Available online at www.sciencedirect.com
ScienceDirect
Journal of Magnetism and Magnetic Materials 311 (2007) 358–362
www.elsevier.com/locate/jmmm

In vitro investigation of the behaviour of magnetic particles by a circulating artery model

Christian Seliger[a], Roland Jurgons[a], Frank Wiekhorst[b], Dietmar Eberbeck[b], Lutz Trahms[b], H. Iro[a], Christoph Alexiou[a,*]

[a]*Department of Otorhinolaryngology, Head and Neck Surgery, Friedrich-Alexander-University Erlangen-Nürnberg, Waldstr. 1, 91054 Erlangen, Germany*
[b]*Physikalisch-Technische Bundesanstalt, Berlin, Germany*

Available online 4 January 2007

Abstract

Magnetic drug targeting is the use of coated magnetic nanoparticles as carriers for cytostatic drugs. After intraarterial application of these carriers, they are attracted with an external magnetic field to, for example, an experimental VX2 tumour. The biological compatibility of this system depends on several physiological and physical parameters. We established an in vitro model to simulate in vivo conditions in a circulating system consisting of a circuit with an intact bovine femoral artery close to an external magnetic field. Nanoparticle suspensions were applied by a side inlet. After the magnetisation procedure particle size, concentration and distribution was examined.

Keywords: Magnetic drug targeting; Mitoxantrone; Magnetic nanoparticle; Chemotherapy; VX2 tumour

1. Introduction

Arterial delivery of cytostatic loaded magnetic nanoparticles is a new approach of side specific drug deposition in tumours. The advantage of arterial delivery of magnetic nanoparticles compared to intravenous application is that considerably less particles are cleared by cells of the mononuclear phagocyte system (MPS) and the particles therefore do not end up in the macrophages of the liver, spleen and lung [1,2]. Furthermore, in previous animal studies (experimental VX2 tumour on rabbits) we could show the great advantage of intraarterial application compared to the intravenous administration resulting in a much higher accumulation of the particles and the drug in the target region, respectively [2,3].

Our nanoparticles for intraarterial drug delivery consist of colloidal dispersed magnetite with a functionalised coating (phosphated starch) which is loaded with the cytostatic agent mitoxantrone via ionic binding to the phosphate groups [4]. The resulting drug loaded compound consists of multidomain particles of magnetite cores with unaltered size embedded in the coating with an average size of about 100–250 nm. In a DLS measurement you can detect only the hydrodynamic diameter of this multidomain particle, further described as nanoparticle [2,3]. After intraarterial application of this compound in the tumour-region supplying vessels, an external magnetic field, focused to the tumour, attracts the nanoparticles into the tumour supplying vessels. The drug is released from the particle surface after a defined time and infiltrates the tumour tissue and the surrounding area. With only 20% of the regular systemic drug concentration total tumour remission was achieved in an experimental VX2-rabbit-tumour model [4–6].

Despite these results, further examinations regarding intracorporal particle stability, interactions of the nanoparticles with blood components and their influence on vascular tissue are necessary. For the supplementation of animal experiments we introduced a circulating artery model for the examination of nanoparticle behaviour in an artificial vessel system. In these examinations we plan to optimise magnetic field, flow rate and duration of the magnetic field application for a prospective clinical feasibility study.

*Corresponding author. Tel.: +49 9131 8534769; fax: +49 9131 8534828.
E-mail address: C.Alexiou@web.de (C. Alexiou).

0304-8853/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jmmm.2006.10.1205

2. Materials and methods

Artery Preparation: Freshly isolated bovine femoral arteries harvested in the local slaughter house were transported in 4 °C PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 pH 7.4,) supplemented with Heparin (500 i.E.). Side branches of the artery were ligated and the artery was flushed with Krebs–Ringer/BSA buffer (0.114 M NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 1 mM KH_2PO_4, 0.8 mM $MgSO_4$, 24 mM $NaHCO_3$, 1 g/l glucose, 6.25 g albumin, Sigma, Germany) supplemented with nitroprusside (10^{-5} M, Sigma, Germany) to prevent spontaneous contraction. The artery was cannulated at the ends (Medtronic, Germany) and mounted in the "holder apparatus" for the circuit.

Circuit conditions: The artery was mounted into the tempered (37 °C) circuit and rinsed with 60 ml Krebs–Ringer buffer + BSA + nitroprusside for 15 min for equlibration of the system (Figs. 1 and 2) The electromagnet (Siemens, Germany) was positioned over a given part of the artery. The flow rate was 6 ml/min under pulsatile flow with 1 pulse/s. After the equilibrating period 1 ml of magnetic nanoparticles were administered in the circuit and the magnet was switched on reaching a magnetic gradient of 10 T/m. After 15 min the magnetic field was switched off and the perfusion medium was collected. The artery was replaced from the circuit and fixed with 4% formaldehyde in PBS buffer for further histological examinations and magnetorelaxometry.

For the evaluation of the model we used two different particle suspensions. The first particle suspension consists of magnetite nanoparticles covered with a phosphated starch surface (Chemicell, Berlin, Germany). The second suspension consists of magnetite nanoparticles covered with a citric acid shell (MagneticFluids, Berlin, Germany). Both suspensions were loaded with 0.7 mg Mitoxantron (GRY, Germany) per ml suspension at a concentration of 25 mg Ferrofluids/ml suspension.

Size determination: Particle suspensions in a dilution of 1:100 in distilled water were analysed by dynamic light scattering (DLS) before and after administration in the circuit with a commercial Zetasizer (Nicomp ZLS 380, CA, USA).

Magnetorelaxometry: The embedded artery was cut into approximately equal segments, dehydrated in increasing ethanol concentrations and embedded in paraffin after a xylol step. The iron content of the segments and the perfusion medium was determined by magnetorelaxometry. Individual parts of the artery of about 1 cm length embedded in stearine were measured using a single-channel superconducting quantum interference device (SQUID) gradiometer. In short, a helium cooled low T_c-SQUID sensor detects the time varying magnetic induction generated by the relaxing magnetisation of the super paramagnetic iron oxide (SPIO) shortly after the magnetic field that has aligned the magnetic moments is switched off. With a close distance of less than 10 mm between sensor and sample, this system is optimised for high-resolution measurements and magnetic field changes down to 1 pT flux density can be resolved. The measured signal amplitudes are directly proportional to the SPIO amount

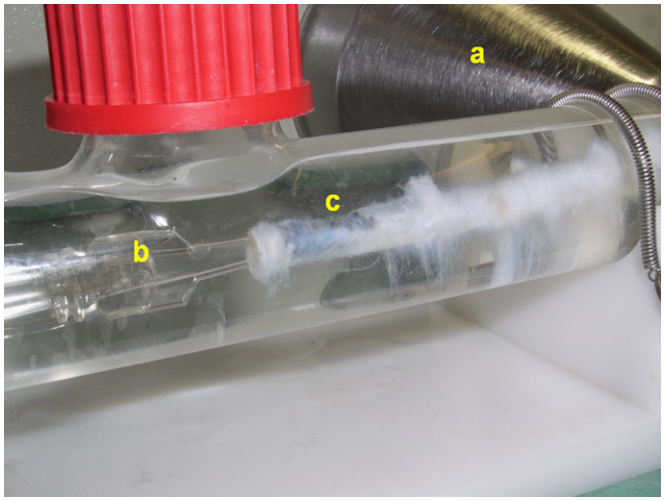

Fig. 2. The circulating model: tip of the magnet (a); artery holding container tempered at 37 °C (b) and artery (c).

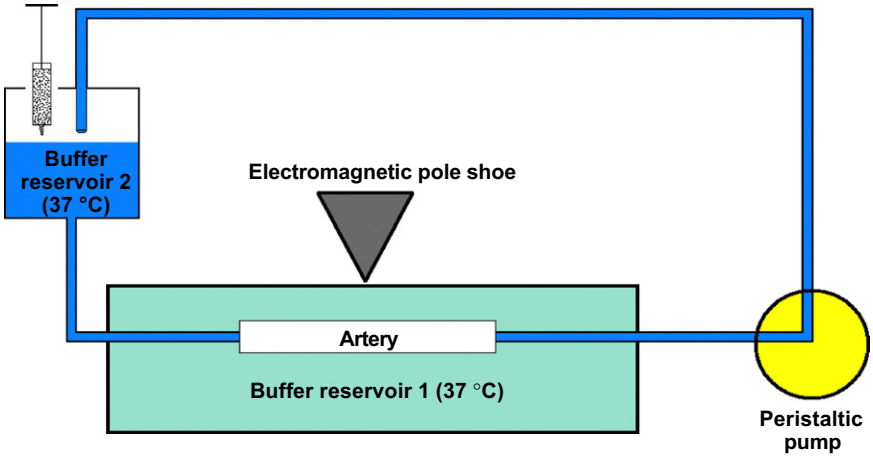

Fig. 1. Schematic drawing of the circuit model. Nanoparticle suspension was administered into the buffer reservoir after the magnetic field was turned on.