Doped Hallyosite Nanotubes as a Drug Delivery Tool for Anti-Cancer Treatment

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Introduction: Osteosarcoma is the most common primary malignancy of bone, representing about 3.4% of all childhood cancers and 56% of malignant bone tumors in children [1]. Metastasis of the tumor before or after medical intervene is the leading cause of death. Current treatment methods include surgery and multiagent chemotherapy, like high-dose methotrexate with leucovorin rescue, adriamycin, cisplatin, ifosfamide and cyclophosphamide [2]. Surgical resection from all sites of detectable metastatic tumor with systemic chemotherapy before and/or after surgery is required for successful treatment. Although there has been some significant progress, the survival rate has not improved. New chemotherapeutic agents and strategies are urgently required to improve treatment and patient outcome. Halloysite nanotubes (HNTs) are aluminosilicate clay nanoparticles, have a hollow tubular structure in the submicrometer range and have been shown to be non-cytotoxic [3].

In this preliminary study, HNTs/methotrexate doped complexes alter osteosarcoma cell behavior and inhibit cell proliferation, showing a potential for using HNTs as a delivery tool to send chemotherapeutic agents to tumor sites. In this way, a focal and sustained concentration of drugs can be delivered to targeted tissues designed to prevent potential tumor metastasis after surgery.

Methods: Osteosarcoma cells (OC, UMR-106, ATCC) were cultured in a 96-well plate as our test model. Halloysite nanoclay (Sigma) was immersed in a solution of NaOH (pH 11) and loaded with methotrexate hydrate (Sigma) under vacuum pressure (28 Hg). The resulting HNTs/methotrexate complex was added to the Dulbecco's Modified Eagle's Medium (D-MEM, Quality Biological INC.) used for osteosarcoma cell culture. The Live/Dead viability/cytotoxicity kit (Invitrogen) and the CellTiter 96 Aqueous one solution reagent (Promega) with LT-4000 Microplate reader (Phenix) was used to test cellular viability and proliferation.

Results: Methotrexate can be loaded into HNTs and released into NaOH (Ph=11, standard control) and D-MEM during a period of 24h. (See ***Figure 1.)***

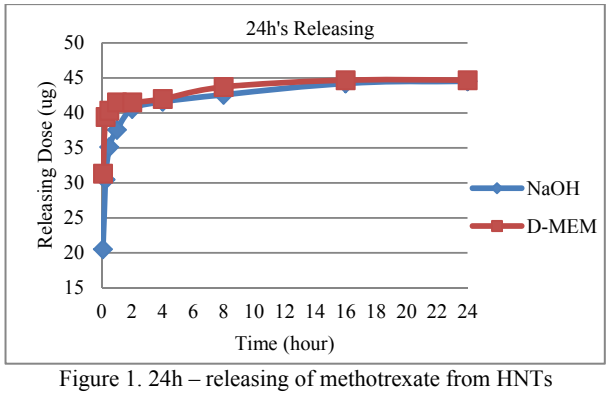
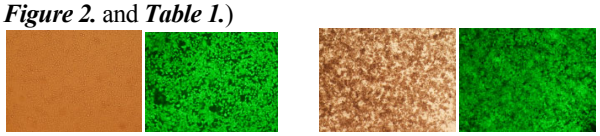


Figure 1. 24h – releasing of methotrexate from HNTs

Osteosarcoma cells (control & experimental) grew well in in all HNT concentrations (up to 2000 μ g/ml HNT). (See ***Figure 2.*** and ***Table 1.***)



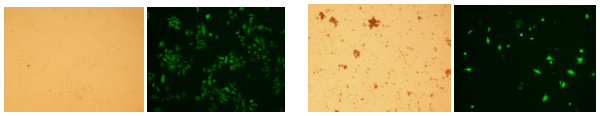
0 μ g/ml HNTs 2000 μ g/ml HNTs

Figure 2. Osteosarcoma cells grew after HNT exposure (Live/Dead)

HHTs (μ g/ml)	0	2000
Absorption	1.954	1.960

Table 1. Proliferation results from LT-4000 microplate reader

HNTs-methotrexate complex inhibited cell proliferation. (See Figure 3. and Table 2.)



0 μ g/ml HNTs/Methotrexate 500 μ g/ml HNTs/Methotrexate

Figure 3. Osteosarcoma cells grow with HNTs (Live/Dead Test)

HHTs/Methotrexate (μ g/ml)	0	500
Absorption	0.765	0.233

Table 2. Proliferation result from LT-4000 Microplate reader

Conclusions: HNTs (w/out methotrexate) had no observable cytotoxic effect on osteosarcoma cells and did not impair their proliferation ability. In contrast, methotrexate loaded HNTs, inhibited cell proliferation, and altered cell morphology. We envision doped HNTs as a tool to deliver chemotherapeutic antitumor drugs to targeted tissues with their sustained release locally to prevent tumor metastasis after surgery. Recent studies in our lab have further shown similar effects (described here) after drug doped HNTs (DNA, RNA, proteins, hormones and antibiotics) were incorporated in polymer scaffolds, sutures, hydrogels, bone cement and as an aerosol spray and applied to in vitro human tissue equivalents and in a rat model.

References:

[1]. Geller, David. "Osteosarcoma: A Review of Diagnosis, Management, and Treatment Strategies."*Clinical Advances in Hematology & Oncology*. 8.10 (2010): 705-718.
[2]. Jaffe, Norman. "Osteosarcoma: Review of the Past, Impact on the Future. The American Experience." *Cancer Treatment and Research*. 152. (2010): 239-262.
[3]. Viviana Vergaro, Elshad Abdullayev, Yuri M. Lvov, Andre Zeitoun, Roberto Cingolani, Ross Rinaldi, and Stefano Leporatti. "Cytocompatibility and Uptake of Halloysite Clay Nanotubes". *Biomacromolecules. 2010* Mar 8;11(3):820-6.