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Response to a magnetic field
Thermal energy
Alter NMR signal
OUTLINE

1- Nanoparticles for medicine
2- Basic principles in magnetism
3- Biomedical applications: Separation, Diagnosis, Therapy
4- Synthesis of magnetic nanoparticles
5- Future
Nanosystems in medicine

**Materials**
- Nano-porous
- Nano-crystals
- Nano-reinforced
- Nano-structured surfaces

**Properties**
- Tissue ingrowth, transport of substances
- Physical, electrical, optical, mechanical properties
- Mechanical properties, Biocompatibility

**Applications**
- Surgery
- Therapy
- Diagnostics
- Biosensors/biodetection
- Implantable materials/devices: Tissue engineering
- Textiles and wound care products
- Drug/gene delivery materials and devices

Bandages with silver nanoparticles Curad® (www.curadusa.com) antibacterial agent.

Patrick Couvreur et al., Chem. Rev. 112, 5818, 2012
He was the first to develop nanometric capsules able to penetrate cells to deliver medicine
Nanosystems in medicine

Nanotechnology and health care, huge potential and some risks.

Global Market for Nanoparticles

- Drug release control
- Drug solubility problems
- DNA carriers
- Tissue regeneration

Areas:
- Cancer
- Neurodegenerative
- Cardiovascular
- Infection

Source: BCC Research (BIO113B), August 2014

Oncology

Consequences: The oncology market is the third largest pharmaceutical market, behind the cardiovascular and central nervous system therapy areas.

Treatment of cancer with traditional medicine involves surgery, ionizing radiation, and chemotherapy.

These treatments affect both tumors and healthy tissue.

Consequences: Multi-billion markets in medical and palliative expenses because systemic toxicity and undesirable side effects.
<table>
<thead>
<tr>
<th>Product</th>
<th>Nanosystem</th>
<th>Application</th>
<th>Status</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Doxil</strong></td>
<td>Doxorubicina encapsulada en liposomas PEGilados</td>
<td>Cáncer de ovarios</td>
<td>Aprobado 11/17/1995 FDA50718</td>
<td>Ortho Biotech (adquirida por JNJ)</td>
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<td>(Barenholz, 2012)</td>
<td></td>
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<td><strong>Myocet</strong></td>
<td>Doxorubicina encapsulada en liposomas No PEGilados</td>
<td>Cáncer de mama metastásico</td>
<td>Europa y Canadá, en combinación con ciclofosfamida</td>
<td>Sopherion Therapeutics, LLC EEUU y Cephalon, Inc. en Europa</td>
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<td>(Waterhouse et al., 2001)</td>
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<td><strong>DaunoXome</strong></td>
<td>Daunorubicina encapsulada en liposomas</td>
<td>Tratamiento de sarcoma de Kaposi avanzado asociado al VIH</td>
<td>Aprobado en E.E.U.U</td>
<td>Galen Ltd.</td>
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<td>(Forssen, 1997)</td>
<td></td>
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<tr>
<td><strong>ThermoDox</strong></td>
<td>Doxorubicina encapsulada en liposomas (liberación mediada por calor)</td>
<td>Cáncer de mama y primeras etapas de cáncer de hígado</td>
<td>Aprobación esperada para el año 2013</td>
<td>Celsion</td>
</tr>
<tr>
<td>(Dromi et al., 2007)</td>
<td></td>
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<tr>
<td><strong>Abraxane</strong></td>
<td>Nanopartículas de albúmina-paclitaxel</td>
<td>Diferentes tipos de cáncer</td>
<td>Aprobado 1/7/2005 FDA21660</td>
<td>Celgene</td>
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<tr>
<td>(Guarneri et al., 2012)</td>
<td></td>
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<tr>
<td><strong>Rexin-G</strong></td>
<td>MicroRNA-122 encapsulado en liposomas</td>
<td>Sarcoma, osteosarcoma, cáncer de páncreas, y otros tumores sólidos</td>
<td>Aprobado en Filipinas, Fase II y III en E.E.U.U</td>
<td>Epeius Biotechnologies Corp.</td>
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<tr>
<td>(Gordon and Hall, 2010)</td>
<td></td>
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<tr>
<td><strong>Oncaspar</strong></td>
<td>Asparaginasá PEGilada</td>
<td>Leucemia linfoblástica aguda</td>
<td>Aprobado 24/06/2006</td>
<td>Enzon Pharmaceuticals, Inc.</td>
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<td>(Avramis and Tiwari, 2006)</td>
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<tr>
<td><strong>Resovist</strong></td>
<td>Nanopartículas de óxido de hierro recubiertas de carboxidextran</td>
<td>Agentes de contraste para hígado y bazo</td>
<td>Aprobado en Europa en 2001</td>
<td>Bayer Schering Pharma AG</td>
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<tr>
<td>(Hamm et al., 1994)</td>
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<tr>
<td><strong>Feridex</strong></td>
<td>Nanopartículas de óxido de hierro recubiertas de dextrano</td>
<td>Agentes de contraste para hígado y bazo</td>
<td>Aprobado por la FDA en E.E.U.U en 1996</td>
<td>Berlex Laboratories</td>
</tr>
<tr>
<td>(Weissleder et al., 1989)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Endorem</strong></td>
<td>Nanopartículas de óxido de hierro recubiertas de dextrano</td>
<td>Agentes de contraste para hígado y bazo</td>
<td>Aprobado en Europa</td>
<td>Guerbet</td>
</tr>
<tr>
<td>(Weissleder et al., 1989)</td>
<td></td>
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</table>
Chemical Design of Biocompatible Iron Oxide Nanoparticles for Medical Applications,
Daishun Ling and Taeghwan Hyeon, Small 2013, 9, No. 9–10, 1450–1466.
MAGNETIC NANOPARTICLES $\Leftrightarrow$ LIVING SYSTEMS

Fe$_3$O$_4$
Polymer

Magnetotactic bacteria
For orientation

Life in Mars?

© 2012 American Soc. for Microbiology
OUTLINE

1- Nanoparticles for medicine
2- Basic principles in magnetism
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5- Future
Basic principles

Particle size

100 nm

5 nm

Magnetic recording

Ferromagnético

Hc

Ms

M

H

Sensors

Superparamagnético

\[ \chi_i \]

M

H
The moments within each particle are ordered (Red arrows).

The net magnetic moment of a system containing MNPs will be zero in zero field and at high enough temperatures.

In the presence of a field, there will be a net statistical alignment of magnetic moments.
Superparamagnetism

Basic principles

\[ \Delta E = K_a V \approx k_B T \]

Small particle size

Jump frequency
\[ \nu = \tau_0^{-1} \exp\left(-\frac{K_a V}{k_B T}\right) \]

Relaxation time
\[ \tau = \tau_0 \exp\left(\frac{K_a V}{k_B T}\right) \]
Superparamagnetism

\[ \chi \approx \frac{V M_s^2}{3 k_B T} \]

Particle size

Magnetic behaviour

Saturation field

\[ M(H) \]

Figure 7: Calculated magnetization behaviour at \( T \approx 300 \) K of Fe particles of various sizes.
Ferromagnetism

Alternating magnetic field

Delivered by the applied field

Characterised by area enclosed by the hysteresis loop

Thermal energy
MECHANISM OF MAGNETIZATION ROTATION

Basic principles

Rotation of the moment within the NP

Mechanical rotation of the NP

Movement of domain walls in multidomain
Néel relaxation = \( \tau_N = \tau_0 \exp\left(\frac{KV_M}{k_B T}\right) \)

Brownian relaxation = \( \tau_B = \frac{3 \eta V_H}{k_B T} \)

Total relaxation
\[
\frac{1}{\tau} = \frac{1}{\tau_B} + \frac{1}{\tau_N}
\]

- No hysteresis $\rightarrow$ no interest for heat
- Analytical calculation: Langevin...

- Open hysteresis loops $\rightarrow$ optimized nanoparticles
- Analytical calculations: Stoner-Wohlfarth model

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Biomedical applications

Separation
- Cellular proteomics

Diagnosis
- MRI
- Stem cell tracking
- Biosensing

Therapy
- Hyperthermia
- Drug delivery

Controlled cellular interactions
- Living cell with bioactive magnetic nanoparticle
Nanometer

- **Size**: Get close to a biological entity of interest
- **Surface**: Bind a biological entity
- **Properties**: Manipulated by a magnet

Requirements for biomedical applications
Requirements for biomedical applications

NANOPARTICLES  →  COLLOIDAL SUSPENSIONS  →  APPLICATIONS  →  REQUIREMENTS

- Size
- Surface
- Properties

In vitro
In vivo

No toxic!!

- Stable
- Biocompatible
- Reversible
Requirements for biomedical applications

**Size**

<table>
<thead>
<tr>
<th>Size</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-100 nm</td>
<td>Small response to a magnetic field</td>
</tr>
<tr>
<td></td>
<td>Detected by the immune system and eliminated</td>
</tr>
<tr>
<td>5-50 nm</td>
<td>Ideal diameter for most forms of therapy</td>
</tr>
<tr>
<td></td>
<td>Remain in the body long enough to be circulated through the blood stream</td>
</tr>
</tbody>
</table>

5-50 nm = Ideal diameter for most forms of therapy
Requirements for biomedical applications

Hydrodynamic size

Core + Molecules around
Surface Modification of the particle’s surface to make it biocompatible and specific

- Hydrophilic coating makes the particle look friendly to the immune system
- Coated with a biological entity to function in a specific manner
- Carrier to transport and deliver a biological active agent
Requirements for biomedical applications

**Surface**

- Recognise specific proteins on the surface of the virus
- Nanoparticles clump with the virus

**Detect virus in body fluids**

**Recognise and destroy a cancer cell**

- Ferrite
- Polymer
- Carboxyl groups
- Drug
- Drug
- Drug
- Drug
Magnetic properties

- They must **constantly and rapidly** “flip” magnetic states.
  
  \[ \Rightarrow \text{Mr}=0 \]

- **Saturation magnetisation** (Ms) should be **strong enough** to be manipulated by an external magnetic field

- **Resonant respond** to a time-varying magnetic field should be enough to heat up.
Biomedical applications

Separation

Cellular proteomics

Cell isolation

Biosensing

MRI

Stem cell tracking

Drug delivery

Hydrotherapy

Diagnosis

Controlled cellular interactions

Bioactive magnetic nanoparticle

Living cell
Goal: Separate/detect/isolate one type of cell from others, often when the target is present in very small quantities

- Reduce the time
- Detect lower concentrations
**Separation/selection**

1. **Functionalized nanoparticles**
2. **Add to sample**
3. **Magnetic nanoparticles bond with targeted cells**
4. **Retain desired cells by applying a magnetic field**
Detection of proteins at $10^{-18}$ M = Prostate-specific antigen (PSA)

Detection of DNA at $10^{-21}$ M

6 orders of magnitude more sensitive

**Magnetic Sorting/Detection**

- High sensitivity
- Multiple analytes at one time
- Hand-held
- Lightweight
- Fast
- Potential for single-bead detection

Separation/selection

Probe DNA → polymer → SiO₂

Analyte DNA + biotin

Wash unbound DNA away

Streptavidin + magnetic particle

Separation/selection

BIOMOLECULE SEPARATIONS

FOOD QUALITY CONTROL

WATER PURIFICATION (Ar, Pb, Hg, Zn...)

884 millones = Personas que carecen de acceso a fuentes de abastecimiento de agua potable (una de cada ocho)


Mohan and Pittman 2007
Biomedical applications
The most powerful technique for diagnosis

Nobel Prize 2003
Paul C. Lauterbur and Sir Peter Mansfield
"for their discoveries concerning magnetic resonance imaging"

Advantage: not use X-Rays nor any other type of "ionizing" radiation

Instead: it is a technique that combines a large magnetic field and some radio frequency antennas

Measure the relaxation rate of protons in the atoms of water within the patient from their excited state to the ground state
protons of water

"line-up"

magnetic field

high-frequency electro-magnetic pulse

protons out of alignment

"resonance" signal as the proton goes back into alignment

image reflects the water protons in the patient and their chemical association with proteins
NMR imaging

Fig 22

Coupling of a T1- and a T2-curve resembles a mountain with a slope. It takes longer to climb a mountain than to slide or jump down, which helps to remember that T1 is normally longer than T2.

MRI made easy
NMR Imaging

(d) \( B_0 \uparrow \)

(e) \( B_0 \uparrow \)

\( m_y \) signal vs. time

- slow \( T2 \) relaxation
- fast \( T2 \) relaxation

Images:

- Negative contrast from iron oxide
- Positive contrast

Images:

- Slow White
- Fast Black
- Slow white
Commercial products = 5-10 nm

NMR imaging

- USPIO
- SPIO
- Resovit
- Sinerem
- Endorem

Hydrodynamic size (nm)

$r_2 (\text{mM Fe. s})^{-1}$

Bibliog
4 nm
6 nm
9 nm
14 nm
The chemistry of contrast agents in medical magnetic resonance imaging, André E. Merbach and Eva Toth, Wiley, 2001

**Shorter** relaxation ($T_2$) => **Darker** in the MRI

$$\frac{1}{T_2} = R_2 = \left(\frac{64\pi}{135000}\right) \gamma^2 N_A M \mu^2 \frac{1}{r D}$$

NP magnetic moment

Concentration (mole.L\(^{-1}\))

NP radio

Diffusion coefficient of water molecules
NMR Imaging


NMR Imaging

Challenges

R. Weissleder et al. 2001 *Angew. Chem.*, Int. Ed. **40** 3204

Basic Res Cardiol 103:122–130 (2008)
NMR Imaging of Rat Brain During 1 Hour

USPIO

NMR IMAGING OF RAT BRAIN DURING 1 HOUR

Arrowheads (Yellow)- Third Ventricle
Arrows (white)- Lateral Ventricle
Arrowheads (blue)- Recess Inferior Colliculus

R. Mejias et al., Nanomedicine 2010
NMR Imaging

Targeted imaging

tumor

C. Sun et al. / Advanced Drug Delivery Reviews 60 (2008) 1252–1265
NMR Imaging

T1 contrast agents based on ultrasmall iron oxide nanoparticles

NMR Imaging

A) Magnetization 100 emu g\(^{-1}\) Fe

B) Magnetization 5 emu g\(^{-1}\) Fe

C) Temperature (K)

-500 500

-5

5

2

4

6

8

Temperature (ºC)

***
n.s.
r\(_2\)/r\(_1\)

B) NMR Imaging

C) NMR Imaging

D) NMR Imaging
Multifunctional contrast agents

<table>
<thead>
<tr>
<th>Imaging technology</th>
<th>Contrast agents</th>
<th>Spatial resolution</th>
<th>Toxicity</th>
<th>Sensitivity</th>
<th>Time Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray CT</td>
<td>Iodinated contrast material</td>
<td>sub-mm</td>
<td>Nephrotoxic</td>
<td>mM</td>
<td>1–2 s</td>
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<tr>
<td>MRI</td>
<td>Gadolinium-based</td>
<td>sub-mm</td>
<td>Nephrogenic systemic fibrosis</td>
<td>mM for Gd-based</td>
<td>1–2 s</td>
</tr>
<tr>
<td>PET/SPECT</td>
<td>Radioactively labelled agents</td>
<td>mm</td>
<td>Dosimetric exposure</td>
<td>pM</td>
<td>min</td>
</tr>
</tbody>
</table>

Core/Shell Magnetite/Bismuth Oxide Nanocrystals with Tunable Size, Colloidal, and Magnetic Properties

Evaluation as CT agent

Nanotechnology 2015
Coronal and axial images taken by CT and MRI after the subcutaneous administration of 100 μL of FeBi@SiPEG (157mM Fe and 14.6mM Bi). The location of the contrast in the left leg of the mouse is marked with an arrow in the CT pictures.
Hybrids

PET + MRI

68Ga-dCNIC-Dextran

dCNIC-Dextran

Pellico et al, Contrast Media Mol Imaging (2016)
DRUG DELIVERY

Targeting of a drug immobilised on magnetic nanoparticles under the action of an external magnetic field.

• Specific
• High local concentration
• Problem

- Reducing side effects
- Reducing the dosage
- Field strength

Skin

Deep tissue

Driven with a magnet

Release drug by photolysis, pH..

Reverse field

IONP ionically or covalently binding a drug

Human preliminary test
Drug delivery

Drug carrier systems

- IONP ionically or covalently binding a drug
- Polymer coated nanosystems loaded with drugs and IONPs
- Drug loaded magnetic micelles
- Lipid vesicles loaded with drugs and IONPs
Cytokine IFN-γ

Cancer immunotherapy: Activating immune response to removal primary tumor and prevent metastases.

Cytokine: small protein produced by macrophages and T lymphocytes

Activity:
- Activate macrophages production
- Induce cancer cell apoptosis

IFN-γ the most effective cytokine in tumor elimination

Magnetic nanoparticles: Controlled local release of cytokines

Drug delivery

Tumor size

Biomaterials 32, 2938, 2011.

Also for induced tumours with 3-methylcholanthrene (MCA)
Drug delivery

Main limitation

- **Large MNPs (> 200 nm)** will be easily detected by the immune system and removed from the blood and delivered to the liver and the spleen.

- **Very small MNPs (< 5.5 nm)** can be excreted through the kidneys.

- Different magnetic biocomposites can be transported to reach the tumor area inside the body thanks to the applied magnetic field.
Drug delivery

Challenges

Nanoparticle-based chemotherapy
*Proof-of-principle, in vivo studies*

Nanoparticle-based radiotherapy
*In vitro and in vivo studies*

Nanoparticle-based phototherapy
*Proof-of-principle*

---

**Drug delivery across blood-brain barrier**

- Treatment of brain tumours, Alzheimer’s, and Parkinson’s – *development phase*
  NanoDel Technologies GmbH, Germany

**Nanovectors for gene therapy**

- Non-viral gene delivery systems – *in vitro studies*
Delivery of genetic materials (siRNA and pDNA) into stem cells

Delivery of siRNA against SOX9 (siSOX9) and CAVEOLIN-1 (siCAV) gens for inducing neural differentiation of NSCs


**Figure Legend:**

- **Experiment (proliferation)**
  - A1: Ki67, Hoechst
  - A2: TuJ1, GFAP, Hoechst

- **Experiment (differentiation)**
  - A1: TuJ1, GFAP, Hoechst
  - A2: TuJ1, GFAP, Hoechst

- **Experiment (with MF)**
  - B1: GFP-NSCs
  - B2: GFP-NSCs

- **Control (without MF)**
  - B1: GFP-NSCs
  - B2: GFP-NSCs

- **Control (only MCNPs)**
  - Control only MCNPs

- **EX1 (MCNP + siSOX9)**
  - EXP (MCNP + siCAV)

**Notes:**

- TuJ1 = Neurons, MBP = Oligodendrocytes
Biomedical applications

- Separation
  - Cellular proteomics
- Therapy
  - Hyperthermia
- Controlled cellular interactions
- Living cell
  - Bioactive magnetic nanoparticle
- Drug delivery
- Cell isolation
- Biosensing
- MRI
- Stem cell tracking
- Diagnosis

Image credit: Nanomedicine © Future Science Group (2009)
Hyperthermia

**HYPERTHERMIA**

Heating of a target tissue to the temperatures between 42-43 °C

- Reduces the viability of cancer cells
- Increases their sensitivity to chemotherapy and radiation

**Conventional Hyperthermia**

Heating methods:
- External heating
- Internal heating

Heating regions:
- Whole body heating
- Local/regional heating

Heating techniques:
- Ultrasound heating
- Electromagnetic field heating
- Hot water and blood flow

Instituto de Ciencia de Materiales de Madrid

CSIC

Consejo Superior de Investigaciones Científicas
Hyperthermia

Magnetic Hyperthermia

Advantages of using magnetic nanoparticles

- Avoid heating healthy tissues
- Combining other therapies
  Targeting radionuclides

42°C / 30 min  Cancer is destroyed

Goya et al, Current Nanoscience 2008, 4, 1-16
Nearly complete regression of tumors via collective behavior of magnetic nanoparticles in hyperthermia, C L Dennis et al., Nanotechnology 20 (2009)
Hyperthermia

What we actually measure...

\[ \text{SAR} = C_m \phi(\Delta T/\Delta t) \]
Hyperthermia

Important parameters

Specific Absorption Rate

\[ \text{SAR} = C_m \phi(\Delta T/\Delta t) \]

Specific Loss Power = Experimental

\[ \text{SLP} = \mu_0 \cdot \pi \cdot f \cdot H^2 \cdot \chi''(f) \]

where \( C_m \) is the specific heat capacity of the sample

Specific hysteresis loss = SHL = Theoretical area

\[ \text{SHL} = \text{SAR} / H^2 \cdot f \]

Intrinsic Loss Parameter = ILP

\[ \text{ILP} = \text{SAR} / H^2 \cdot f \]
Hyperthermia

(A) SAR (W/g) vs. Size (nm)
- Thermal decomposition + seed-growth (26 mT, 700 kHz)
- Thermal decomposition (26 mT, 700 kHz)
- Thermal decomposition (30 mT, 700 kHz)
- Coprecipitation + size-sorting (31 mT, 700 kHz)

(B) SAR (W/g) vs. $\mu_0 H$ (mT)
- 19.7 nm
- 8.9 nm

(C) S1, S2, S3

G. Salas, et al., International Journal of Hyperthermia, 29, 8, 768-776, 2013
EFFECT OF NP CONCENTRATION

Dependence of the SAR values for magnetite colloids, at different HMAX for a given frequency (107 kHz). Arrows depict the concentration at which the SAR value is maximum for each HMAX.

A Single Picture Explains Diversity of Hyperthermia Response of Magnetic Nanoparticles

Non-monotonic concentration dependence of heating efficiency

- Intrinsic features
- Experimental conditions

NANOPARTICLE ASSEMBLING

The highest area $M(H)$ curve is attained for chain-like shape.

Dependence of area $M(H)$ curve on chain length and orientation.

Chain-like arrangements produce higher hyperthermia output (up to 5 times!)
Hyperthermia

HeLa and MDA-MB-231 cells (stained with Hoechst 33258) were incubated with DMSA coated NPs, and exposed to an AC magnetic field (AMF).

24 h after the treatment apoptotic cells (HeLa) and giant and multinucleated cells (MDA-MB-231) were observed.

AMF conditions: 161 kHz, 210 G, 15 min exposure (HeLa cells), 225 kHz, 150 G, 45 minutes exposure (MDA-MB-231 cells).
Hyperthermia could be used to locally modify tumor stroma and thus improve drug penetration.
Hyperthermia

Table 3. Summary of nanoparticle features favoring MRI and/or magnetic hyperthermia applications.

<table>
<thead>
<tr>
<th>MRI (contrast)</th>
<th>Nanoparticle feature</th>
<th>Magnetic hyperthermia (heating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>High magnetization (size and surface coating)</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>SPIO</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>USPIO</td>
<td>-</td>
</tr>
<tr>
<td>+/-</td>
<td>Large size (core diameter &gt;10 nm)</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>Sequestration by MPS</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Long plasma half-life (targeting)</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>Short plasma half-life (targeting)</td>
<td>-</td>
</tr>
</tbody>
</table>

+: Favoring feature/parameter; -: Disfavoring feature/parameter; MPS: Monocyte phagocyte system; SPIO: Superparamagnetic iron oxide; USPIO: Ultsmall superparamagnetic iron oxide.

Ingrid Hilger et al., Nanomedicine 2012

http://www.magforce.de/en
http://www.youtube.com/watch?v=BZLmD3SOR_Y
http://www.clinicaltrials.gov/ct2/show/study/NCT00003052
http://www.mhaus.org/
Fighting cancer more effectively and with fewer side effects

HOW DOES NANOTHERM™ THERAPY WORK?

NanoTherm™ therapy is a new approach to the local treatment of solid tumors. The method is based on the principle of introducing magnetic nanoparticles directly into a tumor and then heating them in an alternating magnetic field. At approximately 15 nanometers in diameter, the nanoparticles, which are suspended in water, are extremely small (a nanometer is one millionth of a millimeter), and comprise an iron oxide core with an aminosilane coating. The particles are activated by a magnetic field that changes its polarity up to 100,000 times per second, generating heat.
### Growing nanomedicine into a cancer therapy of the future

<table>
<thead>
<tr>
<th>TUMOR TYPES</th>
<th>CLINICAL TRIALS</th>
<th>STATUS OF TRIALS</th>
<th>EU Regulatory Approval</th>
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</thead>
<tbody>
<tr>
<td>Glioblastoma multiforme</td>
<td></td>
<td>Phase I Feasibility study</td>
<td></td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td></td>
<td>Phase II Efficacy study</td>
<td></td>
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<tr>
<td>Pancreatic carcinoma</td>
<td></td>
<td></td>
<td>EU Regulatory Approval</td>
</tr>
</tbody>
</table>
Blood interaction

Biological barriers

Effects on coagulation

Nanoparticle-cell interactions

Toxicity

Design a nanoparticle for each application

LIMITATIONS

Blood interaction

Biological barriers

Nanoparticle-cell interactions

Toxicity

Design a nanoparticle for each application

LIMITATIONS

Blood interaction

Biological barriers

Nanoparticle-cell interactions

Toxicity

Design a nanoparticle for each application

LIMITATIONS

Blood interaction

Biological barriers

Nanoparticle-cell interactions

Toxicity

Design a nanoparticle for each application

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LIMITATIONS
Interaction with cells

HeLa cells-DMSA
0,5 mg Fe/mL- 24 horas

MTT TEST

NPs concentration

Surviving fraction (%)

<table>
<thead>
<tr>
<th>NPs concentration (mg/ml)</th>
<th>0</th>
<th>0,05</th>
<th>0,1</th>
<th>0,5</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>50</td>
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<td>30</td>
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<td>20</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fluorescence and optical microscopy show that cytoskeleton is not affected by the presence of the NPs. Scale bar: 10 µm.
Problem: Biodistribution

100 mg Fe => Endorem (1-5 mg/Kg)

Distribution of Iron in Adults

NANCY C. ANDREWS

The New England Journal of Medicine
Volume 341 Number 26, 1986, 1999
Magnetización

Campo aplicado

All materials are magnetic to some extent with their magnetic response depending on their atomic structure and temperature.

Susceptibilidad AC

Temperatura

$x'$

$0$

Temperatura

$x''$

$0$
Liver and brain imaging through dimercaptosuccinic acid-coated iron oxide nanoparticles
Nanomedicine 5(3), 397-408, 2010
Biodistribution: Characterization

AC MAGNETIC SUSCEPTIBILITY

With the appropriated standards it is possible to calculate the amount of the total iron that is in the form of the magnetic nanoparticles.

Biodistribution

Coating

Biodistribution

Anesthesia

Ketamine and xylazine
Intraperitoneal

Isoflurane
Inhaled (0.5% in oxygen)

Biodistribution

External magnetic field

In vivo

R. Mejías et al, Biomaterials 32 (2011) 2938-2952
Quantification of NPs in tumor

Biodistribution in vivo: Magnetic methods

NP-γ-IFN

NP-γ-IFN- Magnet

Susceptibilidad magnética

Nanomedicine 5(3), 397-408, 2010
Long term particle transformations
Future Research

Contrast agents

• More efficient agents
• Targeting
• Multimodal imaging agents
  MRI/TC
  MRI/PET

Transporte de fármacos

• NP-Chemotherapy
• NP-Radiotherapy
• NP-Phototherapy
• Across the blood-Brain barrier
• Gene therapy

Hipertermia

• Optimization of agents and heat generator
• Mechanism of cell death
• i.v. injection
New applications

Pulse Therapeutics

http://scitation.aip.org/content/aip/magazine/physicstoday/news/10.1063/PT.5.5029

Regeneration

Angewandte Chemie International Edition
53, 6369-6373, 16 APR 2014 DOI: 10.1002/anie.201401043

Detecting disease early

A device worn on the outside of the body can detect the nanoparticles and provide useful information to physicians.

http://www.zmescience.com/medicine/google-nanoparticle-pill-5344/
大纲

1- 纳米颗粒在医学中的应用
2- 基本磁学原理
3- 生物医学应用：分离、诊断、治疗
4- 磁性纳米颗粒的合成
5- 未来
Design a Nanoparticle for each application

Magnetic nanoparticle (MNP)

**IMPORTANT PARAMETERS**

**MAGNETIC CORE**
- Different core size
- Different core composition
- Doping

**COATING**
- Biocompatible polymers
- Colloidal stability
- Strong anchoring
- > MNPs blood life-time
- Core protection

- Binding region to MNPs
- Hydrophilic arm
- Functional group for bio-conjugation
UNIFORM NANOPARTICLES

Upon application, only a small number of particles contribute to the desired magnetic effect.
Nanoparticle synthesis routes

Modelo Clásico
LaMer and Dinegar

Concentration

Critical supersaturation

Nucleation

Growth

Time

I

II

III

Synthesis and Characterization of Nanoparticles: Synthesis of Inorganic Nanoparticles,
Gorka Salas, Rocio Costo and Maria del Puerto Morales
Part I, Vol. 4 Nanobiotechnology, Inorganic Nanoparticles vs Organic Nanoparticles
edited by J.M. de la Fuente and V. Grazu, 2012 Elsevier Ltd, FRONTIERS OF
NANOSCIENCE, Series, Editor: R. E. Palmer, UK.
**Nanoparticle synthesis routes**

**NUCLEATION**

- Ions/Complexes
- Clusters
- Nuclei (8-10 Å)

**GROWTH**

- Diffusion growth
- Coagulation

**Stable nanosystems**

- Surfactant

**Homogeneous phase**

- Large crystalline particles
- Large polycrystalline particles or crystalline
Fe$^{3+}$ Fe$^{2+}$

Fe(III) salt

Fe(CO)$_5$

Precipitation

Pyrolysis

Size selection

Pyrolysis

Dispersion Coating

Furnace

Laser

Si-Alkoxide

Colloidal suspensions of magnetic nanoparticles
<table>
<thead>
<tr>
<th>Synthesis Method</th>
<th>Reaction Time</th>
<th>Solvent</th>
<th>Surface-Capping Agent</th>
<th>Sizes</th>
<th>Size Distribution</th>
<th>Shape Control</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coprecipitation</td>
<td>Minutes</td>
<td>Water</td>
<td>No</td>
<td>2–15</td>
<td>Broad</td>
<td>Not good</td>
<td>Medium</td>
</tr>
<tr>
<td>Thermal decomposition</td>
<td>Hours–days</td>
<td>Organic compound</td>
<td>Yes</td>
<td>4–30</td>
<td>Very narrow</td>
<td>Very good</td>
<td>Medium</td>
</tr>
<tr>
<td>Polyol process</td>
<td>Hours</td>
<td>Polyglycol</td>
<td>Yes</td>
<td>5–150</td>
<td>Narrow–broad</td>
<td>Good</td>
<td>Medium</td>
</tr>
<tr>
<td>Microemulsion</td>
<td>Hours</td>
<td>Organic compound</td>
<td>Yes</td>
<td>5–50</td>
<td>Narrow</td>
<td>Good</td>
<td>Low</td>
</tr>
<tr>
<td>Spray pyrolysis</td>
<td>Seconds</td>
<td>Water and volatile solvents</td>
<td>No</td>
<td>2–10</td>
<td>Broad</td>
<td>Not good</td>
<td>High</td>
</tr>
<tr>
<td>Laser pyrolysis</td>
<td>Milliseconds</td>
<td>Gases</td>
<td>No</td>
<td>2–10</td>
<td>Very narrow</td>
<td>Good</td>
<td>High</td>
</tr>
</tbody>
</table>

Synthesis by precipitation in water

Coprecipitation

Sal de Fe(II) y Fe(III)

- Concentration
- Temperature
- Atmosphere
- Stirring

Fe$_3$O$_4$
(Magnetite)

Synthesis by precipitation in water

\[ H = 100 \text{ Oe} \]
\[ f = 522.7 \text{ Hz} \]
\[ c = 50 \text{ mg/ml} \]

\[ \Delta T(\text{C}) \]

\[ \Delta t (\text{s}) \]

\[ \text{SAR vs concentracion} \]
\[ (H = 100 \text{ Oe}, f = 522.3 \text{ kHz}) \]
Synthesis by precipitation in water

- **APS coating**

  ![Graph showing Zeta potential (mV) vs pH for 13 nm and 13 nm_APS](image)

  - **Red circles** represent 13 nm, and **blue triangles** represent 13 nm_APS.

- **Synthesis by precipitation in water**

  ![Diagram of APS coating](image)

- **Intensity versus Size graph**

  - **Red line** for 6 nm and **blue line** for 13 nm.

  ![Graph showing Intensity (%) vs Size (nm) for 6 nm and 13 nm](image)

  - **6 nm**
    - D core = 6.3 nm (PDI = 0.2)
    - D hyd = 47.81 nm (PDI = 0.23)

  - **13 nm**
    - D core = 13.7 nm (PDI = 0.2)
    - D hyd = 106.3 nm (PDI = 0.17)
Synthesis by precipitation in water

Aqueous solution
Fe(II) sulfate

Sal de Fe(II)

Aqueous solution
Na(OH) + KNO₃

90°C ± 0.1
24 hours

Undisturbed system
Control de la oxidación

150 nm 95 nm 70 nm 30 nm

[Fe(II)] concentration decreases

[OH]_{exc} increases from 0.0002 M to 0.02 M

Particle size decreases from 300 nm to 30 nm

Instituto de Ciencia de Materiales de Madrid

Core/Shell Magnetite/Bismuth Oxide Nanocrystals with Tunable Size, Colloidal, and Magnetic Properties

Nanotechnology 2015

Hyperthermia + Dual imaging agent (NMR + CT)
High temperature decomposition of organic precursors

Atmosphere control

Temperature control (200-400°C)

Stirring

condenser

N₂
Surfactante

- El surfactante tiene la habilidad de controlar el crecimiento de la partícula

- Combinando diferentes surfactantes es posible controlar tamaño y forma de partícula.

\[
\text{Co}_2(\text{CO})_8 \quad \text{Oleic acid} + \text{TOPO}
\]

100 nm
High temperature decomposition of organic precursors

**Precursor**

- Fe(CO)$_5$
- Fe(acac)$_3$
- Fe(ole)$_3$

Fe(acac)$_3$ + a.oleico ⇄ (Fe-oleico)$_x$ + subproducts
High temperature decomposition of organic precursors

Instituto de Ciencia de Materiales de Madrid
High temperature decomposition of organic precursors

Fe/Surfactant ratio

Fe: tensioactivo 1:1
Fe: tensioactivo 1:2
Fe: tensioactivo 1:3
Fe: tensioactivo 1:5

Fe(acac)_3 + a. oleico $\leftrightarrow$ (Fe-oleico)_x + subproductos
High temperature decomposition of organic precursors

Size control

Iron oxide nanoparticles showing one nanometer increments in diameter


IEEE TRANSACTIONS ON MAGNETICS, 42, 3025 (2006)
Problems: low Ms at larger sizes


Other phases

wüstit-spinel core-shell structure

FeO $\gamma$-Fe$_2$O$_3$/Fe$_3$O$_4$


Structural imperfections

50 nm

Broad size-distribution

|Chem. Mater. 2011, 23, 4170–4180

High temperature decomposition of organic precursors
Hydrophilic Nanoparticles

The major challenge in the development of nanoparticles for biomedical applications is to make them hydrophilic, stable at physiological conditions and without significant aggregation.

Easy and reproducible experimental set up.

Functional groups for water stability.

Ready for further functionalization.
Hydrophilic Nanoparticles

- Encapsulation in polymers
- Ligand exchange modification


Surface modification
Laser Pyrolysis

The C$_2$H$_4$ absorbs the laser energy, Fe(CO)$_5$, is rapidly heated and decomposed resulting atomic Fe saturated vapour and leads to the nucleation and growth of iron metal nucleus.

To stabilise the powders, a mixture of air and ethylene can be introduced together with the iron pentacarbonyl (hard oxidation) or after the laser pyrolysis (soft oxidation).
Figure 4.35: Field Cooling hysteresis loops of the samples at 5, 15, 30 and 50K. The insets show the low field area where the loop shift and the coercivity increase can be clearly observed.
Microwave

![Microwave device image](image)

**a)**

![Intensity vs. Size plot](image)

**b)**

![Intensity vs. Size plot](image)

**c)**

![Images of materials labeled C*ESION100, C*ESION165, C*ESION180](image)

- C*ESION100
  - Size: 1.5 ± 0.9 nm
  - Scale: 25 nm
  - Image resolution: 10 nm

- C*ESION165
  - Size: 2.5 ± 1.0 nm
  - Scale: 20 nm
  - Image resolution: 10 nm

- C*ESION180
  - Size: 3.4 ± 1.3 nm
  - Scale: 20 nm
  - Image resolution: 10 nm
Polyol Mediated Process

Ethylene glycol = EG

DEG

TREG

TEG

✓ $\uparrow \varepsilon_r, \uparrow T_B$

✓ Hydrophilic coating

✓ Easily dispersed in aqueous media and other polar solvents

✓ $\uparrow T$ favors higher crystallinity and $\uparrow M_s$

✓ Narrow Size distribution

Other reagents present:
Iron precursor, precipitator, surfactant/stabilizer

NanoMag
H. Gavilán et al. (2017) “Formation mechanism of γ-Fe₂O₃ nanoflowers synthesized by polyol mediated process” (Submitted)
Phosphate and amine bond preferentially onto \{100\} and \{110\} facets through covalent bonding rather than on the \{001\} facet.

Silicate anions and citric acid (CA) induce anisotropy growth.

Anisotropic growth along a particular direction \(\Rightarrow\) choosing appropriate capping reagents (e.g. surfactant and additives)

Shape control

Induced anisotropy growth
• There is an urgent for development of adequate **testing protocols and metrology standards** to assess the quality and hazard of, and exposure to nanomaterials.

• **NanoMag project** addresses this task in the field of magnetic nanoparticles for medical applications.

• We have to **defined and standardized ways of analysing these nanostructures**.
  
  ✓ Define the **relevant measurands**
  ✓ Describe the available techniques: their **limits, uncertainty**
  ✓ Summarize existing standards and develop **new standards**
  ✓ Provide **reference materials**
• Thermal decomposition require:
  - Long reaction times and high temperatures
  - Control of the oxygen free atmosphere
  - Toxic reagents and byproducts
  - Particles are hydrophobic = need extra steps

• Hydrothermal/Solvothermal methods produce high quality nanoparticles with relatively lower reaction temperatures (<200ºC), relatively simple equipment and process. Polyol process allow using higher temperatures (~250ºC) and render hydrophilic nanoparticles.

Wrapping up

Toward the standardization of the synthesis of magnetic nanoparticles
- Homogeneous temperature distribution for large scale production
- Short reaction times
Magnetic nanoparticles could help to improve clinical practice in the treatment of cancer, most probably in synergy with other conventional treatments.

Imaging agents

Therapeutic agents

Magnetic nanoparticles advantages

Magnet assisted
CHARACTERISATION

Magnetic properties
Jana Vejpravova (A. of Science)
Daniel Niznansky (Charles U.)
Patricia de la Presa (IMA)

Heating efficiency
Dr. Gorka Salas (IMDEA Nanoscience)
Dr. Francisco Terán (IMDEA Nanoscience)
Prof. Marc Respaud (INSA, Toulouse)
Prof. Ingrid Hilger (UHJ, Jena)

NMR imaging
Dr. Fernando Herranz (CNIC)
Prof. Jesús Ruiz Cabello (CNIC)

BIOMEDICAL
Cell therapy
Dr. Angeles Villanueva (UAM)
Dr. Domingo Barber (CNB)

MODELLING
Dr. Oksana Chubykalo-Fesenko (ICMM)
REFERENCES


• Scientific and industrial challenges of developing nanoparticle-based theranostics and multiple-modality contrast agents for clinical application, Yì Xiáng J. Wáng, Jean-Marc Idée and Claire Corot, Nanoscale, 2015, 7, 16146.


• Magnetite nanoparticles for cancer diagnosis, treatment, and treatment monitoring: recent advances, Materials Today Volume 19, Number 3 April 2016

• Free course on magnetic nanoparticles for biomedical applications, http://www.npl.co.uk/commercial-services/products-and-services/training/e-learning/magnetic-nanoparticles-standardisation-and-biomedical-applications/