interest. From the analysis of AFM topographic profiles of the nanoarray before and after the incubation with a cellular sample, we quantitatively determine the concentration of the protein of interest in the volume. As a proof of concept, we immobilize an antibody specific for Glial Fibrillary Acidic Protein (GFAP), a biomarker that belongs to the family of intermediate filaments, crucial in the differentiation of central nervous system cells. The calibration curve in the nanomolar range obtained with this nanoscale assay will be compared with a standard ELISA assay. Results about the detection of GFAP in cellular lysate will also be presented. The integration of our nanoarray with microfabricated wells for sorting and hosting cells (ideally few cells per well), will be discussed.

References

[1] Bano F, Fruk L, Sanavio B, Glettenberg M, Casalis L, Niemeyer CM, Scoles G, Nano Lett. **9** (2009) 2614
[2] Liu M, Liu GY, Langmuir **21 (**2005) 1972
[3] Mirmomtaz E, Castronovo M, Grunwald C, Bano F, Scaini D, Ensafi AA, Scoles G, Casalis L, Nano Lett. **8** (2008) 4134