5th Colloquium

DFG-Priority Program

„Kolloidale magnetische Flüssigkeiten: Grundlagen Entwicklung und Anwendungen neuartiger Ferrofluide“

September, 27th – September, 29th 2004

Program Overview

Opening	**27.09.04**	*14:00 – 14:30*
Medical & Technical Applications		**14:30 – 16:10**
Postersession I		*16:10 – 17:20*
Preparation of Ferrofluids		**17:20 – 18:35**
Colloquium Dinner		*19:30 – .....*
Excursion	**28.09.04**	*09:00 – 15:30*
Theory & Rheology		**16:30 – 18:10**
Postersession II		*18:15 – 20:30*
Basics	**29.09.04**	**09:00 – 10:40**
Postersession III		**10:40 – 12:30**
Closing		*12:30 – 13:00*

Content

Human plasma increases the magnetic separation of tumor cells from peripheral blood leukocytes

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Magnetic nanoparticles interact with living cells in a cell-type specific manner. We could show, that human plasma as an important fraction of human blood expands the differential labeling of tumor cells and leukocytes. Therefore, we evaluated the enrichment of minimal residual tumor cells from the peripheral blood of tumor patients by magnetic nanoparticles in the presence of optimized concentrations of human plasma.

Methods

Leukocytes were prepared by erythrocyte lysis from whole blood samples from patients. Cells were inoculated with magnetic core/carboxymethyl-dextran nanoparticles with an average magnetite/maghemite core TEM-size varying between 3 and 15 nm. The incubation medium (PBS/EDTA) contained human plasma as indicated. Magnetically labeled cells were separated by MACS using a SuperMACS and MS columns. The separated cells were analyzed by FACS and Laser Scanning Cytometry (LSC). Tumor cells were detected with anti-human epithelium-antigen (HEA)-FITC.

Results and Conclusion

Previously, we could show that the interaction of tumor cells with magnetic nanoparticles was affected by plasma levels above 5%, whereas peripheral blood leukocytes showed a dramatic reduction in binding of nanoparticles already in the presence of 0.5% plasma. The addition of plasma at different time points during plasma-free incubation of leukocytes with the magnetic nanoparticles was able to stop the interaction. Based on these results, we incubated leukocyte fractions from blood samples from patients suffering from various cancers (colon, breast, lung) with magnetic nanoparticles over a period of 20 min. We could show that magnetic labeling of leukocytes reached a maximum of more than 60% independent of the specific disease. Analysis of HEA-positive cells, indicating that these cells are of epithelial origin and thus putative tumor cells by LSC revealed, that all HEA-positive cells were in the positive fraction. The total amount of cells was similar to the cell number estimated by direct LSC. In order to enrich the HEA-positive cell fraction and reduce the amount of leukocytes within the positive fraction, we added none, 1% or 5% plasma for 8 min and separated with our regime. The retained cells (positive fraction) and the flow-through cells (negative-fraction) were quantified. In accordance with the results from healthy volunteers leukocytes from the patients samples showed a considerable reduction in binding of nanoparticles already after addition of 1% plasma. In the presence of 5% plasma less than 20% of the applied cells were separable by MACS. The amount of the tumor cells within the positive and negative fraction was estimated by FACS. In contrast to normal leukocytes the tumor cell fraction increased 2.5-fold in the presence of 5% plasma in comparison to the sample without supplementation of plasma, and thus was enriched. In comparison to the unseparated control more than 70% of the HEA-positive cells were eliminated from the sample with one separation step.

In conclusion, we could show that our approach is efficient in separating putative tumor cells from the peripheral blood of tumor patients.

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