Antifolates-Modified Iron Oxide Nanoparticles for Targeting Cancer Cells

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Iron oxide nanoparticles have been used, in the last years, as a suitable platform for contrast enhancement in magnetic resonance imaging and as a drug carrier. The identification of specific agents or drugs that can be effectively release from the nanoparticles inside the target cells is nowadays a strategy in development[1].

Folic acid (FA) is an effective tumor targeting to conjugate with nanoparticles due to folate receptors which are overexpressed on the cell membranes of many cancer cells[2]. It also has been described the use of antifolates joined to the surface of nanoparticles[3]. Antifolates are antimetabolites structurally similar to FA used in cancer chemotherapy. The best known is Methotrexate (MTX), but there are others like Raltitrexed (RTX) and Pemetrexed (PMX) whose effects on these kinds of cells have not been reported yet.

In this work we report the synthesis of iron oxide nanoparticles functionalized with RTX and PMX using as a linker 3-Aminopropyltrietoxysilane (APTES). As shown in figure 1, the nanoparticles, in a first synthetic step, were modified with APTES following with the conjugation of RTX or PMX respectively, through one of the carboxylic acids, establishing an amide bond. These new nanoparticles have been characterized by TEM, FTIR and MALDI TOF/TOF.

The potential biological applications of the free drugs (RTX, PMX) and the functionalized nanoparticles (NP-APTES-RTX, NP-APTES-PMX) were evaluated via MTT assay using lung carcinoma cells (A549), which are known to be folate-expressing cancer cells. Cells were incubated with different concentrations of the drugs and the respective nanoparticles at 37^{o}C from 24 to 96 hours. The results are presented in figures 2 and 3. Concentrations between 25 nM to 1 μM of the free drugs were used. There is a high in vitro differential citotoxicity from free RTX to PMX. A concentration of 50 nM of free RTX produce after 96 h about a 70% reduction in cell viability, however, a similar cytotoxicity from free PMX is only achieved when using a concentration of 1 μM after the same time. Analogous results were obtained after the incubation of NP-APTES-RTX and NP-APTES-PMX with cancer cells. Concentrations between 0.001 to 0.01 mg Fe/mL were examined. The highest concentration of NP-APTES-PMX used only reduces cell viability in a 10% though the same concentration of NP-APTES-RTX reduces it about a 90%.

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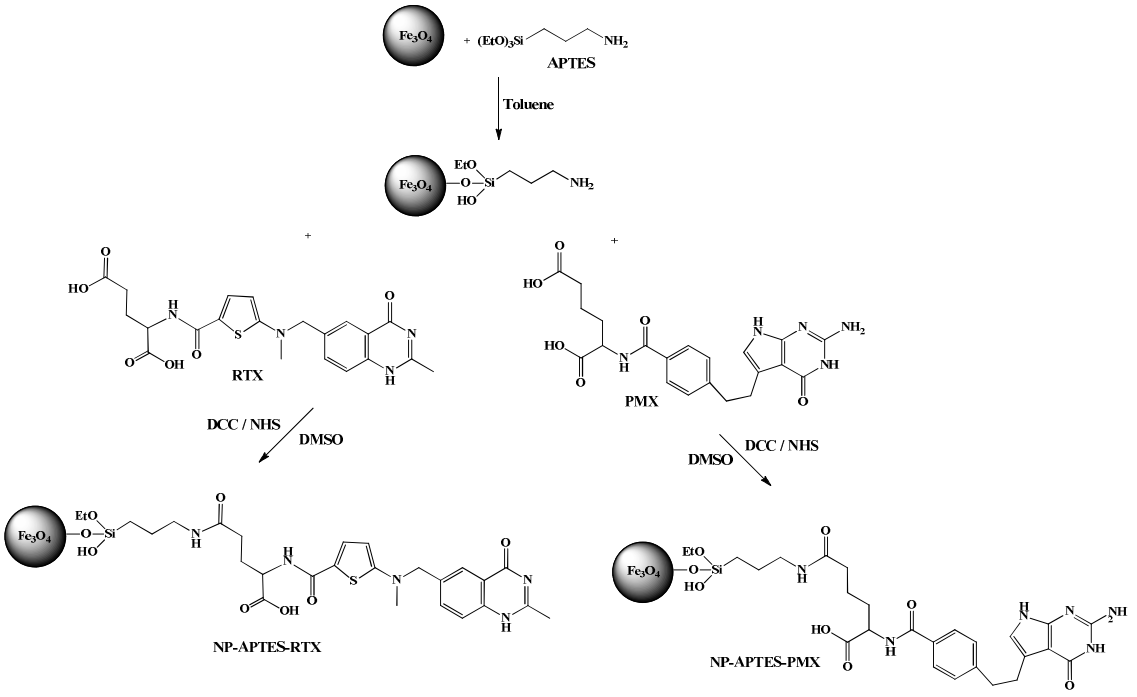

Figure 1. Synthesis of NP-APTES-RTX and NP-APTES-PMX.

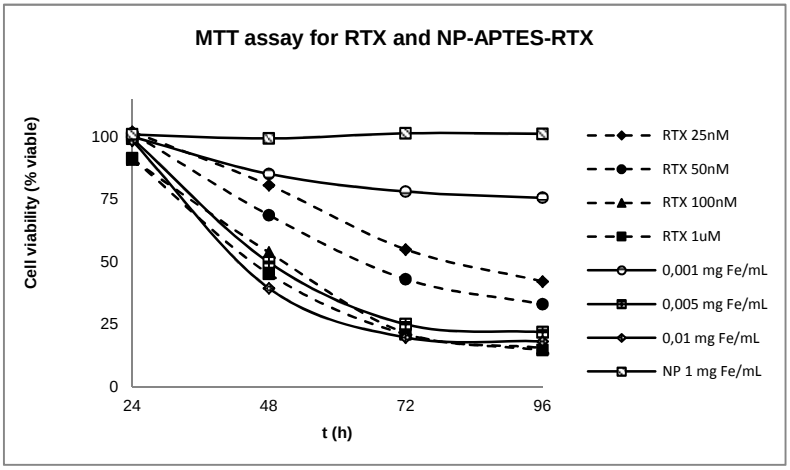

Figure 2. Determination of cytotoxicity of free RTX and NP-APTES-RTX at different concentrations. Nanoparticles without functionalization (NP) were also tested.

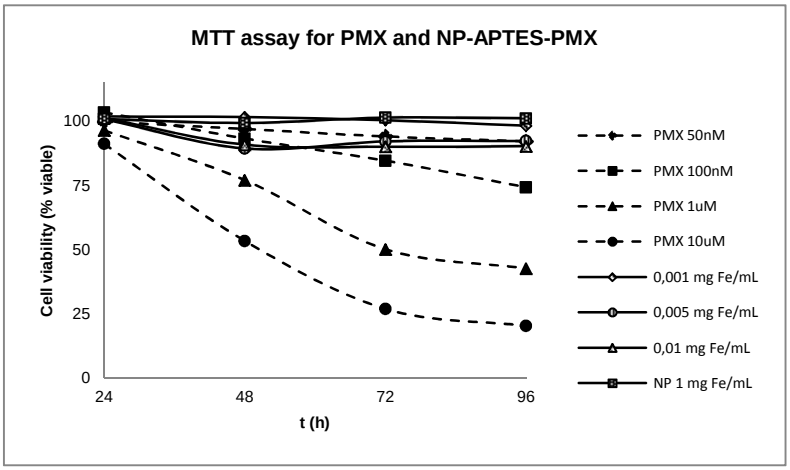

Figure 3. Determination of cytotoxicity of free PMX and NP-APTES-PMX at different concentrations. Nanoparticles without functionalization (NP) were also tested.