Electronic Supplementary Material (ESI) for Nanoscale Advances.

SUPPORTING INFORMATION

Doxorubicin loaded thermostable nanoarchaeosomes: A next-generation drug carrier for breast cancer therapeutics.

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1.1 Effect of NAD on MCF-7 Cells at 24, 48 and 72 h

We also tested NAD cytotoxic effect on MCF-7 at different time period of 48 h and 72 h (Figure SI-1). We observed that the cell viability decreased at 48 and 72 h compared to 24 h. The cell viability at 24 h at 2, 4, 6 , 8 and 10 nM concentration deceased from 99 ± 4 to 90 ± 3 and 80 ±3 %, 98 ±5 to 75 ± 4 and 60 ± 5% , 50 ± 3 to 40 ± 4 and 30 ± 2 %, 42 ± 3 to 31 ± 3 and 25 ± 2%, 31 ± 2.2 to 18 ± 1.5 and 11 ± 1% at 48 and 72 h. No significant differences in the cell viability were observed for cells treated with NAD at the concentration less than or equal to 1 nM for all three-time point.

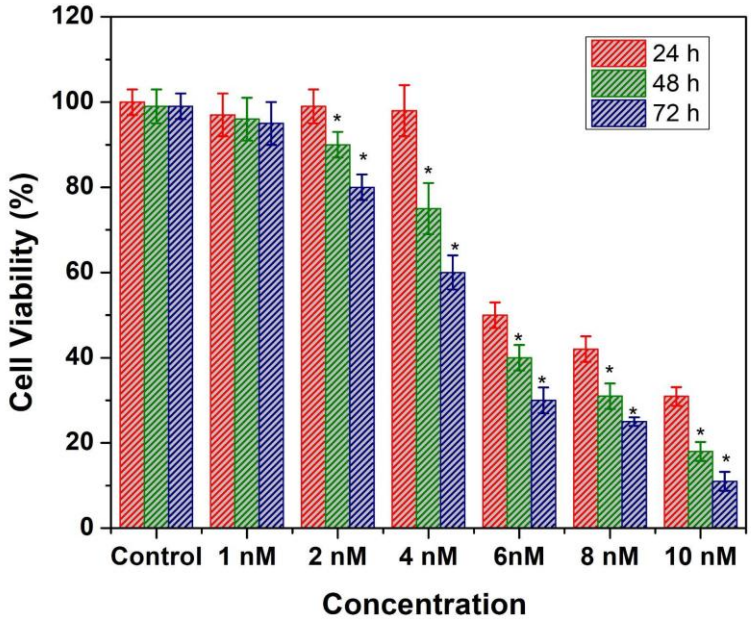

Figure SI-1: MTT assay result depicting the cell viability (%) of control, NAD treated MCF-7 cells at various concentrations after 24, 48 and 72 h incubation (Statistical analysis by One-way ANOVA, Tukey test, *p <0.001, indicates significant difference between 24, 48 and 72 h samples)

1.2 Fluorescent Microscopic images of MCF-7 breast cancer cells treated with Dox alone.

We have carried out the fluorescence microscopic experiments on NAD (100 nM) treated MCF-7 cells without any additional staining to exclude the interference of DOX fluorescence. Figure SI-2 infers that the cancer cells trated with NAD at 100 nM alone without any external staining did not illustrate any fluorescence at excitation and emission wavelengths of 480 and 590 nm. From the result, we infer that red itensity in the Figure 5 vi) corresponds to ethidium bromide signal and there is no interference of Doxorubicin at 100 nM concentration.

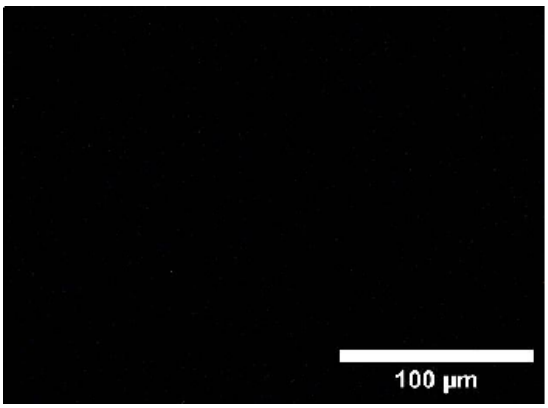

Figure SI-2: Fluorescence microscopy images of MCF - 7 treated with Doxorubicin at 100nM concentration.

1.3 Flow cytometry analysis of Cells treated with Dox alone

We have performed flow cytometric measurements for the cells treated with Doxorubicin **(**100 nM) alone. The control of unstained cells (Figure SI-3a), shows autofluorescence with 99.19% cell viability. For the cells treated with doxorubicin (100 nM), we observed interference of doxorubicin intensity in the PI channel in a meager amount without any additional staining (Figure SI-3b). We have addressed the spillover of Dox intensity in the PI channel and further analysis were performed by background subtraction and fluorochrome compensation. We didn't observe any fluorochrome spill over in the APC channel (Annexin V).

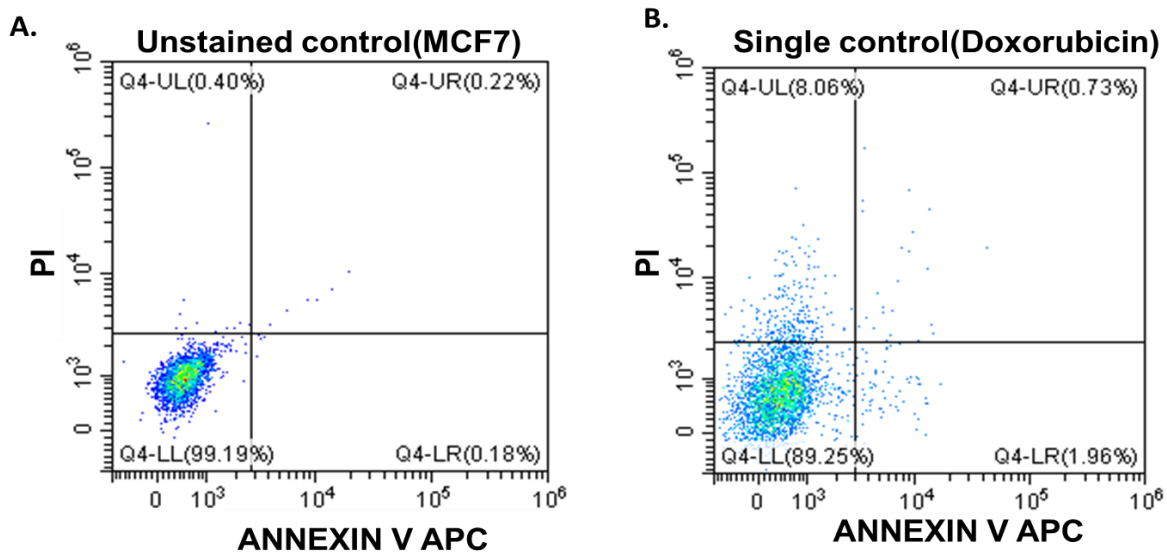

FIGURE SI-3: FACS analysis of (A) MCF-7 unstained cells (B) Cells treated with Dox alone (100 nM) without Annexin V and PI Staining.