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Research Article

Multifunctional SPIONs for Theranostics in Cancer

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Abstract

Due to their magnetic properties, superparamagnetic iron oxide nanoparticles (SPIONs) offer a large variety of possibilities for medical applications. In Magnetic Particle Imaging (MPI) to date SPIONs are the only tracers or imaging agents so far. It is proposed that the optimal tracer for MPI is a single core SPION with a core diameter of around 30 nm. However, such particles seem not to be optimal for magnetically enhanced drug delivery. On the other hand, there are multicore particles equipped with a high loading capacity for drugs, which can be accumulated e.g. in a tumor by a static magnetic field and additionally be heated by an alternating magnetic field. Here we show that such particles cannot be only imaged by magnetic resonance imaging (MRI) but also do show a MPI-signal. Providing, that the sensitivity of MPI for such particles and therefore also the resolution is high enough, this could be exploited to estimate the SPION-mediated drug load in a tumor after Magnetic Drug Targeting (MDT) as a real theranostic approach.

I. Introduction

The unique potential of superparamagmetic iron oxide nanoparticles (SPIONs) is their usefulness in a variety of biomedical applications. Beyond *in vitro* diagnostics, SPIONs could improve the treatment of e.g. arteriosclerosis and cancer [1, 2]. Due to their large surface SPIONs can bind and deliver drugs in high amounts. Targeting of the diseased area can be achieved either by secondary surface modifications capable of recognizing molecular target structures, e.g. on cancer cells, or by magnetic fields, because of their magnetic properties. However, SPIONs also can be visualized with magnetic resonance imaging (MRI) and are to date the only tracers for magnetic particle imaging (MPI). Taking all these properties together, SPIONs can be considered as a unique platform for theranostic applications. In this context, the great advantage of MPI is its capability of imaging and quantifying SPIONs. Therefore, with MPI it could be possible to estimate the drug load in the diseased area after the application by measuring the content of the SPIONs used for delivering the drug. The aim of the Section of Experimental Oncology and Nanomedicine (SEON) is to utilize SPIONs for the treatment of cancer and arte-

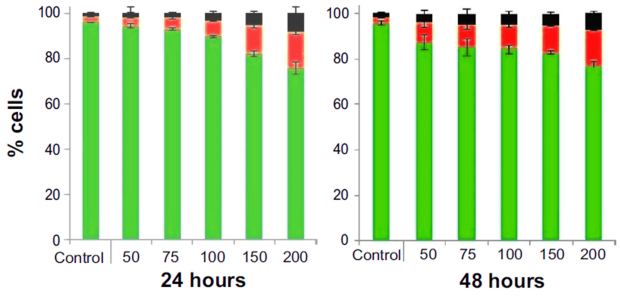

Figure 1: Effect of different concentrations of SEON$^{LA\text{-}BSA}$ nanoparticles on cell viability of Jurkat cells: Flow cytometry measurements show that after 24 h and 48 h no relevant induction of apoptosis (red columns) and necrosis (black) columns can be detected up to a particle concentration of 100 μg/ml.

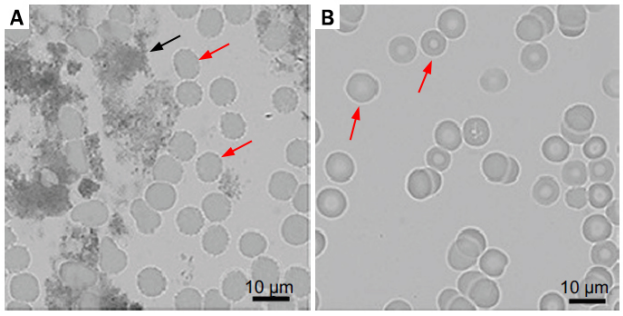

Figure 2: Blood stability of SPIONs for medical application (modified from [3]). SEON^{LA}- and SEON$^{LA\text{-}BSA}$-nanoparticles where incubated in human blood stabilized with EDTA for 60 minutes. A) SEON^{LA}-nanoparticles form agglomerates (black arrow) of several μm upon incubation in blood. These agglomerates are visible under the light microscope next to the erythrocytes (red arrows). B) Under the same conditions, the improved system did not form agglomerates, which are visible in a normal light microscope.

riosclerosis by MDT. Therefore, we developed SPIONs over a period of several years optimized for the purpose of magnetic drug delivery. These particles are very stable in human and animal blood, can carry a sufficient drug load [3], and be accumulated in a target area by magnetic fields and first results show that these particles even can be heated with alternating magnetic fields. These SPIONs were also suitable for MRI-imaging but from the theoretical point of view not optimal for MPI, because they are clusters of a size between 60 nm and 70 nm with a single core diameter of approximately 7.6 nm. The aim of this publication is to show, that multifunctional nanoclusters, which are suitable for magnetic drug targeting, hyperthermia, MR-imaging can also show a signal in Magnetic Particle Imaging.

II. Multifunctional Nanoclusters

II.I. Biocompatibility

Any nanoparticle system, which is dedicated for translation into clinical use, has to prove that it does not harm to living cells, at least in doses, which are relevant for the later use in humans. Unfortunately, classical toxicity assays based on colorimetry, fluorescence or luminescence are prone to interference of the black iron oxide nanoparticles with the measuring method [4, 5]. Therefore, we combined different approaches to assess possible toxic effects *in vitro*. For example, flow cytometry revealed for the particles system SEON$^{LA\text{-}BSA}$ that in doses up to 100 μg/ml did not show relevant induction of apoptosis or necrosis in Jurkat cells after 24 hours or 48 hours, when compared to the untreated control (Figure 1) [3].

Another advantage of flow cytometry is the possibility of performing multiparameter assays in one experiment. This allows an additional screen for effects on e.g. the mitochondrial potential and DNA-degradation [3] and it is even possible to quantify the nanoparticle content in the cells via their granularity [6].

On the other hand, nanoparticles, which are intended for intraveneous or intraarterial administration into a living animal or patient, have to be tested for different aspects of blood compatibility. Therefore, in a next step, we tested the SEON$^{LA\text{-}BSA}$ particles for colloidal stability in blood. Transmission microscopy showed that compared to the precursor particle system (SEON^{LA}), which only has a lauric acid coating for stabilization, the addition of an albumin coating SEON$^{LA\text{-}BSA}$ resulted in a highly improved colloidal stability in whole blood (Figure 2) [3]. Also, hemocompatibility was improved by the protein corona [7].

II.II. *In-vitro* efficiency

The nanoparticle system SEON$^{LA\text{-}BSA}$ was developed as a platform for delivery of a variety of drugs for different applications. For cancer treatment the loading with the chemotherapeutic agent mitoxantrone (MTO) was chosen, since it is an efficient drug and can be analyzed by HPLC to reliably quantify the loading efficiency but also drug content in cells and tissues. In experiments analogous to the unloaded nanoparticles the effect of the uncoupled drug and SEON$^{LA\text{-}BSA*MTO}$ was investigated employing flow cytometry and compared to an untreated control after 24 h and 48 h [3]. These tests showed that the drug loaded nanoparticle system was as effective in killing suspension cells and adherent cells growing in monolayers. To further gain information about the ability of the nanoparticles and/or the drug to infiltrate tumor tissue, we investigated the effect of the pure drug and SEON$^{LA\text{-}BSA*MTO}$ on three dimensional multicellular tumor spheroids [8]. First, we investigated the normal growth behavior, proliferation rate and rate of apopto-

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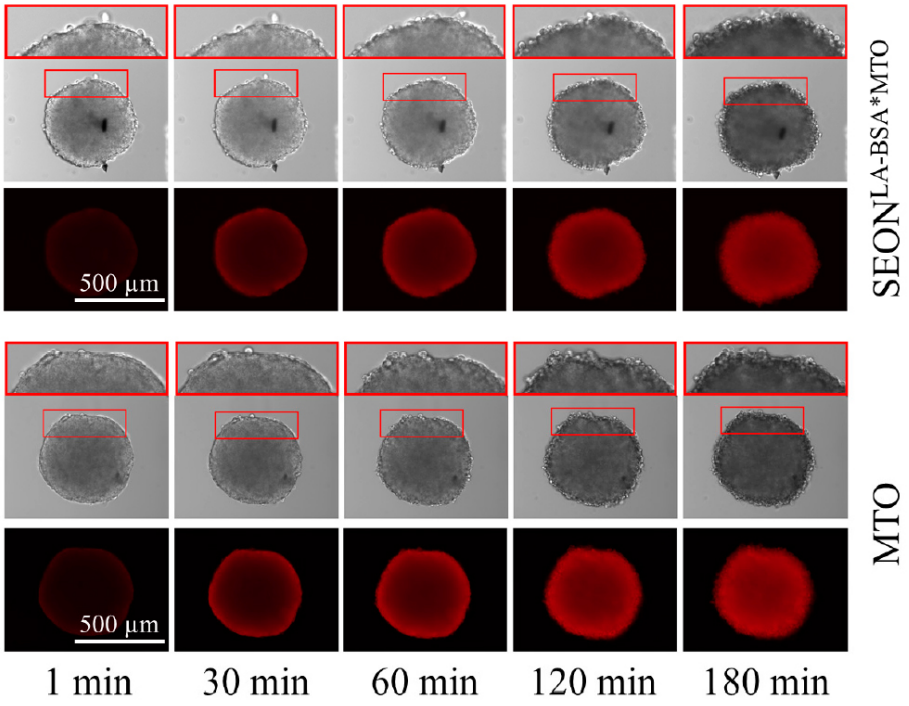

Figure 3: Infiltration of MTO into HT-29 tumor spheroids [8]. The images show the red fluorescence signal of MTO infiltrating into the tumor spheroid over a period of 180 minutes.

sis and necrosis during an unaffected growth of HT-29 tumor spheroids over a period of up to 12 days. It became obvious, that expectedly after a short time the spheroids developed a necrotic core, which is due to limited nutrient supply and gas exchange in the growing structure. This is in concordance with the findings in normal tumors. Next, the HT-29 tumor spheroids were treated with MTO and SEON$^{\text{LA-BSA*MTO}}$ in equivalent MTO doses 72 h after the initial spheroid formation. Fluorescence microcopy showed that both the free and the nanoparticle loaded drug infiltrated efficiently into the tumor spheroids (Figure 3) [8], whereas the penetration of SEON$^{\text{LA-BSA}}$ was only slightly delayed.

In concordance with this finding, the HT-29 spheroids showed a highly reduced proliferation rate and higher rates of apoptosis and necrosis, when they were treated with pure MTO or its nanoparticle loaded counterpart [8].

II.III. Heating properties of SEON$^{\text{LA-BSA}}$

One promising use of magnetic iron oxide nanoparticles is the treatment of tumors with hyperthermia [9]. Since we were interested in a combination of enhancing the outcome of a chemotherapeutic treatment with the support of magnetically induced hyperthermia, we investigated the possibility of heating the SEON$^{\text{LA-BSA}}$ via an alternating magnetic field. It is obvious, that the resulting heat is depended on the content of magnetic nanoparticles in a given volume. Thus, with magnetic field strength of 15.4 kA/m and a frequency of 435 kHz the increase of the nanoparticle concentration from ca. 5mg/ml to ca. 16 mg/ml resulted in an increase of T_{max} from 45 °C to nearly 65 °C (Figure 4) [10]. Increasing the nanoparticle content of the suspension did not affect the SAR value of 180 W/g, which is not surprising, since the SAR is normalized to the iron content. These results show that although the SAR value of the SEON$^{\text{LA-BSA}}$ nanoparticle platform is not tremendously high, effective heating is possible when the particle content in the suspension is adjusted.

II.IV. MRI-Imaging of SEON$^{\text{LA-BSA}}$ *in vivo*

In Magnetic Drug Targeting (MDT) iron oxide nanoparticles are used as drug carriers, e.g. for the chemotherapeutic agent mitoxantrone. The *in vivo* model, we are using for investigating MDT in living animals, is a VX2-tumor, which is implanted subcutaneously at the hind limb of New Zealand White rabbits (NZW). It is known that in MRI imaging iron oxide nanoparticles are able to cause signal extinctions in the area, where they are accumulated. Our hypothesis is, that by using MRI after the accumulation one can use this information in a theranostic approach to monitor the accumulation efficiency and particle distribution in the tumor region. This information, respectively, might enable an estimation of the drug distribution in the tumor region and

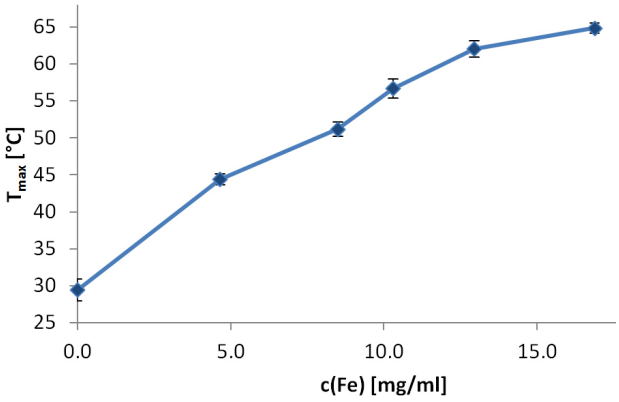

Figure 4: Heating profile of SEON$^{LA\text{-}BSA}$ in dependence of the iron content [10]. The maximum heating temperature T_{max} is dependent on the concentration of the nanoparticle suspension.

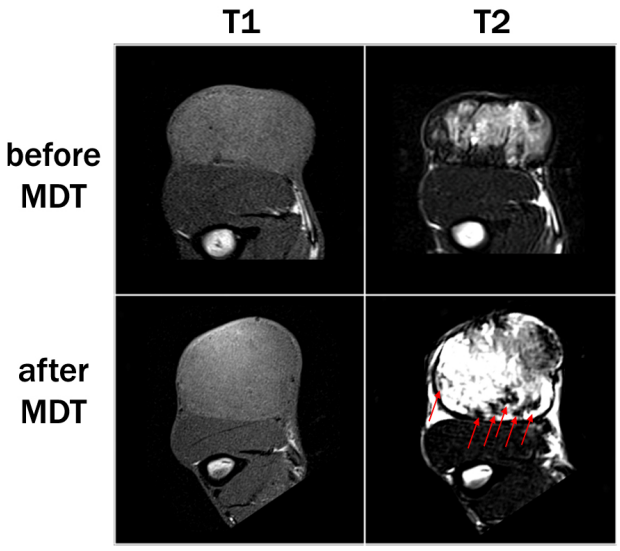

Figure 5: MRI-imaging of a VX2-tumor before and after MDT. The T1 sequence does not show any signal extinction after the administration of SEON$^{LA\text{-}BSA}$, while in T2 signal extinction is caused by nanoparticles as seen at the basis of the tumor (red arrows).

hence an estimation of the expectable outcome of the treatment. To get a first impression, MRI-imaging of one animal was done in a three tesla Siemens Trio Tim^{TM} MRI-device accompany ing to the MDT trials (Animal trial permission 54-2532.1-54/12, government of Mittelfranken, Ansbach, Germany), which we are running currently. As expected, the nanoparticles did not show signal extinction in T1-weighted sequences, while in the used T2-weighted sequence, distinct signal extinction can be detected at the basis of the VX2-tumor and at several other areas (Figure 5). This shows that in principle a theranostic approach is possible with MRI, even if with this technique a quantitative assessment of the iron content in the tumor region is still under investigation.

II.V. MPI-Imaging of SEON$^{LA\text{-}BSA}$

Magnetic particle imaging (MPI) is using iron oxide nanoparticles as tracers. These tracers cannot only be employed for generating an image, but the technique of MPI is promising to extract quantitative information about the iron content in a certain volume. This feature could be tremendously helpful for MDT and the theranostic approach of an online monitoring of the nanoparticle content in the tumor region. Therefore, we wanted to know, if it is in principal possible to image the SEON$^{LA\text{-}BSA}$-nanoparticles with a currently available preclinical MPI-device (Bruker/Philips). A dilution series of these particles was measured with Magnetic Particle Spectroscopy (MPS) at Universitätsklinikum Hamburg-Eppendorf. The MPS-spectrum showed that in comparison to the MPS-signal of Resovist^{TM} the signal of the SEON$^{LA\text{-}BSA}$-nanoparticles was weaker at higher frequencies but comparable at frequencies below 100 kHz (Figure 6a). Subsequently, a sample of 20 μl (2 mm × 2 mm × 1 mm) was measured with MPI. (Figure 6b-d) show, that it was possible to get an image of this point sample by MPI. While the spatial resolution of the reconstructed sample is only about 2 mm in z-direction and about 4 mm in x- and y-direction, the images illustrate the excellent contrast to noise ratio of MPI images.

III. Discussion and Conclusion

During the last years SEON developed a multifunctional nanoparticle platform SEON$^{LA\text{-}BSA}$, which is capable of carrying a variety of drugs. We were able to show that this nanosystem is very biocompatible *in vitro* and has excellent blood compatibility. *In vitro*, SEON$^{LA\text{-}BSA}$ are also very effective in transporting the chemotherapeutic mitoxantrone to cancer cells in 2d cell culture, suspension and 3d cell culture revealing a similar effectivity as the unbound drug. Additionally, this particle system can be heated via alternating magnetic fields in a concentration depended manner up to ca. 65 °C and be used for imaging in MRI. Taken this together, the nanoparticle system SEON$^{LA\text{-}BSA}$ could be a promising candidate for an effective theranostic approach of cancer and arteriosclerotic diseases. This preliminary study shows that in principal it is possible to image SEON$^{LA\text{-}BSA}$ using MPI. Further experiments will demonstrate how effective and quantitative this imaging is and if modifications on the particle platform or the technical equipment will be able to improve the imaging properties of this system. If this can be realized without impairing therapeutic efficiency, the combination of MDT and MPI could open new doors in the field of theranostics.

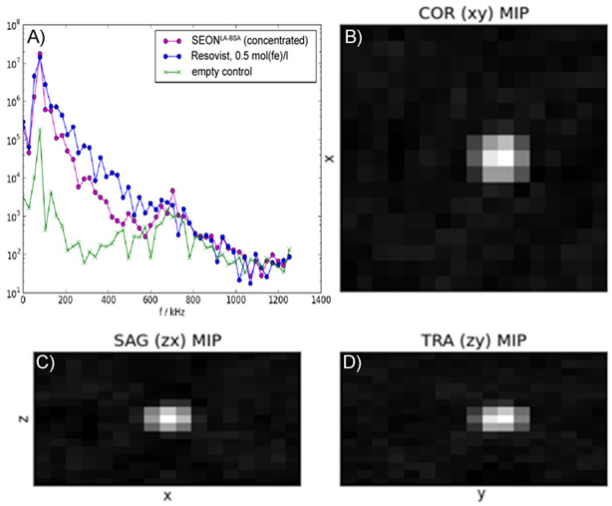

Figure 6: a) Comparison of the MPS signal of SEON$^{\text{LA-BSA}}$-nanoparticles and Resovist$^{\text{TM}}$ at different frequencies. b)–d) MPI-signal of a point sample of SEON$^{\text{LA-BSA}}$-nanoparticles.

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