Radiolabeled Nanoparticles and Recent Approaches

Serap Teksöz, Çiğdem Îçhedef, Eser Uçar, Kadir Arı
Ege University
Institute of Nuclear Sciences
Izmir-TURKEY
Nanoparticles have more surface area.

This makes them more reactive since chemical reactions happen on the surface.

• More reactive means potentially more useful.
Nanotechnology is a multidisciplinary subject as it incorporates many different areas of science and industry.
The radiolabeled nanoparticles are useful for:

- diagnosis,
- therapy,
- control of biological systems.
In NP-radiolabeling there are several ways depending on:

- the radionuclide that is used and
- the NP type.
## Radiolabeled Nanoparticles used in SPECT-CT

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<th>Nanoparticles</th>
<th>Radionuclides</th>
<th>Applications</th>
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<td>Liposomes</td>
<td>Tc-99m, In-111, Ga-67</td>
<td>Tumor diagnosis, Infection, Inflammation, Lymphoscintigraphy</td>
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<td>Liposomes</td>
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<td>Polimers</td>
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<td>Iron oxide</td>
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<td>Perflurocarbon</td>
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<td>Immunoliposomes</td>
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<td>Carbon nanotubes</td>
<td>In-111</td>
<td>In-DOTA-CNT-Rituximab</td>
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The advantages of using nanoparticles as a drug delivery system include the following:

- The size and surface characteristics of nanoparticles can be easily manipulated. This could be used for both passive and active drug targeting.
- Nanoparticles can be made to control and sustain release of the drug during the transportation as well as the location of the release.
- When an appropriate matrix is chosen:
  - efficacy increases and
  - reduced side effects.
- Targeted drugs may be developed.
- Various routes of administration including oral, nasal, injection, intra-ocular (within the eyes) etc. can be used.
Emerging role of radiolabeled nanoparticles as an effective diagnostic technique, EJNMMI Research 2012, 2:39.
Lipid-based nanoparticles
Liposomes

- Liposomes are small vesicles consisting of one or more concentric lipid bilayers enclosing discrete aqueous spaces.

- Phospholipids and cholesterol are main components of liposomes.

- Various approaches have been developed to radiolabel liposomes with gamma-emitting, PET and SPECT radionuclides, such as $^{18}F$, $^{64}Cu$ $^{67}Ga$, $^{111}In$, and $^{99m}Tc$. 
Advantages

- Carrier systems consist of phospholipids.
- They can carry substances dissolve both in water and lipid.
- They show therapeutic effects in low doses.
- They can decrease or even eliminate side and toxic effects.
- They can be used for targeting and controlled release purposes.
<table>
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<td>Doxorubicin (PEG liposomes)</td>
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<td>DepoCyt</td>
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<td>Depo-Dur</td>
<td>Morphine Sulfate XR Liposome</td>
<td>Pain therapy</td>
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<td>Thermodox</td>
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<td>Liver Cancer</td>
<td>Phase III</td>
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<td>MM-398</td>
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<td>LEP-ETU</td>
<td>Paclitaxel</td>
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<td>Aroplatin</td>
<td>Oxaliplatin</td>
<td>Colorectal Cancer</td>
<td>Phase II</td>
</tr>
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<td>SPI-077</td>
<td>Cisplatin (PEG-Liposomal)</td>
<td>Platinum-sensitive ovarian cancer</td>
<td>Phase II</td>
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Solid lipid nanoparticles (SLNs) are composed of physiological lipids (i.e., fatty acids; mono, di, and triglycerides; phospholipids; etc.),

- SLN core can be modified to incorporate either hydrophobic or hydrophilic carriers.
Metal based particles
Magnetic nanoparticles (5-20 nm)
Iron oxides (Fe$_2$O$_3$, Fe$_3$O$_4$), doped iron oxides (CoFe$_2$O$_4$, MnFe$_2$O$_4$) or metal alloys (FePt)

Quantum dots (2-10 nm)
Semiconductor nanocrystals composed of a core (CdSe, CdTe) embedded in a layer of shell (i.e. ZnS).
Gold nanoparticles (<50 nm)
A kind of metal nanoparticles with a wide variety of geometries (spheres, rods, hollow spheres, cages..)

Silica nanoparticles (<100 nm)
A way to access mesoporous materials (drug encapsulation), increasingly used for encapsulating other types of nanoparticles (core-shell)
Radiolabeled Nanoparticle Approach

Selection of the appropriate radionuclide depends on radiolabeling technique and NP structure.

These techniques can be;

- Direct
- Chelator mediated
- Encapsulation

They depend on the time of NP synthesis.
Approaches for radiolabeling NPs with $^{99m}$Tc:

(i) direct labeling;
(ii) labeling of chelator-functionalized NPs (chelator: HYNIC)
(iii) encapsulation of lipophilic radiocompounds ($^{99m}$Tc–HMPAO)
The following slides represent our research group studies of radiolabeled nanoparticles

- Assoc. Prof Dr. Serap Teksöz
- Prof. Dr. Perihan Ünak
- PhD Çiğdem İçhedef
- Assoc. Prof. Dr. E. İlker Medine
- PhD Student Eser Uçar
- Msc. Student Kadir Arı
- Msc. Seniha Özyüncü
- Msc. Onur Büyükok
The magnetic nanoparticles were prepared by a co–precipitation method using ferric chloride as starting material.

Surface modification with TEOS
- to prevent aggregation
- readily modified with other functional groups

TEOS coated magnetic nanoparticles were modified with an amino silane coupling agent (AEAPS).
- to be able to give reactions with biological ligands

\[
6\text{Fe}^{3+} + 3\text{SO}_3^{2-} + 18\text{H}_2\text{O} \rightarrow 2\text{Fe}_3\text{O}_4 + 3\text{SO}_4^{2-} + 18\text{NH}_4^+ + 9\text{H}_2\text{O}
\]
Characterization of MNPs

**SEM (Scanning electron microscopy)**

**TEM (Transmission Electron Microscopy)**

**XRD (X-Ray Diffraction)**

The diffraction peaks of the MNPs, (220), (311), (400), (422), (511), and (440) were matched to reference magnetic crystal peaks, which all agree with the literature data.

It is obvious that the aminosilane-coated MNPs exhibit spherical morphology and the particle size is distributed uniformly in the range of 40–60 nm.

Figure show that the silica coated nanoparticles have good uniformity in spherical shape. The particle diameter shown in the TEM image is approximately agreed with the SEM observation.

4th International Nuclear Chemistry 2014-Brazil
Coupling of Guanine to MNPs

Glutaraldehyde linked MNPs

Guanine coupled MNPs

4th International Nuclear Chemistry 2014-Brazil
Radiolabeling of Guanine coupling MNPs

A 60 MBq/200 μL of $^{99m}$Tc(H$_2$O)$_3$(CO)$_3]^+$ solution was added to 100 μL of guanine coupled MNPs in a glass vial.

Incubated at 80°C for 45 min

Radiolabeling yield was determined to be 72 ± 4%.
Cell Culture Study

In this work;

- Human lung adenocarcinoma epithelial (A-549),
- Normal Human Bronchial Epithelial (NHBE)
- Human breast adenocarcinoma (MCF-7),
- Human epithelial colorectal adenocarcinoma cells (Caco-2),

Cell lines were obtained from ATCC (American Type Culture Collection)
• We performed parameter studies on Lung cancer cell line. These parameters are time, specific activity, concentration.

• Guanine and Tc-99m have no significant incorporation. However when they conjugated to magnetic particles, their cellular incorporation significantly increased. Also under magnetic field cellular incorporation gradually increased. The results show that the optimum cellular accumulation conditions were at 30 min, 2 microcuri activity and 3 microgram per 500 micro liter ligand concentration.

All radiolabeled complexes have higher cellular accumulation in cancer cell line than normal cell line.
% cellular accumulation vs time graphs for Caco-2 and MCF-7 cell lines

Caco-2

- [99mTc(CO)3(H2O)3]+
- 99mTc(CO)3-Gua
- 99mTc(CO)3-MNP-Gua
- 99mTc(CO)3-MNP-Gua (Applying magnetic field)

MCF-7

- [99mTc(CO)3(H2O)3]+
- 99mTc(CO)3-Gua
- 99mTc(CO)3-MNP-Gua
- 99mTc(CO)3-MNP-Gua (Applying magnetic field)
Scintigraphy studies on Rabbits

Three New Zealand Albino Rabbits were used for scintigraphic imaging.

Static images were performed within 24 hours. 500,000 counts static images (at 3rd, 30th, 60th, 120th minutes and 24 hours) were obtained on supine position.

30 Min scintigrames

24 h scintigrames
In this study, it is aimed to obtain a new diagnostic agent for targeted delivery.

➤ Guanine coupled MNPs were synthesized and radiolabeled using $[^{99m}\text{Tc}(\text{CO})_3]^+$. 

➤ While guanine have no significant incorporation for all cell types, it conjugated to magnetic particles, their cellular incorporation significantly increased. The radiolabeled MNPs accumulate especially in lung and liver, and show increasing uptake in the targeted region by applying external magnetic field. 

➤ The radiolabeled MNPs with purine base of DNA could be expected to contribute in the future development of new diagnostic and therapeutic agents.
Preparation and characterization of radiolabeled magnetic nanoparticles as an imaging agent

Çiğdem İşçedef · Serap Teksöz · Perihan Ünak · Emin İ. Medine · Türkan Ertay · Recep Bekiş

Preparation and bioevaluation of $^{99m}$Tc–carbonyl complex of guanine

Çiğdem İşçedef · Serap Teksöz · Kamile Şenocak · Eser Uçar · Ayfer Yurt Kılıçar

Bioevaluation of $^{99m}$Tc(CO)$_3$–Guanine in vitro and in vivo

Çiğdem İşçedef · Serap Teksöz · Perihan Ünak · Kamile Şenocak · Emin İ. Medine · Türkan Ertay · Recep Bekiş

4th International Nuclear Chemistry 2014-Brazil
The aim of this study is to synthesize D-Penicillamine (D-PA) magnetic nanocarriers for targeted purposes. Penicillamine is a powerful chelating agent and is used largely in medicine such as rheumatoid arthritis and Wilson’s disease for the removal of copper, in heavy metal poisoning. Magnetic nanoparticles were prepared by partial reduction method and surface modification was done with an amino silane coupling agent’s (structural properties), AEAPS.

This study was supported by Ege University Research project numbered 2008NBE009
Particle size and morphology of samples were determined by Scanning Electronic Microscopy (SEM, Phillips XL-30 S FEG). The magnetic properties were analyzed by Vibrating Sample Magnetometer (VSM).

Particle size of MNP’s was found in a range of 40-60 nm

The saturation of silica coated MNPs was found to be equal to 26.45 emu/g, while that of the amino-silane coated MNPs was 23.73 emu/g.
A 11 MBq/ 200 µL of $^{99m}$Tc(H2O)3(CO)3]+ solution was added to 50 µL of Penicillamine coupled MNPs in a glass vial

Incubated at 70°C for 30 min

Radiolabeling yield was determined to be 97.05%.
In Vitro Cell Uptake study

As for in vitro study,

- MCF7 cell line (human, Caucasian, breast, adenocarcinoma) was obtained from ATCC (American Type Culture Collection)
- Three different time interval were examined for incorporation (15, 60, 120 min)

- Radiolabeled magnetic nanoparticles showed more incorporation on MCF7 cells
- When external magnetic field was applied to the radiolabeled magnetic nanoparticles high incorporation was observed.
To Investigate The Effect of Aminoacid modification on MNP synthesis
Multistep polymer or silica coating can cause:
- an increase in the particle's size
- reduce its magnetic saturation value.

Aminoacids are suitable because they play a very important role in the body and also biocompatible.

The aminoacid coating not only stabilize the nanoparticle in solvents, they also provide potential reaction sites for further modification.
Synthesize of Alanine coated MNPs

Molar ratio of $2\text{Fe(III)}:1\text{Fe(II)}:8$ Alanine were stirred at $40^\circ\text{C}$ for 30 minutes

Concentrated $\text{NH}_4\text{OH}$ was added drop by drop

Mixture was stirred at $80^\circ\text{C}$ for 2 hours under Ar atmosphere

Particles were washed with water several times
Characterization of Alanine coated MNPs were done by XRD and SEM.

- The XRD pattern indicates the product mostly consists of magnetite, Fe₃O₄.

- Particles size was found as 30-40 nm by SEM.
Radiolabelling of Alanine-MNPs with $^{99m}$Tc(CO)$_3$

A 37 MBq/100 μL of $^{99m}$Tc($H_2O$)$_3$(CO)$_3$ solution was added to 100 μL of alanine coated MNPs in a glass vial.

Incubated at 80°C for 30 min.

Radiochemical purity
$^{99m}$Tc(CO)$_3$-M-Alanine
% 97.68 ± 1.06
Biodistribution studies on Rats

- After radiolabeling biodistribution study was performed on rats.
- Three time interval was selected such as 30, 90, 180 minutes.
- Intravenous injections were done.
- After sacrifice all major organs were removed weight and counted by Cd(Te) detector.
Biodistribution studies on Rats

- Radiolabeled magnetic nanoparticles, $^{99m}$Tc(CO)$_3$-M-Alanine, mainly accumulated in the liver, kidney, lungs and bladder.

- Relatively low uptakes were noted in the thyroid and stomach, supported that there is no free Tc-99m.

- The clearance was occurred by the reticuloendothelial system (RES).

- All other tissues also showed a minimal uptake.
Formulation of Radiolabeled Paclitaxel Encapsulated Solid Lipid Nanoparticles and Determination of Radiopharmaceutical Potential

Folic acid derivative was synthesized at Instituto Tecnologico e Nuclear Lisbon / Portugal as described by Xiang G. et al.

Radiolabeling studies are being performed at present.

Preparation and characterization of lipid nanoparticles will be studied.

Financial support provided by TUBITAK Research Project numbered as 113S369.
Our another ongoing study is related on lipid particles

Temozolomide is being used.

- It is an alkylating agent for the treatment of brain tumor and melonoma.
- Active substance Temozolomide was separated from Temodal.
- Temozolomide encapsulated SLN’s were synthesized by using temperature sonification method.
Particle size of TMZ-SLN were analyzed by SEM. In addition size distribution analyzes were determined using Dynamic Light Scattering.

The partial size distributed in the range of 100-200 nm. Average particle size was found as 121 nm according to DLS.
Further studies;

- Temozolomide encapsulated Solid Lipid Nanoparticles will be radiolabeled with $^{99m}\text{Tc}(\text{CO})_3^+$
- Drug content of Solid Lipid Nanoparticles will be determined.
- In vitro studies will be established.
IN CONCLUSION

- Radiolabeled nanoparticles have the advantage;
- to deliver high concentration of radioactivity to the target area, without damaging normal surrounding tissue as well.

- These new complexes will find a wide spread field in creation of materials besides medicine in near future.

Acknowledgement
Financial support provided by TUBITAK Research Project numbered as 113S369.
THANK YOU...