Control of cancer cell fate by magnetically driven treatments

M. Angelakeris, Associate Professor

Magnetic Nanostructure Characterization Group:
Technology & Applications
Department of Physics, Aristotle University of Thessaloniki,
54124 Thessaloniki, Greece

http://magnacharta.physics.auth.gr
email: magnacharta@physics.auth.gr
Magnetically Driven Therapies

1. **Alternative:** with the possibility to obtain stable colloids using MNPs, they can be administered through a number of drug delivery routes.

2. **Selective:** MNPs can be targeted through specific binding agents making the treatment much more selective and effective.

3. **Cancer-specific:** cancer cells absorb MNPs thereby increasing the effectiveness of treatment.

4. **Brain tumors:** MNPs can also effectively cross blood-brain barrier (BBB) and hence can be used for treating brain tumors.

5. **Homogenous:** compared to macroscopic implants, MNPs provide much more efficient and homogeneous treatment.
Magnetically Driven Therapies

Optimizing the carrier
- Material Choice
- Size
- Shape
- Magnetic profile
- Concentration
- Colloidal Stability

Choosing the proper agent

Adjusting the conditions

From lab to clinical trials

Optimizing the treatment
- biocompatibility
- toxicity
- In-vivo efficiency
- multifunctionality

the conditions
- Frequency
- Field intensity

Short & Long term side effects

the side-effects
- Short-term
- Long-term
- extraction
Choosing the proper agent

Alloys
- FeCo
- FePt
- CoPt

Ferrites
- Maghemite
- Magnetite
- Ni-ferrite
- Co-ferrite
- Mn-ferrite

Elements
- Ni
- Fe
- Co

Nanoparticle Diameter (nm)

CoFe
- 10
- 16
- 1.5 kJ/m³, 201 A m²/kg

FePt
- 4
- 7000 kJ/m³, 75 A m²/kg

CoPt
- 6
- 4000 kJ/m³, 46 A m²/kg

Maghemite
- 43
- 6.2 kJ/m³, 47 A m²/kg

Magnetite
- 30
- 9 kJ/m³, 89 A m²/kg

Ni-ferrite
- 30
- 200 kJ/m³, 85 A m²/kg

Co-ferrite
- 30
- 4.7 kJ/m³, 85 A m²/kg

Mn-ferrite
- 43
- 4.7 kJ/m³, 85 A m²/kg

Ni
- 10
- 1.5 kJ/m³, 201 A m²/kg

Fe
- 20
- 48 kJ/m³, 222 A m²/kg

Co
- 10
- 412 kJ/m³, 163 A m²/kg
Magnetic nanoparticles: A multifunctional vehicle for modern theranostics
M. Angelakeris Biochimica et Biophysica Acta 1861 (2017) 1642–1651
Hyperthermia Optimization

- dimensions
- coercivity
- remanence
- saturation field

heat ⇔ SLP$_{\text{max}}$

Theory predictions

- $10 < D < 30$ nm
- $60 < M_s < 100$ A m$^2$/kg
- $5 < K_{\text{eff}} < 40$ KJ/m$^3$

- Colloidal Stability
- Concentration
- Toxicity
Hyperthermia Optimization

Phases
- MnFe$_2$O$_4$: Dalton Trans. 44 5396 (2015)

Shapes
- Scientific Reports, 3:1652 (2013)

Core-Shell
- RSC Adv. 6, 72918 (2016)

Multi-core

Mix & Match

Arrays
- Scientific Reports 6, 37934 (2016)
- Scientific Reports 6, 38382 (2016)
Tuning the magnetism of ferrite nanoparticles

Metallic Fe hard/soft interface

Fe$_3$O$_4$ or γ-Fe$_2$O$_3$

<table>
<thead>
<tr>
<th>Sample</th>
<th>Morphology*</th>
<th>Core/Shell(s) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F01</td>
<td></td>
<td>44/3</td>
</tr>
<tr>
<td>F02</td>
<td></td>
<td>42/2/3</td>
</tr>
<tr>
<td>F03</td>
<td></td>
<td>25/1/3</td>
</tr>
<tr>
<td>F04</td>
<td></td>
<td>24/4/8</td>
</tr>
<tr>
<td>F05</td>
<td></td>
<td>30/24</td>
</tr>
<tr>
<td>F06</td>
<td></td>
<td>43/-</td>
</tr>
</tbody>
</table>

Adjusting the conditions
- Spherical iron nanoparticles of 75 nm.
- Biocompatible MgO shell and bcc Fe core.
- Significant uptake for the different cell lines (42 -126 pg Fe/cell).
- Concentration-dependent cytotoxicity profile
- SLP was estimated to be in the region of 100-500 W/g Fe.
- Fast thermal response (15 °C/ 10 min)
Fe/MgO nanoparticles

Dose-dependent proliferation assay on 3T3 cells by monitoring the 24-hour cell growth. Normal proliferation rates were obtained at all concentrations tested.


T2-weighthed MR images of mouse body before (left) and after injection of NPs. Because of the negative contrast properties of the solution, the liver appears hypointense in images after contrast injection (see arrow). (B) Color-coded T2 maps, from yellow (high T2) to green (low T2). (C) Comparison of magnetic signal from targeted liver at the same time points as imaged by MRI. Maximum concentrations (~ 90% of the injected dose) were observed 24 hours after injection.
Case Study 02: Thermoresponsive Actions

- Highly faceted iron oxide nanoparticles
- Encapsulation in biodegradable block copolymers
- Hyperthermia & Taxol drug release

Case Study 03: Soft Ferrites+Multiple Pulses

MnFe$_2$O$_4$ CoFe$_2$O$_4$ spherical nanoparticles

Comparative viability for the three cell lines: S1h1: primary bone marrow-derived osteoblasts, S1h2: 3T3-L1 fibroblast-like preadipocytes, C1 and S1c: Saos-2 osteoblasts control and sample (b). (c) Optical microscope images (36x) of Saos-2 osteoblast cell line control sample and MNPs after Prussian blue staining.

Case Study 04: Focusing Hyperthermia

The advantage of such an application scheme is minimization of heat side-impact on surrounding healthy tissues.

MPH a much more effective therapeutic method with

- better focusing capabilities
- tunable heat localization
Case Study 05: Heating cures?

“What medicine cannot cure, iron (the knife) cures; what iron cannot cure, fire cures; what fire does not cure, is to be considered incurable”

A very popular method that is used to provoke brain stimulation is Transcranial Magnetic Stimulation (TMS) which represents major advances of the state of the art in brain stimulation, but the focusing depth and locality are limited.

The temperature range (41-45°C) has been indicated to activate the central nervous system.

Incorporation of MNPs within such a protocol may lead to an increase in the regional temperature facilitate manipulation of brain’s electric fields, limit the high intensity magnetic fields side effects
Case Study 06: Magnetic Activation of cell signals

Hetero-nanocomposites: 30 nm Cu$_2$O nanoparticles with antifungal properties functionalized with 9 nm NiFe$_2$O$_4$ magnetic nanoparticles as a platform for magnetomechanical stress in Saccharomyces cerevisiae.

*J. Mater. Chem. B*, 2015, 3, 5341
Magnetic Activation of cell signals

Magnetic nanoparticles may be used to generate mechanical stimulations on cells which can induce changes in cell activity such as differentiation, growth and death.

By magnetomechanical stimulation we can achieve:

- Remote activation of drug release
- Selective induction of physical cell injury
- Modulation of enzyme activity
- Lysosomal damage
A series of commercially available magnetic iron oxide nanoparticles (FluidMag-DX from Chemicell) uses for MRI-diagnostics and drug delivery applications.

The major advantage of such a study is that an optimum system directly addresses the multifunctional role in modern theranostics and may be further implemented in therapies with faster steps since major tasks have already been undertaken.

<table>
<thead>
<tr>
<th>Size</th>
<th>50 nm</th>
<th>100 nm</th>
<th>200 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Particles</td>
<td>$1.3 \times 10^{16}$/g</td>
<td>$1.8 \times 10^{15}$/g</td>
<td>$2.2 \times 10^{14}$/g</td>
</tr>
<tr>
<td>Density</td>
<td>1.25 g/cm$^3$</td>
<td>1.25 g/cm$^3$</td>
<td>1.25 g/cm$^3$</td>
</tr>
<tr>
<td>Type of Magnetization</td>
<td>Superparamagnetic</td>
<td>Superparamagnetic</td>
<td>Superparamagnetic</td>
</tr>
<tr>
<td>Functional Group</td>
<td>Hydroxyl groups</td>
<td>Hydroxyl groups</td>
<td>Hydroxyl groups</td>
</tr>
<tr>
<td>Storage Buffer</td>
<td>ddH$_2$O</td>
<td>ddH$_2$O</td>
<td>ddH$_2$O</td>
</tr>
</tbody>
</table>
Experimental Steps

Can the generated magnetic fields affect movement of endocytosed MNPs in a way that leads to inhibition of cancer cell growth?

If so, what are the critical parameters governing this effect?

MNPs
Fe$_3$O$_4$
100 nm / 200 nm
Starch coating

Colon cancer cell lines
HT29

3D print out set up

Cell proliferation assay

Fe$_3$O$_4$
Magnetic array setups to create variable magnetic flux modes in sample region. Series of block magnets for variable magnetic field configurations. Support tables for cell samples.

- **Amplitude**: 40-200 mT
- **Frequency**: 0-16 Hz
- **Type**: Static, AC, Rotating

Cell sample in 3.5 cm culture petri dish
Mechanical Forces

Collective forces (/cell) range from 10 to 750 pN, when the corresponding nanoparticle concentration varies from 10 to 250 pg/cell, respectively.

Forces of such strength (tens to hundreds of pN) acting on different cell types can reprogram and cause an effect on cell’s differentiation.

1-10 pN: internal cytoskeleton stress
10-100 pN: stretch a DNA molecule
5-70 pN: neuron cells, mechanical sensitivity
50-460 pN: intracellular transport of the macrophages in the cytoplasm

«Magneto-mechanical action of multimodal field configurations on magnetic nanoparticle environments», N. Maniotis et al. JMMM 2017
Prior to the cellular uptake experiments, we determined the non-cytotoxic concentration of nano-screenMAG/R and fluidMAG-D (100 nm) nanoparticles for HT29 cells, after a co-incubation period for up to 48 hours, with the SRB assay).

100 μg/mL of MNPs do not inhibit proliferation of HT29 cells, compared to control, untreated cells.

Cytotoxicity & Uptake

The concentration of iron [Fe] in cells after MNPs treatment (100 μg/ml for 48h), was measured in an ICP-OES (inductively coupled plasma - optical emission spectroscopy) apparatus: OPTIME 2100DV from Perkin Elmer (USA) and normalized to control, untreated cells.

(a) ICP-OES quantification of intracellular iron of HT29 cells incubated with 100 μg/ml of MNP-100 for various time points.

(b) Number of HT29 cells that successfully endocytosed MNPs after co-incubations for various time points.

*Nanotechnology 29, 17, 175101 (2018).*
The maximum magnetic force ($F_m$) on a single nanoparticle can be estimated as:

$$F_m = m \cdot \nabla B$$

where $m$ is the saturation moment of an individual nanoparticle and $\nabla B$ the magnetic field gradient, for the applied field mode.

Accordingly, the maximum magnetic force on particle ensemble is

$$\sum (m \cdot \nabla B)$$

Cell growth dependence on magnetic flux density magnitudes and subsequent applied forces, exerted from external static magnetic fields of various strengths on endocytosed magnetic nanoparticles.

*Nanotechnology 29, 17, 175101 (2018).*
Rotation effects

Rotational magnetic field effect on cell growth of cells without (blue curve) and with (black curve) MNPs of 100 nm. All magnetic field intensities rotate at the frequency of 2 Hz.

Outlook-Perspectives

Manipulate magnetic nanoparticles within cellular environment

- DC rotating motor
- different NdFeB block magnets in size, shape, magnetic force
- unique magnetic field patterns

Static, AC and rotating magnetic field

- a uniform average force was applied on a large number of HT.29 cells
- The force’s magnitude was quantified experimentally and computationally
- Cells responded both to magnetic forces applied externally and/or generated internally by magnetic nanoparticles
Acknowledgements

Group members

▪ K. Simeonidis, Dr.
▪ D. Sakellari, Dr.
▪ A. Makridis, PhD student
▪ E. Mirovali, PhD student
▪ N. Maniotis, PhD student

Colleagues

▪ M. Farle, M. Spasova, U. Wiedwald, Germany
▪ Ll. Balcels, C. Boubeta, M. P. Morales, D. Serantes, Spain
▪ K. Chliclia, K. Spriridopoulou, Greece
▪ K. Dendrinou-Samara, O. Kalogirou, T. Samaras, AUTh-Greece

Magnetic Nanostructure Characterization Group:
Technology & Applications
Department of Physics, Aristotle University of Thessaloniki,
54124 Thessaloniki, Greece

http://magnacharta.physics.auth.gr
email: magnacharta@physics.auth.gr

Thank you for your attention