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2008 J. Phys.: Condens. Matter 20 204152

(http://iopscience.iop.org/0953-8984/20/20/204152)

J. Phys.: Condens. Matter **20** (2008) 204152 (4pp) doi:10.1088/0953-8984/20/20/204152

Microcomputed tomography analysis of ferrofluids used for cancer treatment

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Received 22 November 2007, in final form 22 February 2008
Published 1 May 2008
Online at stacks.iop.org/JPhysCM/20/204152

Abstract
In order to reduce the side effects generated by the most common cancer treatment therapies, chemo- and radiotherapy, two new approaches are being investigated. These new approaches are magnetic drug targeting (MDT) and magnetic hyperthermia, and are based on the use of magnetic nanoparticles. In the first one, these magnetic nanoparticles are used as drug carriers and the success of the treatment depends on the correct distribution of the drug within the tumour tissue. Computed tomography analysis has been performed on tumour tissue after MDT in order to find out the distribution of the nanoparticles. The measurements have been carried out in two different laboratories, one based on a synchrotron beamline and another one with a cone x-ray source. First results show that the drug carriers form clusters within the tumour tissue.

1. Introduction

Cancer, the uncontrolled growth of cells due to damage of the DNA, is one of the most dangerous and frequently appearing diseases. The institution for health protection, the Robert Koch Institute (RKI), reported in their last study the frequency of occurrence of breast carcinoma in Germany in recent years. In 2002 the number of documented breast cancer cases amounted to 17.8% of the female population, although the estimated number of unreported cases is much higher (26.8%) [1]. The most frequent ways for cancer treatment are chemo- and radiotherapy, two methods that, although effective, have also negative side effects and risks that make them not very attractive.

Chemotherapy is the use of cell-toxic drugs to treat cancer. Chemotherapeutic agents inhibit the growth or multiplication of cells, thereby killing them. However, these drugs also harm healthy tissue especially other fast-dividing cells (e.g. bone marrow, hair) and cause negative side effects, such as hair loss and depression of the immune system.

Radiotherapy is a directed irradiation of the tumour with x-rays and it is often used in combination with chemotherapy. The precise localization of the tumour tissue is very important, as well as the immobilization of the patient during the therapy. Special care needs to be taken to deliver the desired intensity to the tumour for a successful therapy [2, 3]. Nevertheless, the radiation always affects some healthy tissue and therefore damage of healthy cells can occur.

Using magnetic nanoparticles, two promising methods are now being investigated to try to reduce these side effects. In one method, magnetic hyperthermia (MH), these particles are used to induce local heat transfer, leading to a regression of the tumour. In the second method, magnetic drug targeting (MDT), these particles are used as drug carriers with the aim of increasing the local concentration of the chemotherapeutic agent within the tumour. In the present study, samples from tumours subjected to MDT have been analysed.

Suspensions of magnetic iron oxide nanoparticles coated with starch polymers have been proven as a capable carrier system for drug delivery. The advantage of the intra-arterial injection of these magnetic nanoparticles has also been shown [4–9]. Once injected, by applying a strong inhomogeneous magnetic field of up to 1.7 T, the nanoparticles can be directed towards the desired area. For a successful treatment, it is of great importance that the nanoparticles end up distributed within the tumour tissue.

A number of different analytical techniques have been performed to study the distribution of these nanoparticles within the tumour. Histological cross sections have been prepared and analysed by means of light and electron microscopy [7]. In the present study, a non-destructive method of computed image processing by means of microcomputed

Table 1. Preparation of ferrofluid as drug carrier for MDT.

	Animal I	Animal II
Volume of ferrofluid injected (ml)	1	1
Concentration of nanoparticles (mg ml^{-1})	25	24.46
Concentration of mitoxantrone (mg ml^{-1})	1	0.8
Cancer treatment method	MDT	MDT

tomography (μCT) has been used to obtain three-dimensional (3D) information [10].

2. Materials and methods

2.1. Tumour model

For our investigations, VX-2 squamous cell carcinomas were placed on the hind limb of two female New Zealand white rabbits. Magnetic nanoparticles were coated with a starch polymer to make the iron oxide core tolerable by the body and coupled to the chemotherapeutic agent mitoxantrone [7, 9]. Details of the nanoparticles' administration are shown in table 1. These nanoparticles, acting then as drug carriers, were injected into the artery supplying the tumour, and directed towards it and fixated by means of a strong magnetic field gradient. The rabbits were sacrificed 1.5 h after the drug intake and the tumours were resected from the animals. Subsequently the tumours were fixated in formalin and embedded in paraffin [7].

2.2. Microcomputed x-ray tomography

The tumours have been measured in two different μCT laboratories, one with an x-ray tube source and another based on a synchrotron beam line. These laboratories and the technique itself are briefly described in the following sections. Main technical data is cited in table 2. For the analysis of the reconstructed data, the software tool MyVGI has been used [11].

When energetic x-rays travel through matter, the intensity of the beam decreases according to the density, thickness and attenuation coefficients of the different materials. This principle is used in radiography for examining large objects, where the region of interest is well below the surface. X-ray tomography follows the same principle, with the additional advantage of being a 3D investigation method, as either the sample, or the combination of the detector and the source, is rotated at least 180°, depending on the geometry of the x-ray beam. The result is a 3D representation of the different density zones in the object. The method is non-destructive and when a mono-energetic source is used it can provide quantitative data.

In our CT systems, the sample is placed between the x-ray source and the detector. When the sample is irradiated, a projection image of the attenuated beam is recorded. Then the sample is rotated by a small angle, which depends on the achievable resolution of the system, and another projection image is taken. The process is repeated until the sample has been rotated by at least the minimum angle necessary to get information from the whole sample. This minimum angle is 180° when a parallel beam is used, while when a cone beam is used, the beam geometry has to be taken into consideration. From all the individual images, the reconstruction procedure builds up a 3D representation, which can be analysed to study the different density zones of the sample.

Table 2. Main technical data of the different microcomputed tomography laboratories.

	TU Dresden	DESY
Source	X-ray tube	Synchrotron
Beam geometry	Cone	Parallel
Maximum intensity	0.99 mA	140 mA
Energy range	0–50 keV	20–250 keV
Spatial resolution	20 μm	2 μm
Exposure time	2 s	1 ms
Rotation angle	220°	180°
Angle step	0.1°	0.09°

2.2.1. X-ray tube laboratory. This laboratory is located at the Chair of Magnetofluiddynamics of TU Dresden. It consists of an x-ray source (Apogee 5000) that emits a cone beam with a maximum intensity of 0.99 mA and a maximum acceleration voltage of 50 kV, and a combination of a scintillator screen (100 cm^2, Proxitronic) and a CCD camera (Apogee) acting as detector. This system has been described in detail elsewhere [10]. In this CT laboratory we can achieve a spatial resolution of 20 μm by using the highest magnification of 5. The cone angle is 20°, and thus 220° of projection images are necessary for a tomographic reconstruction.

In the energy ranges used for CT, the linear attenuation coefficient for all kinds of materials decreases with energy. For a polychromatic x-ray beam, this causes the artefact known as 'beam hardening', where low energy photons are preferentially absorbed and the remaining beam becomes proportionately richer in high energy photons. To reduce these beam hardening effects, we have used aluminium filters.

2.2.2. Synchrotron laboratory. Our second system is located at the HARWI 2 beam line in Hasylab at DESY (Deutsches Synchrotron, Hamburg). This system is called DiTo and it is a combination of diffractometry and tomography systems. The main feature of the tomography component of DiTo is the detector unit, which consists of two different cameras. With the high resolution camera (PCO 4000) we can achieve tomograms with a spatial resolution of up to 2 μm. The second camera (PCO 2000) can be used for ultra-fast measurements. The combination of the short readout time of this camera and the precise and fast sample manipulator system makes it possible to acquire whole tomograms within just 5 s.

There are a few advantages for using synchrotron radiation in comparison to the conventional x-ray sources. Thanks to the high intensity of the beam, the exposure time is shorter and the measurements are nearly noise free; and thanks to the monochromatic nature, the beam hardening artefacts do not occur and thus quantitative analysis can be performed. Also due to the parallel beam geometry, only 180° are necessary for the reconstruction of the complete tomograms.

The beam cross section is limited here by a slit system, and this limits the size of the sample to be analysed, which

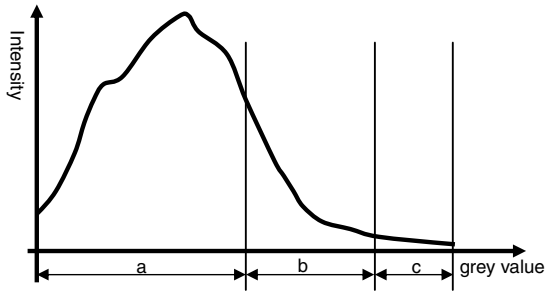

Figure 1. Frequency distribution of grey values of animal I. The histogram has been divided into three different sections, which correspond to three different components of the sample.

is usually around 16 mm wide and 2 mm high. For bigger samples, tomograms are taken at different heights and afterwards stacked together to reconstruct the whole image.

3. Results and discussion

The aim of the CT analysis of these tissue samples is to find out the distribution of the drug carriers within the sample, and especially to see whether they are contained within the tumour region.

As stated previously, the tomography analysis is based on the intensity of an x-ray beam transmitted through an object, and the preferential attenuation of the x-rays when they pass through high density materials. Our samples consist mainly of three different materials: biological tissue, the paraffin where the tissue is embedded and the starch-coated nanoparticles with the attached drug. The least dense of these three is the paraffin, with a density of up to 0.93 g cm^{-3}, followed by the tumour tissue, with a density of 1.04 g cm^{-3}, while the water-based ferrofluid has a density of up to 1.23 g cm^{-3}. A good contrast is therefore expected in the absorption images.

The grey values in the recorded image are directly connected to the absorption of the x-rays within the sample, with the highest grey value corresponding to the maximum absorption, and therefore to the most dense materials within the sample. For a clearer visualization of the image, the histogram can be classified in different zones of grey values and each zone displayed in a different colour. Figure 1 shows a real histogram, from a tomogram of one of the tumours treated with MDT, divided into three different sections. It can be seen that most of the voxels of the tomogram have a low mean grey value (zone a), while the number of voxels with a high grey value (zone c) is very small.

This classification of the histogram can be transferred to the 3D representation of the sample. By disabling one or more zones in the histogram, only the enabled zone or zones will be displayed in the tomogram. This has been applied for the histogram obtained from the sample shown in figure 2. In the slice shown in figure 2(a) all grey values are presented, and the biggest area corresponds to low grey values (zone a in the histogram, yellow area in figure 2(a)). In figure 2(b) the medium grey values are represented (zone b in the histogram), and finally in figure 2(c), only the highest grey values in the histogram are displayed (zone c). By connecting this to the density values of the three main components, and also the information from the preparation of the sample, we can come to the conclusion that figure 2(a) shows mainly the paraffin where the tissue is embedded. Figure 2(b) shows the tissue, and in this display the surrounding paraffin cannot be seen because the lower grey values have been disabled. Finally, figure 2(c) shows the densest materials, which are the magnetic nanoparticles used as drug carriers. By comparing figures 2(b) and (c), we can observe that the ferrofluid is contained within two isolated areas within the sample. It can therefore be concluded that the nanoparticles are locally concentrated within the tumour.

The same tumour sample has been analysed with DiTo. A 1.2 mm high and 16 mm wide slice is presented in figure 3, with the same histogram classification as explained before. The area of highest grey value, which is displayed in blue and red clarification, corresponds to the densest material, the magnetic nanoparticles used as drug carriers. These particles are contained within a well-defined tubular shape, which is part of the vascular system of the tissue. The vessel system can be abstracted from the tissue and paraffin due to the higher attenuation of x-rays caused by higher density of the magnetic nanoparticles. Therefore, with this high resolution analysis, we can identify the vessels—a discrimination that could not be done from the low resolution analysis performed at Dresden (figure 2). We can conclude that the nanoparticles are not only

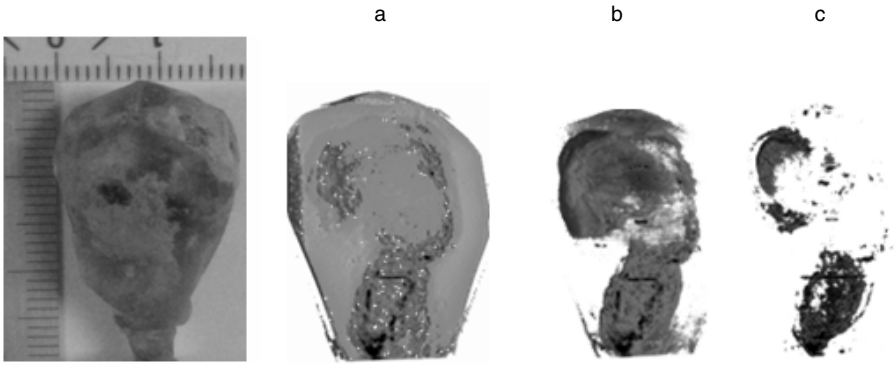

Figure 2. Optical picture of animal I and three representations of a tomogram slice. (a) Light grey area represents paraffin. (b) Dark grey area represents biological tissue. (c) Densest material: nanoparticles within the tumour.

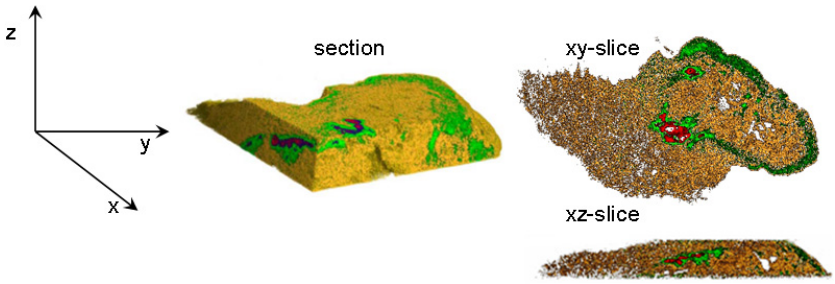

Figure 3. 3D representation of a 1.2 mm high and 16 mm wide slice of animal I and frontal and axial cross sections. The blue and red areas (black and grey in the greyscale version) represent the nanoparticles within the tumour.

(This figure is in colour only in the electronic version)

contained within the vessels but have been transferred by the magnetic force to the tumour tissue.

4. Conclusions and outlook

It has been shown that CT is a useful tool to examine tissue materials treated with the aid of magnetic nanoparticles. Tumour tissue treated with MDT has been analysed in two different laboratories, with two different resolutions, and compared. The results have shown an accumulation of the nanoparticles, used as drug carriers, within the vessels in the tumour and they are not lost outside, which is essential for the success of this kind of cancer treatment.

The new experimental CT setup introduced in this paper, DiTo, will allow the examination of bigger samples, including whole animals, in the near future. This will offer three-dimensional information about particle propagation through different parts of the body. The performance of tomographic measurements of *in vivo* processes is also planned in order to analyse the time-dependent behaviour of particles within the tissue.

Acknowledgment

This project is funded by DFG with OD18/9 within SPP1104.

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