Double Functionalization of Magnetic Colloids with Electroactive Molecules and Antibody for Platelet Detection and Separation

Authors: Feixiong Chen, Naoufel Haddour, Marie Frenea-Robin, Yves MéRieux, Yann Chevolot, Virginie Monnier

Abstract: Neonatal thrombopenia occurs when the mother generates antibodies against her baby's platelet antigens. It is particularly critical for newborns because it can cause coagulation troubles leading to intracranial hemorrhage. In this case, diagnosis must be done quickly to make platelets transfusion immediately after birth. Before transfusion, platelet antigens must be tested carefully to avoid rejection. The majority of thrombopenia (95 %) are caused by antibodies directed against Human Platelet Antigen 1a (HPA-1a) or 5b (HPA-5b). The common method for antigen platelets detection is polymerase chain reaction allowing for identification of gene sequence. However, it is expensive, time-consuming and requires significant blood volume which is not suitable for newborns. We propose to develop a point-of-care device based on double functionalized magnetic colloids with 1) antibodies specific to antigen platelets and 2) highly sensitive electroactive molecules in order to be detected by an electrochemical microsensor. These magnetic colloids will be used first to isolate platelets from other blood components, then to capture specifically platelets bearing HPA-1a and HPA-5b antigens and finally to attract them close to sensor working electrode for improved electrochemical signal. The expected advantages are an assay time lower than 20 min starting from blood volume smaller than 100 µL. Our functionalization procedure based on amine dendrimers and NHS-ester modification of initial carboxyl colloids will be presented. Functionalization efficiency was evaluated by colorimetric titration of surface chemical groups, zeta potential measurements, infrared spectroscopy, fluorescence scanning and cyclic voltammetry. Our results showed that electroactive molecules and antibodies can be immobilized successfully onto magnetic colloids. Application of a magnetic field onto working electrode increased the detected electrochemical signal. Magnetic colloids were able to capture specific purified antigens extracted from platelets.

Keywords: Magnetic Nanoparticles, Electroactive Molecules, Antibody, Platelet

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