Bio-piezoelectric Phenylalanine-αβ-dehydrophenylalanine Nanotubes as Potential Modalities for Combinatorial Electro-chemotherapy in Glioma Cells

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Supporting Information:

Synthesis of Phe-ΔPhe (FΔF):

FΔF was successfully synthesized using solution phase peptide synthesis method.[1] The obtained dipeptide was purified and analysed by reverse phase high performance liquid chromatography (RP-HPLC) and was characterized using mass spectrometry.

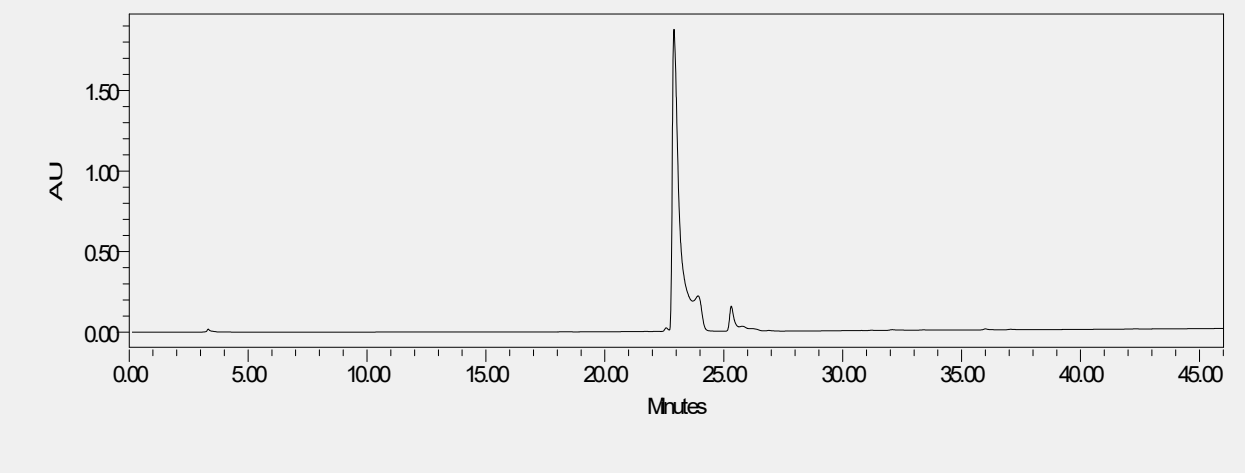

Figure S1: HPLC chromatogram of FΔF dipeptide.

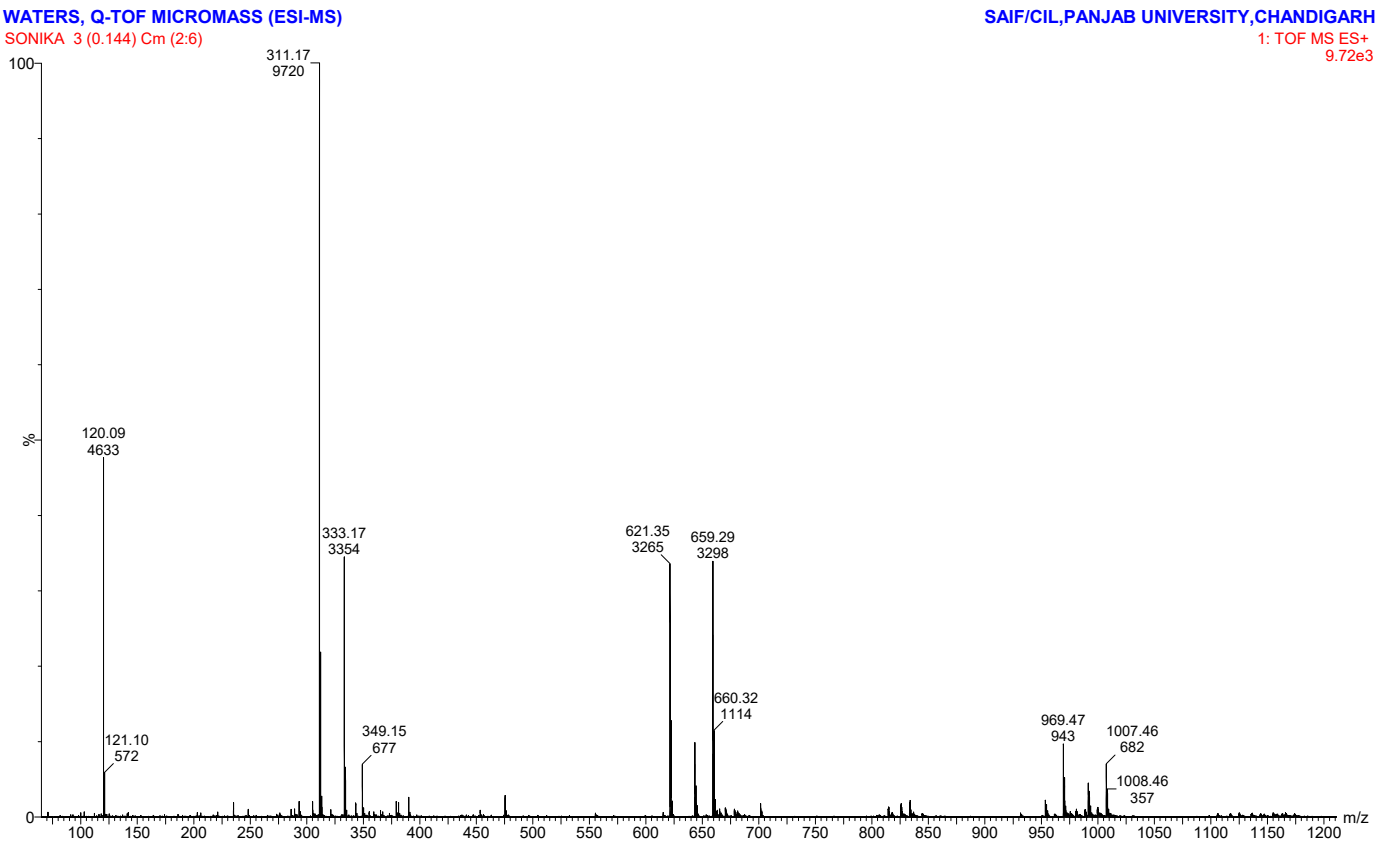

Figure S2: Molecular weight (m/z ratio) of FΔF determined by mass spectrometric analysis.

Table S1: The calculated dielectric and elastic tensors of FΔF dipeptide.

Dielectric tensor

$$\begin{pmatrix} 3.83 & 0 & 0.22 \\ 0 & 3.41 & 0 \\ 0.22 & 0 & 5.64 \end{pmatrix}$$

Elastic tensor (GPa)

$$\begin{pmatrix} 40.69 & 22.91 & 12.47 & 0 & 0 & 3.58 \\ 22.91 & 10.95 & 12.91 & 0 & 0 & 1.57 \\ 12.47 & 12.91 & -7.28 & 0 & 0 & -0.32 \\ 0 & 0 & 0 & -28.24 & 37.84 & 0 \\ 0 & 0 & 0 & 37.84 & -63.21 & 0 \\ 3.58 & 1.57 & -0.32 & 0 & 0 & 1.20 \end{pmatrix}$$

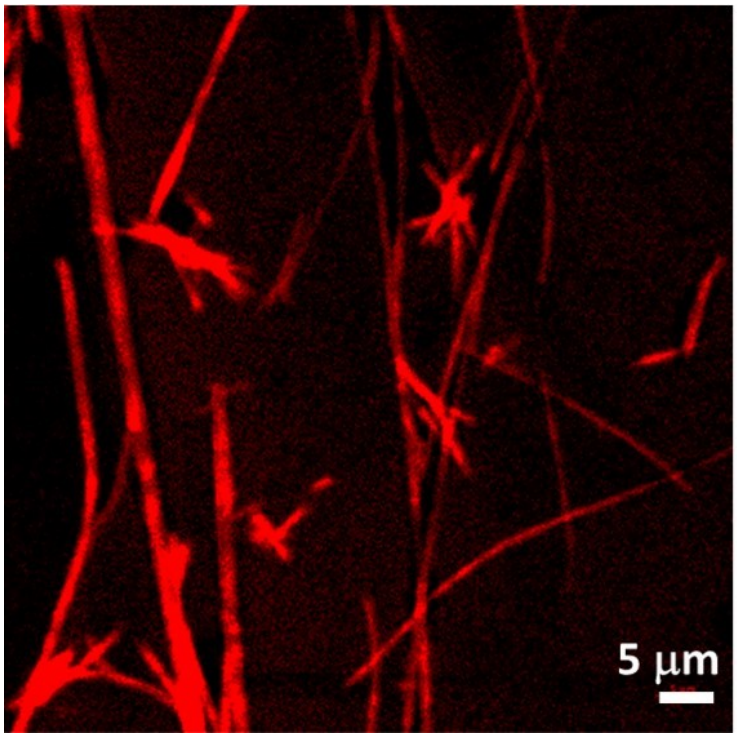

Figure S3: Confocal microscopic images of Dox encapsulated nanotubes; red fluorescence exhibited by the tubes confirmed Dox encapsulation.

Drug encapsulation in FΔF nanotubes:

After successful confirmation of the piezo-response of FΔF nanotubes using PFM, we next explored the encapsulation of the anti-cancer drug Doxorubicin (Dox) in the FΔF nanotubes under mechanical activation. After characterization of the tubular structures, we next investigated their ability to encapsulate Dox using pre loading method (**Figure S3**). The dipeptide nanotubes showed successful encapsulation of Dox, which was confirmed using confocal microscopic images as shown in **Figure S3**. Afterwards, percent encapsulation of Dox was calculated and found to be approximately 25 %.

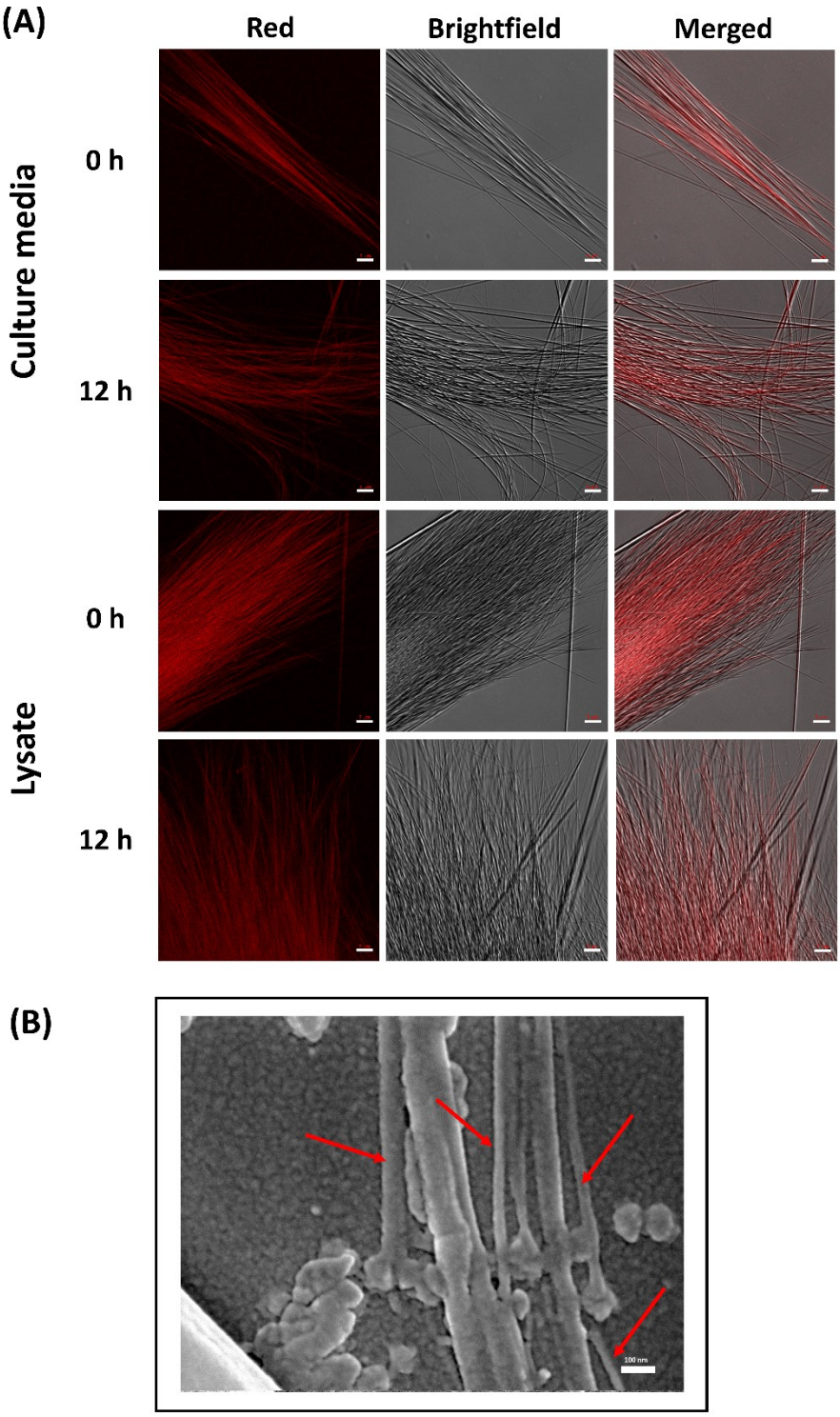

Figure S4: (A) Confocal images showing the structural integrity of FΔF-Dox nanotubes in culture media and cell culture lysate determined for upto 12 h. Images depict high structural

stability of the tubes in the presence of cellular enzymes and factors (Scale 5 μm). (B) FE-SEM images of FΔF-Dox nanotubes in cell culture lysate determined after 12 h incubation (Scale 100 nm). Red arrows demonstrate the intact nanotubular morphology.

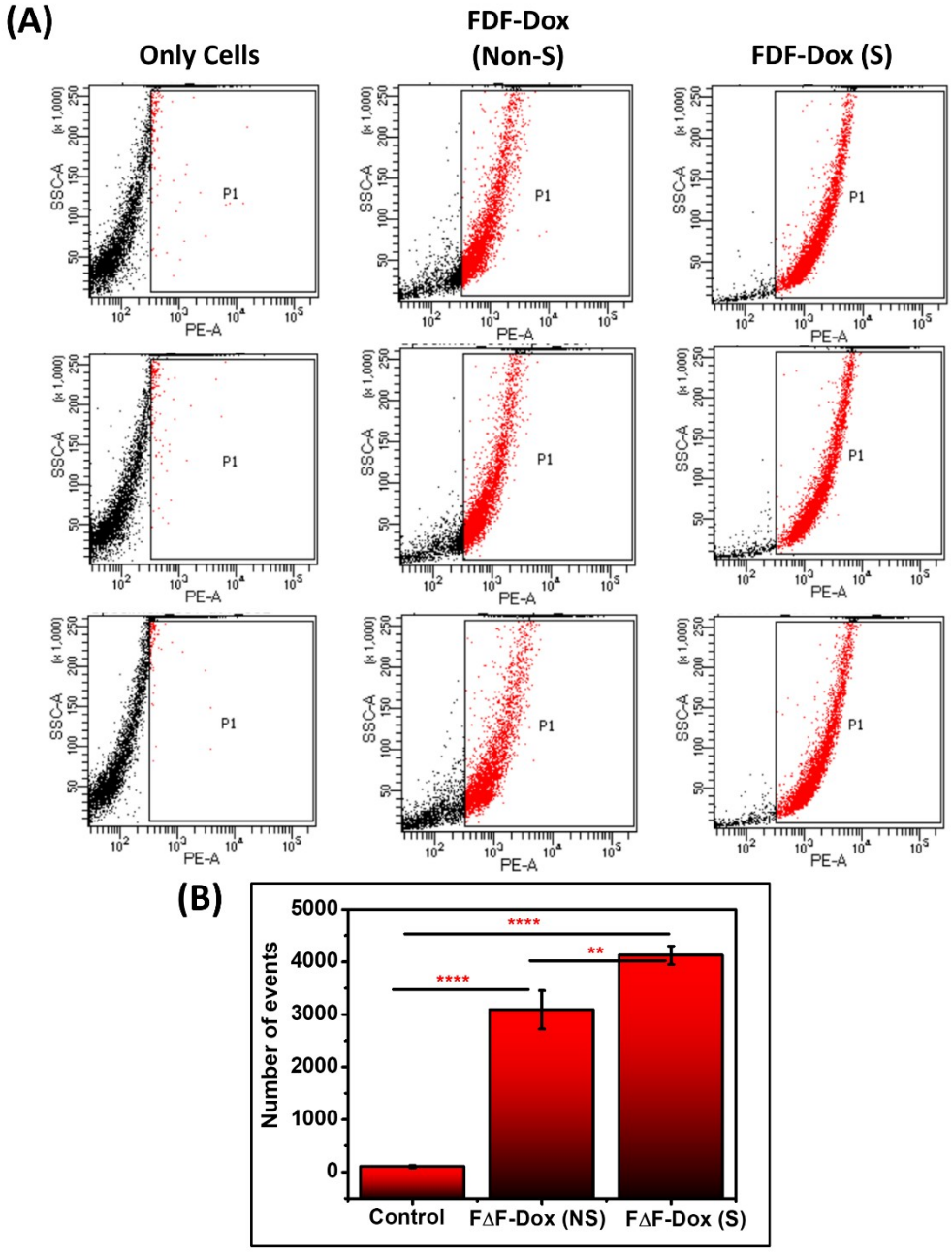

Figure S5: (A) The corresponding flow cytometry dot plots of C6 cells obtained after treatment with FΔF-Dox nanotubes under non-shaking (Non-S) and Shaking (S) conditions for 6 h. (B) Flow cytometry graph demonstrating the cellular uptake of FΔF-Dox nanotubes in C6 cells. The flow cytometric analysis was carried out in triplicates. ****, ***, ** and * represent levels of significance $P < 0.0001$, $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

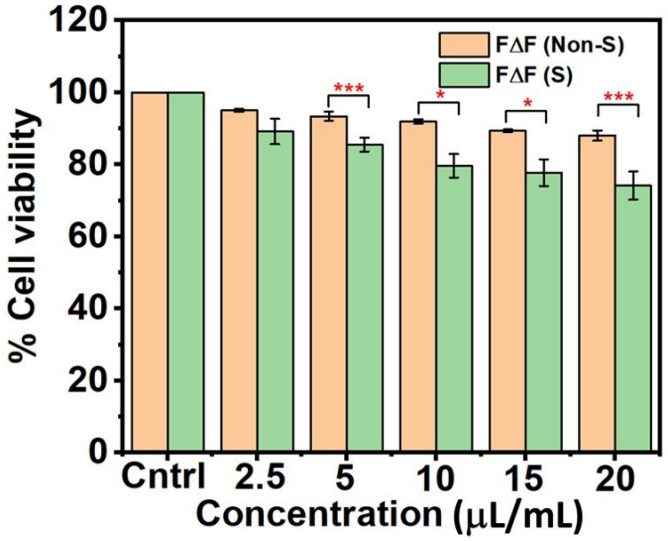

Figure S6: Biocompatibility of bare FΔF nanotubes determined in C6 cells under mechanical shaking (S) and non-shaking/static (Non-S) conditions.

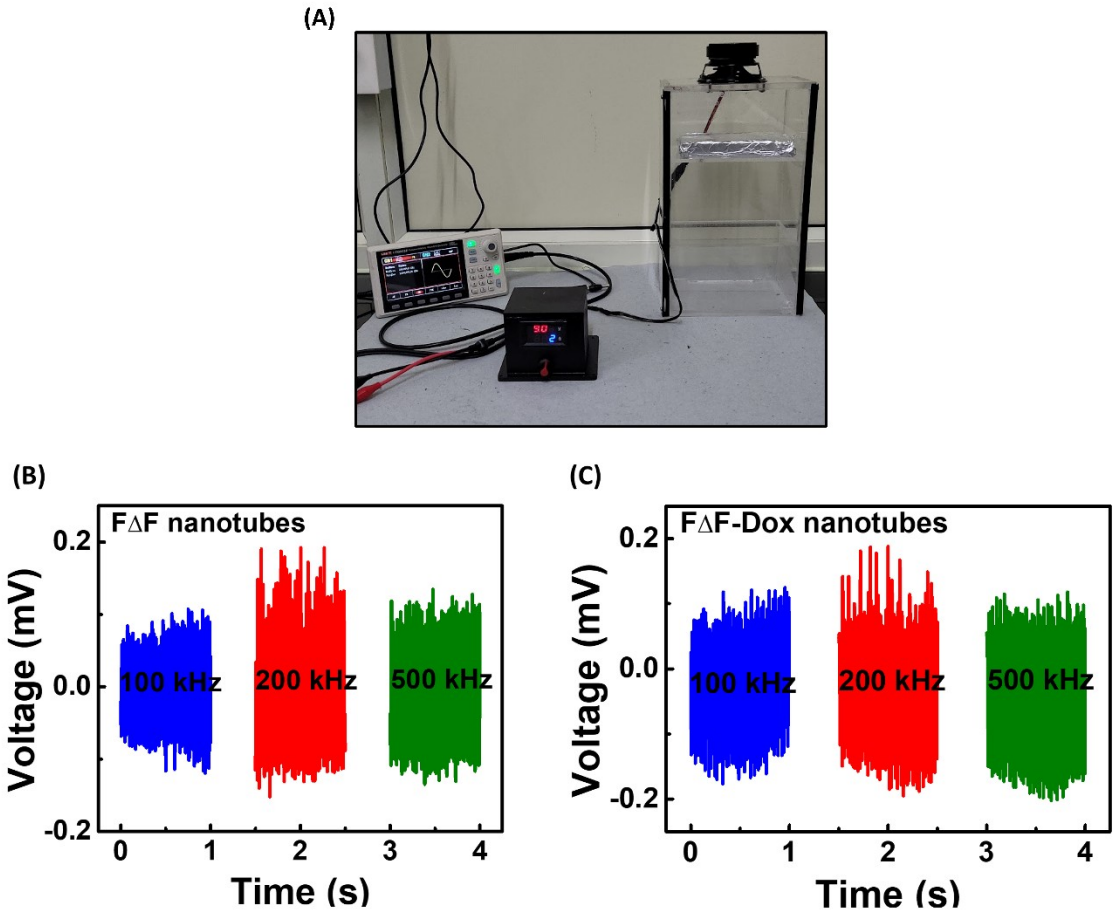

Figure S7: (A) Demonstration of customized acoustic compartment used for inducing acoustic stimulations in in-vitro conditions for generating piezoelectric polarization. Output voltage of (B) FΔF nanotubes and (C) FΔF-Dox nanotubes under acoustic stimulus of frequency 100 kHz, 200 kHz and 500 kHz.

References:

1. M. Gupta, A. Bagaria, A. Mishra, P. Mathur, A. Basu, S. Ramakumar and V. S. Chauhan, *Adv. Mater.*, 2007, **19**, 858–861.