

2. PUBLISHABLE SUMMARY

The project objectives for the MuCoSiNT project can be summarized in two sections; **1)** Mobility and Integration objectives, and **2)** Experimental objectives. Experimental objectives are; **a)** Fabrication of silica nano test tubes (SiNTs) **b)** Creating a responsive gel formulation and filling of SiNTs with this gel to form composite SiNTs (CoSiNTs), **c)** Biofunctionalization and related characterization of SiNTs with Folic Acid (FA) moieties, **d)** Investigation of the in vitro performance of SiNTs with various compositions.

It can be easily concluded that the mobility and integration objectives of the MuCoSiNT project are fulfilled. The researcher made the 3 proposed US university visits, participated in four international conferences and three national conferences, and made several presentations. He made various collaborations (see section 7) which yielded 9 journal publications within the project period. Among these, one article is related with the MuCoSiNT project and the direct results of the project are in preparation for a full journal article. The project also greatly enhanced the reintegration of the researcher. He is currently a faculty member at the top biomedical engineering department in Turkey as an Associate Professor.

In terms of experimental progress, AAO membranes were synthesized by two-step anodization¹ and SiNTs of different dimensions were produced by the surface sol-gel method² and characterized by electron microscopy (Fig. 1A, B). A responsive gel formulation was created using the following constituents: hydroxyethylmethacrylate (HEMA), poly(ethylene glycol) ethylether methacrylate (PEG-EEM), 2-aminoethylmethacrylate hydrochloride (AEM), trimethylolpropane ethoxylate triacrylate as the crosslinker, doxorubicin (DOX) hydrochloride as the drug, 2,2-diethoxyacetophenone as the photoinitiator, and finally water and isopropanol as solvents for AEM, DOX and the initiator. After showing the pH responsive release of DOX from bulk gels, fabrication of gel-filled SiNTs, namely, CoSiNTs were conducted (Figure 1C, D).

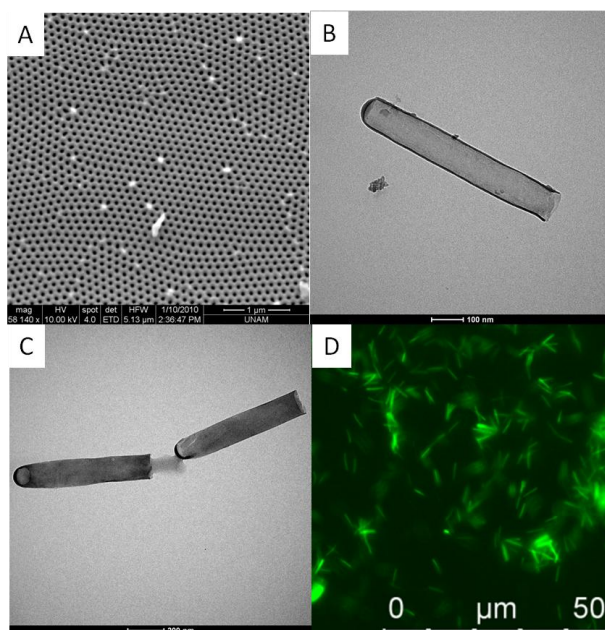


Figure 1. (A) SEM of AAO membrane. (B) TEM of naked SiNTs after template removal. (C) TEM of CoSiNTs. (D) Fluorescence micrograph of CoSiNTs. For visualization purposes ~ 4 μm long tubes with fluorescein-acrylate containing gel matrix was imaged.

Obtaining drug release profiles from the CoSiNTs at different pH values was a problematic task either by the originally proposed UV-Vis or electrochemical detection of the released DOX although SiNTs with largest proposed dimension were utilized. Hence, the drug release performances of drug-filled SiNTs are solely based on cell viability studies. Prior to this in vitro analysis part, SiNTs were targeted by FA conjugation via carbodiimide activation³, and here, each step was characterized by X-Ray Photoelectron Spectroscopy (XPS), Fourier Transform Infrared Spectroscopy (FT-IR) as well as Zeta Potential measurements (Fig. 2).

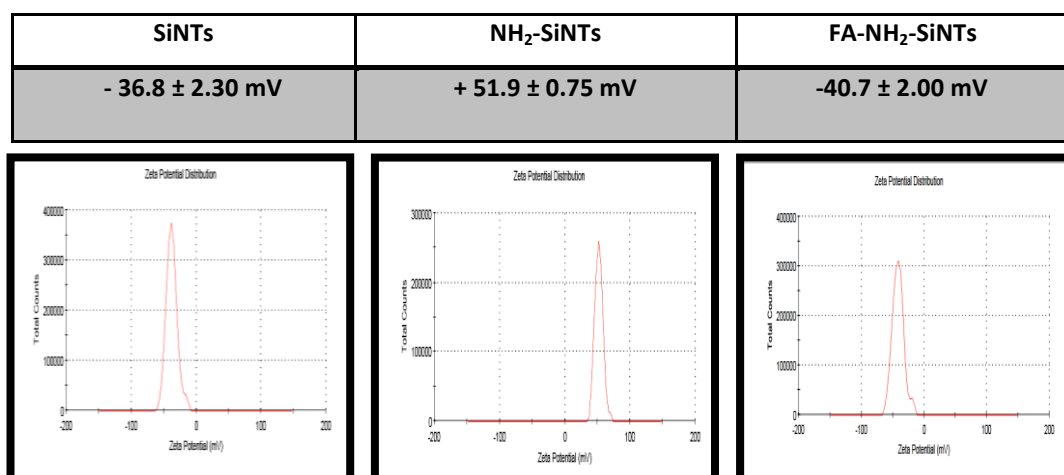


Figure 2. The zeta potential values for naked, amine and FA modified SiNTs

After the optimization of cell viability assay and incubation times, cell viability of tumor (SK-BR3) and healthy breast cell lines (MCF-12A) were investigated against SiNTs of various compositions and concentrations (Fig. 3) and further confirmed by apoptotic index calculations. The results demonstrate that MuCoSiNTs (SiNT 5) are effective killers of tumor cells, larger viabilities are obtained for MuCoSiNT treated healthy cells, and to induce cell death for both cell lines, the presence of DOX together with the targeting moiety are required. Formulations that do not contain DOX does not cause significant cell death (SiNT 1-3). Our results also suggest that when packaged within MuCoSiNTs, much smaller DOX concentrations are required to cause comparable cell death that originates from the exposure to free drug.

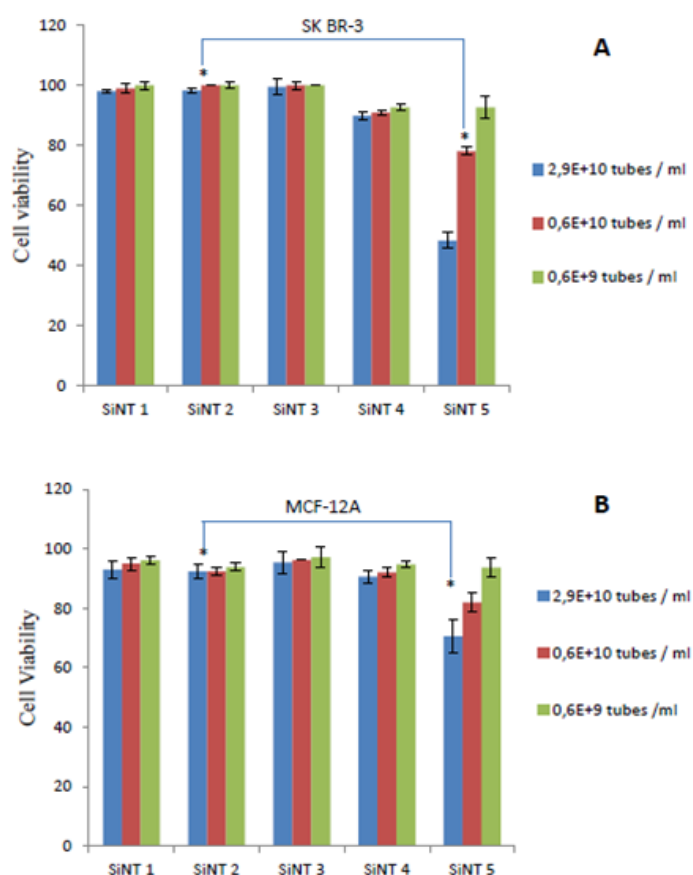


Figure 3. Cytotoxicity experiments of A) SK-BR3, and B) MCF-12A by using WST-1 Kit Assay (*P<0.05). Here, SiNT 1 is naked SiNTs, SiNT 2 is FA-SiNTs without gel nor drug, SiNT3 is FA conjugated SiNTs with gel matrix but does not involve DOX, SiNT 4 contains drug and gel but no FA functionality on their surface, and finally SiNT 5 (MuCoSiNTs) contains gel, drug as well as FA functionality.

References:

1. Masuda H.,Fukuda K., Science, 268, 1466-1468, (1995).
2. Kovtyukhova N. I., Mallouk T. E., Mayer T. S., Advanced Materials, 15, 780-785, (2003).
3. Wissink M., Beernink R., Pieper J., et.al., Biomaterials, 22, 151-163, (2001).