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The Carboxymethyl Dextran Shell is an Important Modulator of Magnetic Nanoparticle Uptake in Human Cells

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In oncology and hematology the separation of tumor cells from healthy cells in peripheral blood is a vital problem. We could show previously, that enrichment of tumor cells from peripheral blood is possible by using magnetic nanoparticles with a carboxymethyl dextran (CMD) shell. Long-term storage of CMD nanoparticles eliminated the differential labeling of tumor cells and leukocytes which might be due to an alteration of the carboxymethyl dextran shell. Incubation of stored CMD nanoparticles with freshly prepared carboxymethyl dextran restored the differential labeling. In contrast, enzymatic degradation of the carboxymethyl dextran shell with dextranase abolished the cell-type specific labeling. Thus, an intact carboxymethyl dextran shell is crucial for the cell-type specific interaction of the CMD nanoparticles and living cells.

1. Introduction

Cancer is one of the most severe diseases of mankind. A serious aspect of cancer is its ability to spread and form metastases. Once the primary tumor is established, tumor cells may dissociate from the tumor and disseminate to other parts of the body via the circulation. Multiple research efforts are focused to the treatment of the primary tumor or the metastases using magnetic nanoparticle technologies (*e.g.* hyperthermia or drug targeting).

We focus on the tumor cells circulating in the peripheral blood. The frequency of circulating tumor cells among normal blood cells is assumed to

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be in the range of 10^{-3}–10^{-7}. In oncology and hematology the detection and quantification of these disseminated tumor cells become an important diagnostic tool in order to monitor the primary tumor and to guide therapeutic decisions. Circulating tumor cells are routinely assayed by using highly sensitive methods, *e.g.* polymerase chain reaction, immunohistochemistry, laser scanning cytometry [1–3]. An efficient, inexpensive and universal method for the enrichment of circulating tumor cells from the peripheral blood of patients is therefore highly recommended. A successful approach to separate cells from suspensions is to use magnetic nanoparticles covered with various coatings. The most popular are dextran and its derivatives [4]. It has been shown from several groups, that carboxymethyl dextran (CMD) coated magnetic nanoparticles can interact with living cells [5–7] and that the interaction is cell-type specific [8]. In order to understand the differential interaction of tumor cells and leukocytes with CMD coated magnetic nanoparticles, we analyzed the role of the carboxymethyl dextran shell in more detail.

2. Materials and methods

2.1 Magnetic nanoparticles

The nanoparticles were produced by one of us (N.B.) and consisted of a superpara-(ferro)-magnetic magnetite/maghemite core. The TEM-size diameter of the core varied between 3 and 15 nm (average diameter 5 nm). The nanoparticles were initially coated with carboxymethyl dextran (MW 15 000–20 000) to yield a hydrodynamic diameter of the nanoparticle clusters of 200–300 nm. The saturation magnetization ranged from 4.5 to 6.2 mT. The Fe (II) content was in the range of 5–10 mg/ml and the Fe (III) content from 45–55 mg/ml.

2.2 Repetitive incubation of magnetic nanoparticles with carboxymethyl dextran

The re-incubation of the magnetic nanoparticles with CMD was performed as follows: CMD (MW 15 000–20 000; Sigma-Aldrich, Deisenhofen, Germany) was dissolved in water and added to the magnetic nanoparticle solution. After incubation for 2 h at 37 °C the magnetic nanoparticles were collected by magnetic force and washed 2 times with tap water. Finally, the nanoparticles were treated with ultrasonic to minimize aggregate formation.

2.3 Dextranase treatment of CMD nanoparticles

25 µl of the nanoparticle solution were treated overnight with 5 U dextranase (1.6-α-D-glucan 6-glucanohydrolase, Sigma-Aldrich, Deisenhofen, Germany) at room temperature.

2.4 Cell line and peripheral blood leukocytes

The breast cancer cell line MCF-7 was obtained from American type culture collection (ATCC, Rockville, USA). The cell line was cultivated under standard conditions with DMEM with 10% fetal calf serum in humidified air. For incubation experiments the adherent MCF-7 cells were detached with Trypsin/EDTA. Peripheral blood leukocytes were prepared by erythrocyte lysis (Qiagen, Hilden, Germany) from whole blood samples of healthy volunteers. The leukocyte pellet was washed with erythrocyte lysis buffer twice and then resuspended in PE buffer (phosphate-buffer saline (PBS), 2 mmol EDTA).

2.5 Incubation of cells with magnetic nanoparticles

MCF-7 cells (1×10^6 cells per 500 µl) or peripheral blood leukocytes (2.5×10^6 cells per 500 µl) were inoculated in short term incubation (0 to 20 min) with 2.5 µl CMD coated magnetic nanoparticles in PE at 37 °C. After treatment magnetically labeled cells were separated using a SuperMACS device and MS columns (Miltenyi-Biotech, Bergisch-Gladbach, Germany). The separated cells were designed as positive fraction (retained in column) and the efflux as negative fraction. Cells from both fractions were quantified automatically (Particle Count & Size Analyser Z2, Beckman-Coulter, Krefeld, Germany).

3. Results and discussion

Carboxymethyl dextran coated magnetic nanoparticles are widely used for labeling and detecting cells. During application the nanoparticles attach to the cell surface and are incorporated into the cell. Intracellular particles are surrounded by membranous structures, which points to endocytotic mechanisms of uptake [9–13]. The attachment and subsequent incorporation of the nanoparticles allows the separation of the cells in a magnetic field. The interaction of the CMD nanoparticles with various cell line cells and primary cells showed, that it is cell-type specific. Most of the tumor cells from various origins showed a more pronounced interaction over an incubation time up to 20 min than primary cells like leukocytes from peripheral blood [8].

During our experiments we observed that the CMD magnetic nanoparticles lost their ability to differentiate between tumor cells and leukocytes (Fig. 1a). After a storage period of 3 to 5 months the kinetic behavior of the stored CMD nanoparticles was similar to magnetic nanoparticles without any shell. These nanoparticles interact within a few minutes with nearly all tumor cells. The same holds true for the leukocytes prepared from peripheral blood showing that the cell-type specificity was lost, too (Fig. 1b). We designated this phenomenon as "aging" and suppose that it is caused by the disruption of the carboxymethyl dextran shell.

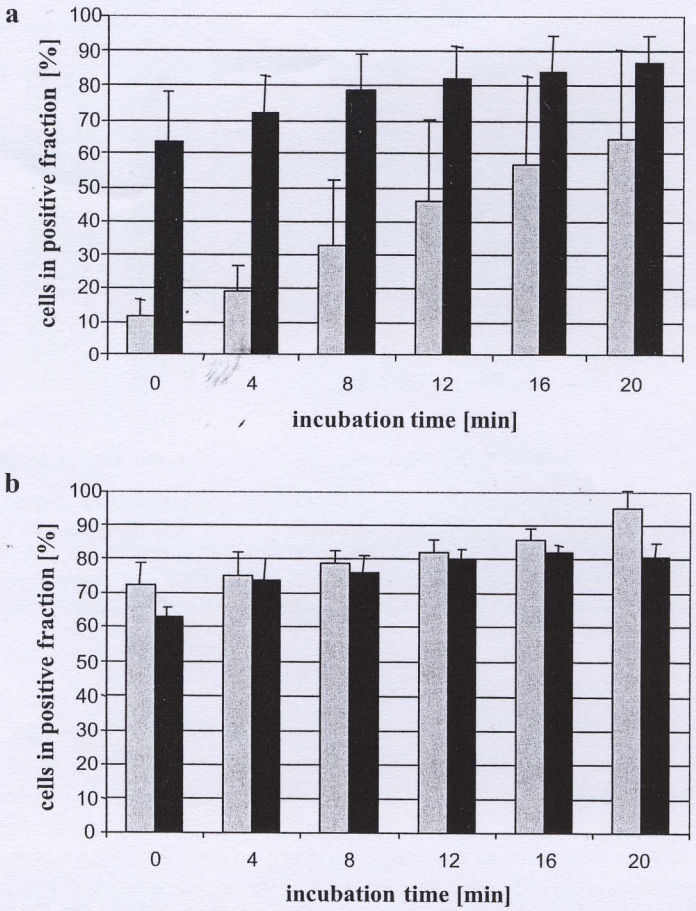

Fig. 1. CMD coated magnetic nanoparticles interact differentially with MCF-7 cells and leukocytes in a time-dependant manner. **a** MCF-7 cells show an immediate interaction with CMD nanoparticles with a further increase of cells in the positive fraction up to nearly 90% after 20 min. Leukocytes from peripheral blood exhibit a delayed interaction with a maximum of cells in the positive fraction of 65% after 20 min. **b** After 3 to 5 month of storage CMD nanoparticles loose their ability to differentially label MCF-7 cells and leukocytes. Bars represent 4 experiments ± SD. MCF-7 ■; leukocytes □.

Dextran is a natural product. Because of the size of the molecule and the free hydroxyl groups complex high-molecular and three-dimensional structures are formed. Thus, it is highly improbable, that each single nanoparticle is surrounded by its own carboxymethyl dextran shell. It is more likely, that single nanoparticles or nanoparticle aggregates are embedded in the complex carboxymethyl dextran network. During storage, the network might be degraded over time and more and more nanoparticles are liberated. In order to proof the

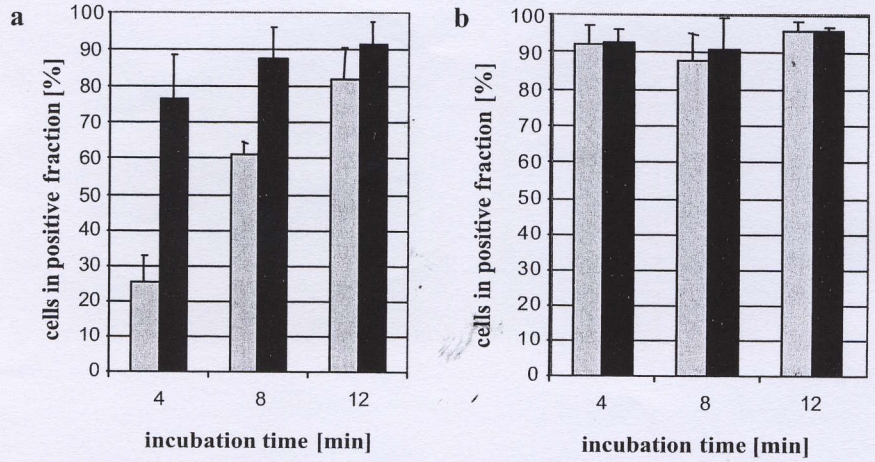

Fig. 2. The differential interaction of CMD magnetic nanoparticles with tumor cells and leukocytes can be restored. **a** "Aged" nanoparticles were reincubated with freshly prepared CMD. They show the same time-course of separable cells as newly obtained CMD nanoparticles (Fig. 1a). **b** After long-term storage untreated CMD nanoparticles showed a similar maximum labeling of leukocytes and MCF-7 cells within a short time. Bars represent 4 experiments ± SD. MCF-7 ■; leukocytes □.

hypothesis, that the carboxymethyl dextran shell is responsible for the cell-type and time-dependant interaction of the nanoparticles and the cells we incubated the "aged" nanoparticles with a freshly prepared carboxymethyl dextran solution and used these nanoparticles for labelling experiments. The reincubated nanoparticles led to a similar labelling kinetic as newly obtained CMD nanoparticles. The tumor cells showed a more rapid uptake of the nanoparticles than the leukocytes with the most pronounced difference after 4 min of incubation (Fig. 2a). In contrast, the presence of nanoparticles without a CMD shell caused a rapid paralleled labelling of tumor cells as well as leukocytes (Fig. 2b). If the carboxymethyl dextran is responsible for the differences between tumor cells and leukocytes, a degradation of the CMD shell of newly obtained nanoparticles should cause the same results. Therefore, the CMD coated magnetic nanoparticles were treated with dextranase in order to enzymatically degrade the carboxymethyl dextran. After the digest the treated nanoparticles showed the same behaviour as the "aged" nanoparticles and the nanoparticles without CMD shell. The tumor cells and the leukocytes were labelled rapidly and to a high extent (Fig. 3).

In conclusion, we could show that the carboxymethyl dextran shell plays an important role for the interaction of magnetic nanoparticles with cells. The CMD shell is sufficient to enrich tumor cells from leukocytes without the use of antibodies coupled to the nanoparticle shell and destruction of this shell leads to loss of this property. The [illegible]

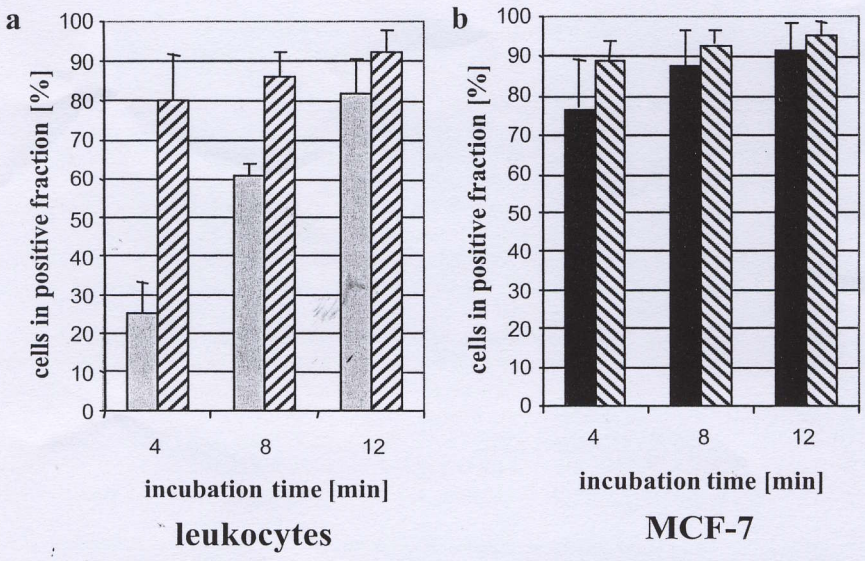

Fig. 3. Breakdown of the carboxymethyl shell abolishes the differential interaction kinetics of magnetic nanoparticles with cells. CMD nanoparticles were treated with dextranase overnight and used for incubation experiments. After that treatment leukocytes do not show the delayed interaction kinetic as with untreated CMD nanoparticles. MCF-7 cells were more rapidly labeled with dextranase-treated nanoparticles. Bars represent 3 experiments ± SD. **a**. Leukocytes: ▨ dextranase treatment; □ control; **b**. MCF-7: ▧ dextranase treatment; ■ control.

type specific labelling could be overcome by reincubation with carboxymethyl dextran. Long-term studies are necessary to evaluate the effect of repeated incubations of CMD nanoparticle charges with freshly prepared carboxymethyl dextran.

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