SYNTHESIZATION, CHARACTERIZATION OF FOLIC ACID AND QUERCETIN FUNCTIONALIZED MAGNETIC NANOPARTICLES
RESEARCH ON BREAST CANCER CELL LINE FOR HYPERTHERMIA AND TARGETED DRUG DELIVERY APPLICATIONS

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Content

• A. Brief information about SPIONs
  – Superparamagnetic iron oxide nanoparticle (SPION) in Medicine
  – Modification of SPIONs
  – Internalisation of SPIONs into the cells
  – SPION and cancer therapy
• B. Our project
  – Synthesis and characterization of SPION@APTES@FA-PEG@CQ
  – Biological applications of SPION@APTES@FA-PEG@CQ on MCF-7 and L929 cell line.
    • Cytotoxicity assays
    • Apoptotic and necrotic assays
  – Conclusion
**SPIONs**

- **Superparamagnetic iron oxide nanoparticles (SPION)**
  - a superparamagnetic iron core
  - small synthetic $\gamma$-Fe$_2$O$_3$ (maghemite),
  - $\text{Fe}_3\text{O}_4$ (magnetite) particles
  - a core ranging from 10 nm to 100 nm in diameter.

- **Physicochemical properties of SPIONs affect**
  - Distribution
  - Toxicity
  - Residual time in blood
  - Magnetic properties
  - Stability
  - Internalization into the target cell

Medical Applications of SPIONs

- Modified SPIONs are very useful tools for numerous applications such as
  - magnetic resonance imaging (MRI)
  - cancer treatment
  - magnetic fluid hyperthermia
  - targeted drug delivery
  - tissue repair and catalysis
  - magnetic separation technologies

Drug Delivery

Varhosaz J. And Farzan M. Nanoparticles for targeted delivery of therapeutics and small interfering RNAs in hepatocellular carcinoma, W. J Gastr. 2015, 14; 21, 12022-12041
SPIONs in Cancer Therapy

Targeting agent
Gene therapy agent
Therapeutic protein
Chemotherapy drug
Magnetic hyperthermia

SPION core
Biocompatible coating
Table 1: Approved nanoparticles for onco-clinical trials

<table>
<thead>
<tr>
<th>Year initiated</th>
<th>Description of nanoparticles [NPs]</th>
<th>Cancer targeted by the NPs</th>
<th>Study phase</th>
<th>Sponsor</th>
<th>Clinicaltrials.gov identifier no./others</th>
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<tbody>
<tr>
<td>2014</td>
<td>Paclitaxel polymeric micelles for injectable suspension (other)</td>
<td>Breast cancer</td>
<td>Not provided</td>
<td>Sorrento Therapeutics, Inc.</td>
<td>NCT02064829</td>
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Sentinel Lymph Node Biopsy With Superparamagnetic Iron Oxide for Breast Cancer Patients After Neoadjuvant Treatment.

**Status:** Recruiting

**Study Phase:** Phase 3

**Start Date:** April 2014  |  **Completion Date:** June 2016

**Condition(s):** Breast Cancer

2006  
TNF-α bound to colloidal gold nanoparticles
endometrial, solid tumors
1
Switzerland
National Institutes of Health Clinical Center (CC)
NCT00356980

2013
Platinum-based albumin-bound paclitaxel (nab-paclitaxel)
Lung cancer
2
Zhejiang University
NCT02016209

2012
Docetaxel-loaded polymeric nanoparticle
Prostate cancer
2
BIND Therapeutics
NCT01812746

2011
Genexol PM
Solid tumors
2
Asan Medical Center
NCT01425126

2014
Superparamagnetic iron oxide (SENTINAC®)
Bladder and Ureter
3
Hospital Universitari Vall d’Hebron Research Institute
NCT02249208

2012
Paclitaxel polymeric micelles
Breast cancer
3
Nippon Kayaku Co., Ltd
NCT01644890
Hyperthermia

Modification of SPION

• The aim of modification of SPIONs with biocompatible polymers are:
  – Solubility
  – Targetting
  – Drug delivery
  – Imaging
  – Internalisation into the cell

• The main objective today is optimization of the properties of these magnetic particles to:
  – provide an increase in magnetic nanoparticle concentration in blood vessels;
  – reduce early clearance from the body;
  – minimize nonspecific cell interactions, thus minimizing side effects; and
  – increase their internalization efficiency within target cells, thus reducing the total dose required
Surface Modifications for Targeting

Muller C. and Schibli R., Folic Acid Conjugates for Nuclear Imaging of Folate Receptor–Positive Cancer, J Nucl Med, 2011 vol. 52 no. 1 1-4
Castro E. and Mano JF. Magnetic Force-Based Tissue Engineering and Regenerative Medicine, J. Biomedical Nanotechnology, Volume 9, Number 7, 2013, pp. 1129-1136(8)
Role of Folate (Vitamin B9)

Wilson PM., Danenberg PV., Johnston PG. Et all. Standing the test of time: targeting thymidylate biosynthesis in cancer therapy
NATURE R. CLINICAL ONC, 2014, 11, 282–298
In this study

• We aimed to synthesize SPION conjugated with folate and quercetin and to determine its biological activities on breast cancer cell.
KEY Elements of our project

• Folate (Folic acid) is B9 vitamin which is essential for nucleotide and DNA synthesis
  – Cells can be classified as folate receptor positive (FR+) and folate receptor negative (FR-).
  – FR+ cells express FR
  – FR- cells have less FR on surface of the cell membrane
  – Folat conjugated drugs affect FR+ cells more than FR- cells

• Quercetin, a kind of flavonoid is produced by plants.

• MCF-7 (Human Breast Cancer Cell Line, FR+) and L929 (Mouse fibroblast as a standard cell, FR-) were used in this study.
Scheme: Schematic illustration for the suggested pattern approach of SPION@APTES@FA-PEG@CQ and its anticancer activity on MCF-7 and L929 cells.

Akal Z.U. Alpsoy L., Baykal A., "Biomedical applications of SPION@APTES@PEG-Folic Acid@Carboxylated Quercetin nanodrug on various cancer cells", Applied Surface Science, Vol. accepted

Figure 1. FT-IR analysis of
i) SPION,
ii) SPION@APTES,
iii) COOH-PEG-NH₂,
iv) FA-PEG,
v) SPION@APTES @FA-PEG

Figure 2. FT-IR analysis of
i) Choloroacetic acid,
ii) Carboxylated Quercetin, (CQ),
iii) Quercetin
iv) SPION@APTES@FA -PEG@CQ
Figure 3 and 4. TEM micrograph and particle size distribution histogram of SPION@APTES@ FA- PEG@CQ nanocomposite

Figure 5. Room temperature M-H curve of SPION@APTES@ FA- PEG@CQ nanocomposite

Figure 6. TG curves of (a) SPION@APTES@PEG-FA and (b) SPION@APTES@FA- PEG@CQ

Figure 7. XRD powder pattern and line profile fitting of SPION@APTES@ FA- PEG@CQ
Figure 8. Quercetin release from SPION@APTES@ FA-PEG@CQ at pH 4.4 and 7.4

- The release of the drug at pH 7.4 is slower compared to pH 4.4

Figure 9. Prussian blue staining micrographs of L929 and MCF-7 cells treated with SPION@APTES@ FA- PEG@CQ at concentration 100 µg/ml.

- Prussian blue staining images showed that there was a dramatically increase of intracellular iron, as visualized by blue granules in Prussian blue staining of MCF-7
They do not have any toxic effect on cell lines in the concentration range of 0-200μg/ml significantly.

**Figure 10:** Cytotoxicity of (A) SPION@APTES@FA-PEG nanoparticles at different concentrations on cell lines for 48h (*p<0.05 compared to untreated group)

All concentrations of SPION@APTES@FA-PEG@CQ influenced cell viability in folic acid positive cells (U87) more than folic acid negative cells (L929).

- Akal Z.U. Alpsoy L., Baykal A., "Biomedical applications of SPION@APTES@PEG-Folic Acid@Carboxylated Quercetin nanodrug on various cancer cells", Applied Surface Science, Vol. accepted.
Figure 12. Effect of SPION@APTES@FA-PEG@CQ on cell index of L929 cells, measured by RTCA

Figure 13. Effect of SPION@APTES@FA-PEG@CQ on cell index of MCF-7 cells, measured by RTCA

SPION@APTES@FA-PEG@CQ inhibited cell number of MCF-7 more than L929

- Akal Z.U. Alpsoy L., Baykal A., "Biomedical applications of SPION@APTES@PEG-Folic Acid@Carboxylated Quercetin nanodrug on various cancer cells", Applied Surface Science, Vol. accepted
Figure 14. Annexin-V Cy5 results exposed SPION@ APTES@ FA-PEG@CQ to L929 and MCF-7 cells.

Cell viability decreased and necrosis and apoptosis increased in MCF-7 cells.
Figure 15. TUNEL results exposed SPION@APTES@ FA- PEG@CQ to L929 and MCF-7 cells

SPION@APTES@ FA-PEG@CQ caused apoptotic effect on MCF-7
Figure 16. Caspase 3/7 activity of SPION@APTES@ FA-PEG@CQ on L929 and MCF-7 cells

Caspase 3/7 activity was higher in MCF-7 than L929
In vitro Magnetic Fluid Hyperthermia Application

• We will apply hyperthermia on cancer cell lines by using BNC
• Hyperthermia studies are going on still.
Conclusion

• In summary, we have synthesized MNC as a multifunctional bionanosystem for drug delivery and cancer treatment.
• The monodisperse MNC size about 13 nm have successfully synthesized.
• The result showed that the SPION@APTES@FA-PEG have lower toxicity both MCF-7 and L929 cell lines. However, SPION@APTES@FA-PEG@CQ have higher cytotoxic effects on MCF-7 cell line.
• The xCELLigence, MTT and Prussian blue analysis showed significant association of SPION@APTES@ FA-PEG@CQ with the FR-positive MCF-7 cells but not with the FR-negative L929 cells.
• Cy3-labeled annexin, Caspase 3/7, TUNEL apoptosis assays showed occurrence of profound apoptosis selectively within the FR-positive MCF-7 cells treated with SPION@APTES@ FA-PEG@CQ but not within the L929 cells.
What do we study?

- We synthesize SPIONs and modify them by coating with biomaterials including flavonoids, and other bio-active components.
- We study on their inhibition activities on cancer and healthy cell lines.
- Also toxic effects of Nanoparticles on the cells are studied.

- We are ready for collaboration in these issues as a research group.
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