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Synthesis and Characterization of Tumor Targeted Peptide (TTP) Conjugated with Magnetic Nanoparticles for Thermo-ablation

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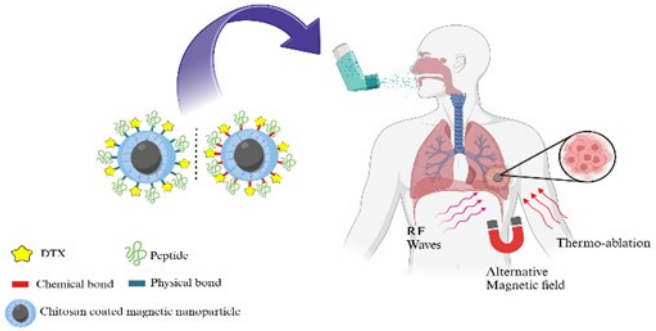
Introduction :

Nanotechnology is transforming various industries by enabling the manipulation of materials at the nanometer scale, leading to breakthroughs in drug delivery, water purification, data management, and the creation of advanced nanoscale materials for healthcare and industrial applications. By leveraging the unique properties of nanometric materials—such as their small size, large surface area, and customizable surfaces—scientists are developing innovative solutions with significant potential, particularly in targeted drug delivery [1-3].

Magnetic nanoparticles (MNPs), especially superparamagnetic iron oxide nanoparticles (SPIONs), have emerged as key players in biomedical research due to their promising applications in cancer treatment and other medical fields. These nanoparticles can be precisely engineered to enhance the delivery of therapeutic agents directly to cancer cells, reducing side effects and improving treatment efficacy.

Peptides are an attractive alternative to antibody-based targeted therapies. Unlike antibodies, peptides are easy to synthesize in large quantities, and their smaller size improves tissue penetration while preventing nonspecific uptake by the reticuloendothelial system. In addition, peptides can be chemically modified to alter affinity, charge, hydrophobicity, stability, and solubility, and can be optimized for in vivo use through modification. Importantly, peptides can exhibit receptor affinity similar to antibodies. Tumor-targeting peptides (TTPs) are an effective alternative for selectively targeting specific tumor receptors. Compared to antibodies, TTPs have better tumor/tissue penetration and are easy to synthesize and chemically modify to improve stability and pharmacokinetics. Peptides can be used for targeted drug delivery. They can be designed to have the ability to destroy cancer cells and inhibit tumor growth. Examples of TPPs are [4]: RGD-4C, iRGD, NGR, which act on: B16 melanoma, MDA-MB-435, HL-60, MMTV-PyMT, KRIB, C8161, K14-HPV16/E2 RIP1-Tag2, K14-HPV16.

This project focuses on synthesizing and characterizing novel hybrid nanomaterials (MNP-CPP-TM-drug) that combine MNPs with active anti-cancer compounds (EGF fragments connected with cytostatic). The goal is to develop a multifunctional nanostructure capable of not only delivering drugs with high specificity but also enabling targeted thermo-ablation for enhanced cancer.



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Methodology :

In the first stage of the research, magnetic nanoparticles (size: 14-29 nm, 99.5%) were coated with chitosan (low molecular weight) physically and chemically modified by different cross-linkers. Chitosan coating is biodegradable and helps attach other elements, such as anticancer drugs and targeting peptides by cross-linker. Targeting molecules were EGF fragments capable of interacting with the EGF receptor (EGFR). Docetaxel was used as an anticancer drug in this research.

EGF fragments in this research were prepared by solid-phase peptide synthesis (SPPS) with Fmoc/tBut strategy using triazine coupling reagent (4-(4,6-dimethoxy-1,3,5-triazin-2-yl) -4-methylmorpholinium toluene-4-sulfonate (DMT/NMM/TosO-))[5]. All EGF fragments were characterized by a high ability to interact with EGFR (microscale thermophoresis (MST)) [6].

Results and discussions :

Nanoparticles were characterized with SEM, DLS, FTIR, and TGA. Additionally, the peptides were characterized by LC-MS.

Table 1. LC-MS analysis of EGF fragments.

peptide	Sequence	Molecular wight	M/Z	HPLC Purity%
EGF fragment	^{10}HDGYCL^{15}	706.263	$[M+H]^{1+}$=707.2867 $[M+2H]^{2+}$=354.1477	96%
EGF fragment	^{43}QYRDL^{47}	693.334	$[M+H]^{1+}$=694.3590 $[M+2H]^{2+}$=347.6831	90%

Comparing coated magnetic nanoparticles with different cross-linkers: Dynamic light scattering (DLS) measurement was used to compare the sizes of MNPs (table 2), and simple Acid-base titration was used to evaluate the number of primary amine groups on the surface of MNPs (table 3).

Table 2. DLS measurement of MNPs (pH=7).

No.	MNPs	Size (nm)
1	Fe_3O_4@CS(300CPS)/TPP	70-71
2	Fe_3O_4@CS(30CPS)/TPP	55
3	Fe_3O_4@CS/Citric acid	90-130
4	Fe_3O_4@CS/Squaric acid	105
5	Fe_3O_4@CS/glutaraldehyde	253-263

Table 3. Result from Acid-base titration method.

sample	Cross-linker	$V_{ave./mL}$	NH_2 group (mol /mg)
1	TPP	1.73	0.293
2	Citric Acid	1.96	0.383
3	Squaric acid	1.85	0.272
4	Glutaraldehyde	1.63	0.065

Magnetic nanoparticles were also characterized by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FT-IR) (Figure 1).

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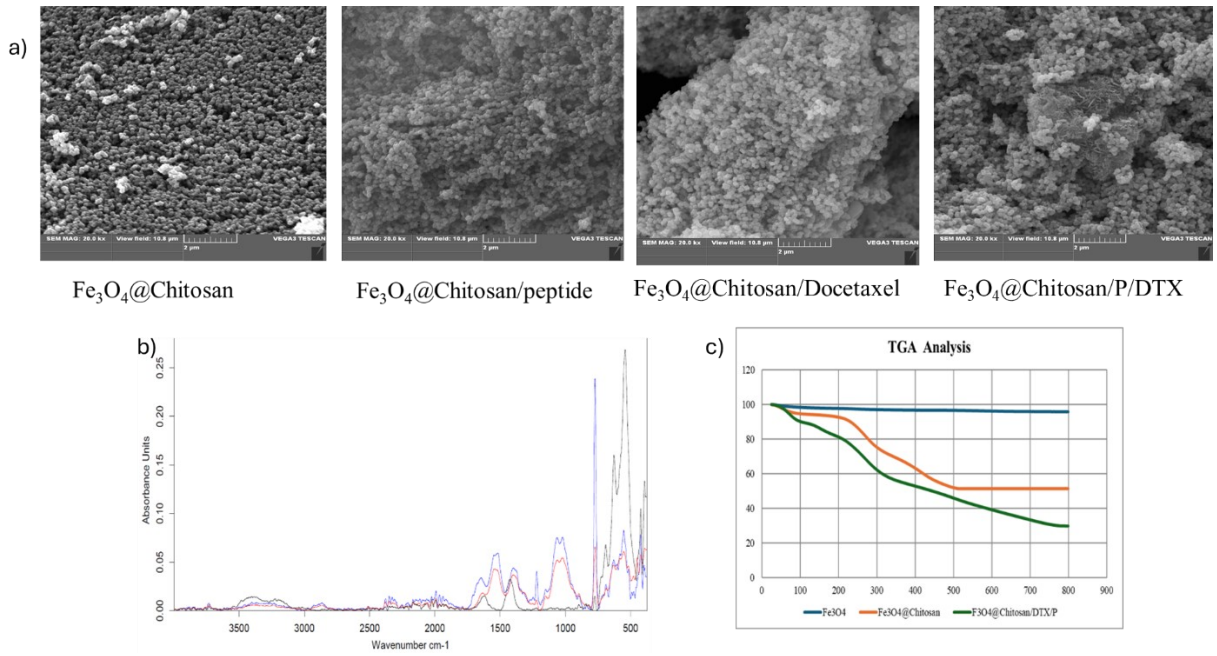

Figure 1. a) SEM images of chitosan-coated magnetic nanoparticles, b) FT-IR spectra of 1)Fe3O4, 2)Fe3O4@Chitosan, 3)Fe3O4@Chitosan/DTX/P, and c) TGA spectra of synthesized nanoparticles

Conclusion :

In this research, an effective method for coating magnetic nanoparticles with chitosan was developed. Tripolyphosphate (TPP) was chosen as the optimal cross-linker for synthesizing these coated MNPs. In the second step, an anticancer drug (DTX) and a peptide derived from EGF fragments were attached to the MNPs both physically and chemically. The thermo-ablation properties of these nanoparticles were also studied, and they are currently in their preliminary phase of research.

The nanoparticles were characterized using SEM, DLS, FTIR, and TGA. Additionally, peptides were characterized by LC-MS. The biological activity of the tumor-targeting peptide conjugated with magnetic nanoparticles and the cytostatic agent was confirmed.

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