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Microscopy study of magnetic fluid hyperthermia on breast cancer cells

A A. Khan[a] · A. Amiri[a] · K. Riahi[a] · C. Dietz[a] · O. Gutfleisch[a] · I. Dirba[a,*]

[a]Materials Science, Technical University of Darmstadt, Darmstadt, Germany
*Corresponding author, email: imants.dirba@tu-darmstadt.de

Abstract

Magnetic fluid hyperthermia (MFH) using magnetic nanoparticles that produce heat in response to an external alternating magnetic field is a promising therapy in cancer treatment offering the possibility to deliver targeted treatment, focusing the energy source to achieve doses that are selectively localized within the tumor volume. In this work, we systematically study MFH treatment at various alternating magnetic field (AMF) frequencies and iron oxide nanoparticles (30 nm) concentrations on breast cancer cells *in vitro* using optical microscopy. Calorimetry measurements show that therapeutic temperature range in the ferrofluid is reached after about 30 s at 402 kHz and 300 G. Microscopy results reveal that at sufficient ferrofluid concentrations > 25 µL, cancer cell death is caused by the AMF treatment at 300 G and 402 kHz for 30 min. Further experimental development towards *in situ* temperature and microscopy monitoring is planned.

I. Introduction

Cancer is among the leading causes of mortality worldwide. Despite significant advances, one of the main common challenges for cancer treatments remains - to deliver the right treatment to the target region and at the necessary dose, in order to reduce damage to the normal tissue. Magnetic fluid hyperthermia (MFH) using magnetic nanoparticles that produce heat in response to an external alternating magnetic field is a promising therapy in cancer treatment [1] offering the possibility to deliver targeted treatment, focusing the energy source to achieve doses that are selectively localized within the tumor volume. In this work, we systematically study MFH treatment at various alternating magnetic field (AMF) frequencies and ferrofluid concentrations on breast cancer cells *in vitro* using optical microscopy.

II. Methods and materials

BT-20 breast cancer lines were purchased from CLS Cell Lines Service GmbH. BT-20 cell lines were cultured in a flask containing DMEM/F-12 medium suspended with 10% (v/v) Fetal bovine serum (FBS) and 1% Pen- Strep at 37°C and 5% CO2 in the incubator. A well-dispersed cell solution was prepared. 1.2 ml of the solution was transferred to the petri dish and rest to the flask. Both the petri dish and flask were kept in the incubator. The cells were split into petri dish for further experimentation and observations. After 48h, cells in the petri dish were ready to be subjected to AMF. Iron oxide nanoparticles (30 nm) with COOH groups on the surface and iron concentration of 6 mg/ml were purchase from micromod Partikeltechnologie GmbH and added to the petri dish in different concentrations (0-100 µL) followed by incubation for 24 h. An AMF applicator (DM100 series, nB nanoScale Biomagnetics) was used for the heating rate measurements on the initial ferrofluid and treatment of cancer cells in a petri dish. Frequencies of 98kHz, 203.5kHz and 402kHz

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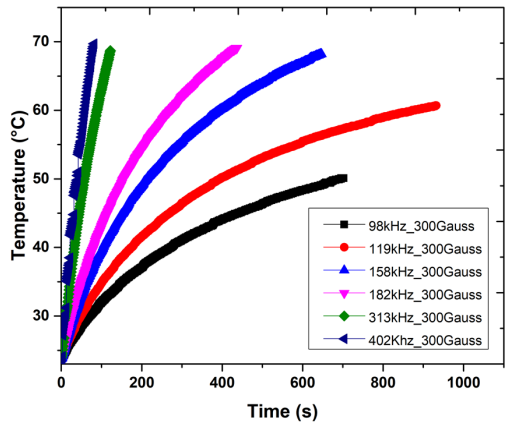

Figure 1: Calorimetry measurements at 300 G applied AMF demonstrate increase in heating rate with frequency.

at a fixed magnetic field intensity of 300G were studied. A Zeiss Axio Observer microscope was used to observe the cancer cells before and after 30 min AMF exposure.

III. Results and discussion

First, measurements using vacuum-insulated calorimeter were conducted on a ferrofluid sample (1 mL) by inserting an optical temperature probe directly into the liquid. The results in Fig. 1 show that the heating rate increases significantly with frequency, in agreement with previous experimental and modelling results [2]. Moreover, therapeutic temperature range of 42-46 °C is reached within 8 min in the case of 98 kHz and already after about 30 s for 402 kHz.

To study the effect of MFH treatment on breast cancer cells, the setup was modified enabling insertion of a petri dish directly in the middle of the AMF applicator coil. A mask was used to ensure that the same area is observed before/after the AMF application. First, the breast cancer cells without magnetic nanoparticles were subjected to AMF at different frequencies for 30 minutes as a reference to confirm that any effects observed later can indeed be attributed to the MFH treatment instead of the AMF. Fig. 2 (a) and (b) show microscopy results before and after the treatment at 300 G and 402 kHz. No statistically significant differences are observed, the cells remain intact. The situation changes completely once the magnetic nanoparticles ferrofluid is added. Fig. 2 shows the results for selected concentrations, 25 µL (c-d) and 100 µL (e-f). From our study, 25 µL appears to be the threshold for observable cancer cell damage. At higher concentrations, e. g. 100 µL in Fig. 2f, most of the cancer cells have been destroyed by the AMF treatment at 300 G and 402 kHz for 30 min. We also investigated the effect of the AMF amplitude and frequency (not shown here) and as expected from Fig. 1, higher values result enhanced degree of cancer cell elimination.

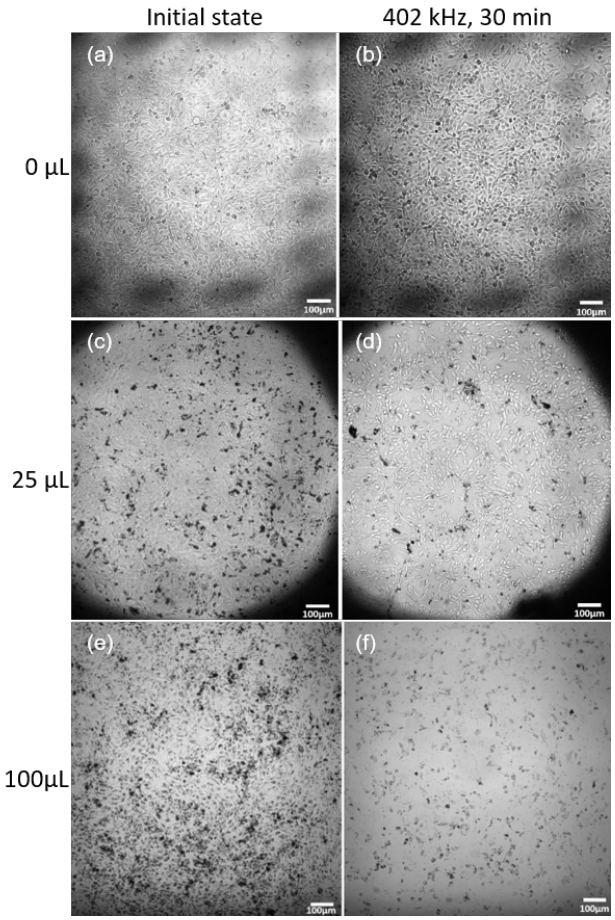

Figure 2: Microscopy images of the breast cancer cells in a petri dish before/after AMF application at 300 G, 402 kHz for 30 min for 0, 25 µL, 25 µL and 100 µL ferrofluid concentrations.

IV. Conclusion

This work successfully demonstrates the effectivity of magnetic fluid hyperthermia on breast cancer cells. Further development towards *in situ* temperature and microscopy monitoring is ongoing.

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Author's statement

Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study.

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