# Supplementary data

**Stimuli-responsive graphene oxide and methotrexate loaded magnetic nanoparticles for breast cancer targeted therapy**

*Mitra Dolatkhah 1,2,3, Nastaran Hashemzadeh 1,2,3, Jaleh Barar 1,2, Khosro Adibkia 1,2, Ayuob Aghanejad1, Mohammad Barzegar-Jalali 2, Hossein Omidian4, Yadollah Omidi4*\*

1 Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran

2 Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

***3*** Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

4 Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, Fort Lauderdale, Florida 33328, USA

**Short title:** GO-SPION-MTX for breast cancer targeting

\* Corresponding author: Y. Omidi, College of Pharmacy, Nova Southeastern University, Fort Lauderdale, Florida 33328, USA. Email: [yomidi@nova.edu](mailto:yomidi@nova.edu)

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**Fig. S1.** The cell viability assay of (a) the 2D and (b) 3D cultured MCF-7 cells, and (c) the 2D cultured MDA-MB 231 cells. Cells were exposed to the different concentrations (10,20,40 and 80 μg/mL) of NPs for 24 h with and without NIR laser [810 nm, 16 J/(s/m2)] in the 2D and 3D cultured MCF-7 cells, and the 2D cultured MDA-MB231 cells. Data present means ± SD of three separate experiments. (\*\*p < 0.05). NIR: Near-infrared laser irradiation, NPs: Nanoparticles.

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**Fig. S2. C**ytotoxic effects of (a) NPs, (b) MTX, (c) MTX-NPs, (d) ACZ as single treatments in breast cancer cell lines *in vitro*, in both buffered and unbuffered growth media to consider the acidic TME role in cancer therapy. MCF-7 cells were incubated for 4 h in the buffered or unbuffered media, and after 4 h of incubation different doses of the treatments (NPs, MTX, MTX-NPs, and ACZ) were added. Cytotoxic effect was evaluated after 48 h. The data present means ± SD of three separate experiments. (\*\*p < 0.05). MTX: Methotrexate, NPs: Nanoparticles, ACZ: Acetazolamide.

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**Fig. S3. C**ytotoxic effects of (a) NPs, (b) MTX, (c) MTX-NPs, (d) ACZ as single treatments in breast cancer cell lines *in vitro*, in both buffered and unbuffered growth media to consider the acidic TME role in cancer therapy. MDA-MB231 cells were incubated for 4 h in the buffered or unbuffered media, and after 4 h of incubation different doses of the treatments (NPs, MTX, MTX-NPs, and ACZ) were added. Cytotoxic effect was evaluated after 48 h. The data present means ± SD of three separate experiments. (\*\*p < 0.05). MTX: Methotrexate, NPs: Nanoparticles, ACZ: Acetazolamide.