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*Development of biohybrid immuno-selective membranes for target antigen recognition*

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***Abstract***

Membranes are gaining increasing interest in solid-phase analytical assay and biosensors applications, in particular as functional surface for bioreceptors immobilization and stabilization as well as for the concentration of target molecules in microsystems.

In this work, regenerated cellulose immuno-affinity membranes were developed and they were used for the selective capture of interleukin-6 (IL-6) as targeted antigen. Protein G was covalently linked on the membrane surface and it was successfully used for the oriented site-specific antibody immobilization. The antibody binding capacity of the protein G-coupled membrane was evaluated. The specific anti IL-6 antibody was immobilized and a quantitative analysis of the amount of IL-6 captured by the immuno-affinity membrane was performed. The immobilization procedure was optimized to eliminate the non-specific binding of antigen on the membrane surface. Additionally, the interaction between anti IL-6 antibody and protein G was stabilized by chemical cross-linking with glutaraldehyde and the capture ability of immuno-affinity membranes, with and without the cross-linker, was compared. The maximum binding capacity of the protein G-coupled membrane was  $43.8 \mu\text{g}/\text{cm}^2$  and the binding efficiency was 88 %. The immuno-affinity membranes showed a high IL-6 capture efficiency at very low antigen concentration, up to a maximum of 91 %, the amount of captured IL-6 increased linearly as increasing the initial concentration. The cross-linked surface retained the antigen binding capacity demonstrating its robustness in being reused, without antibody leakage or reduction in antibody binding capacity. The overall results demonstrated the possibility of a reliable application of the immuno-affinity membrane developed for biosensors and bioassays also in multiple use.

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