Cellular uptake, imaging and pathotoxicological studies of Novel Gd [III]-DO3A-butrol Nano-Formulation

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Running title (Novelty): Cellular uptake and imaging studies of Novel Gadobutrol-ALGD-G2

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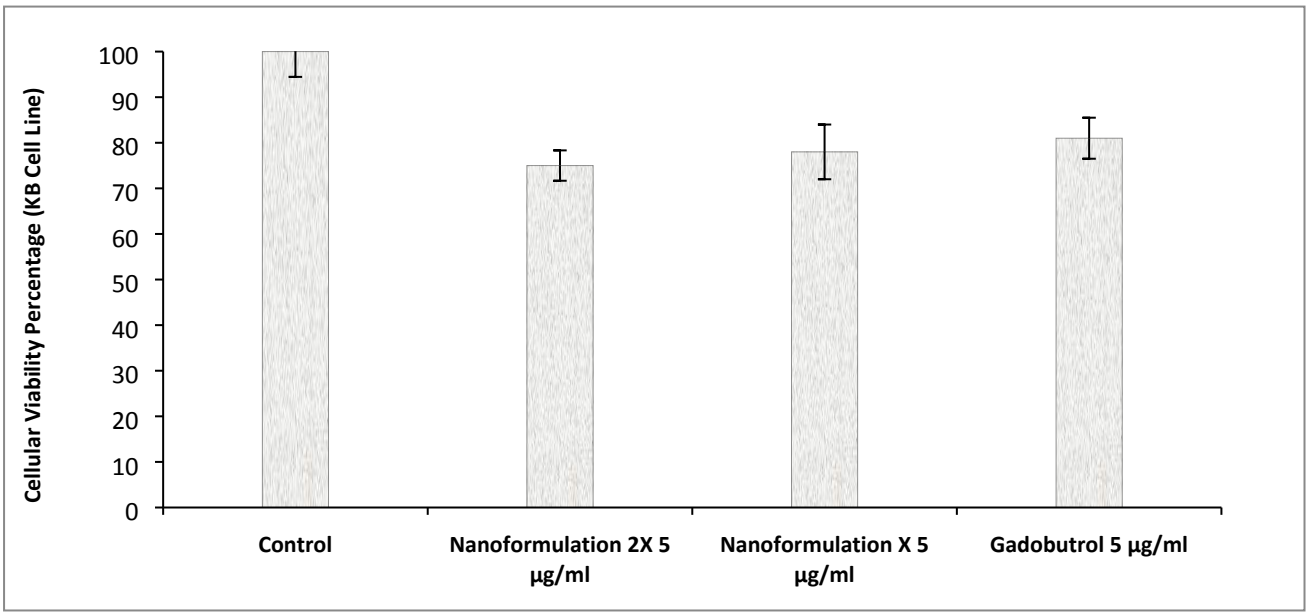
Supplementary Materials:

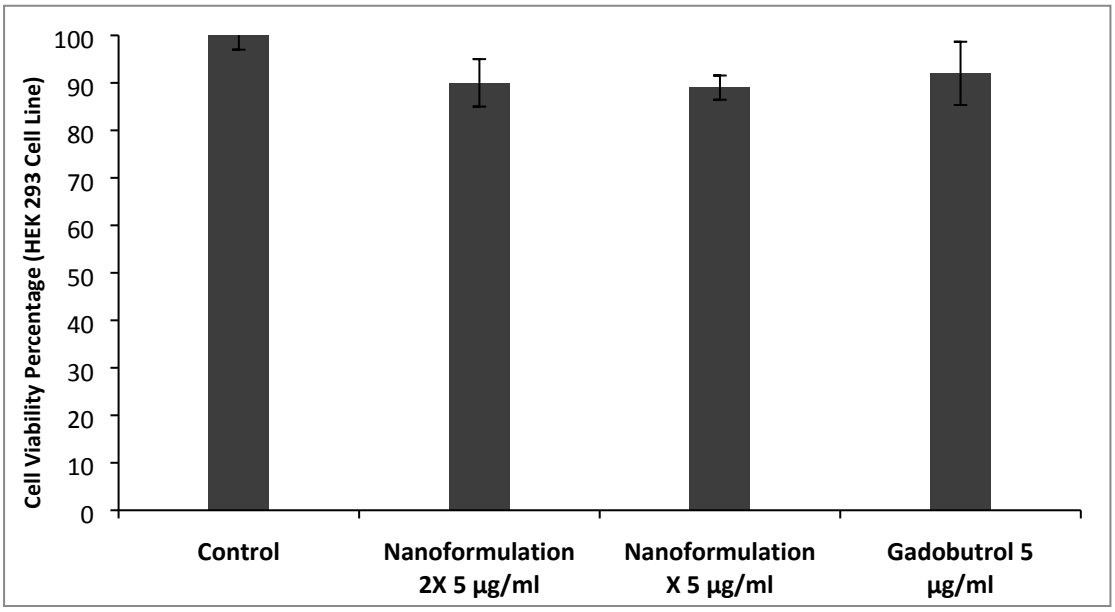
Cellular Toxicity Assay (MTT)

Based on the previously published literature [14-17] both tumor (MCF-7) and normal (HEK-293) cells lines, provided from National Cell Bank of Pasteur Institute of Iran, were cultured and exposed to the same concentration (5µg/ml) of the different nano-formulated contrast agent and gadobutrol for a period of 24 hrs and OD was obtained from ELISA reader at 570 nm respectively. The MCF-7 cellular exposures showed a mild significant $p<0.05$ cellular toxicity for Nano-formulation 2X as well as a not significant cellular toxicity $p>0.05$ for those of Nano-formulation X and gadobutrol. Furthermore, not any HEK-293 cellular toxicity was observed from the nano-formulations and gadobutrol exposures. (See original data at S-1_{a-b}) Briefly, gadobutrol was shown to be safe and insert both normal and cancer cell line but the gadobutrol nanoformulation was found safe on normal human kidney cell line (as a major toxicity target of gadolinium based contrast agent) and toxic on cancer cell lines.

S-1_a: Results of the same concentration (5µg/ml) of the different nano-formulated contrast agent and gadobutrol for a period of 24 hrs on MCF-7 cell lines.



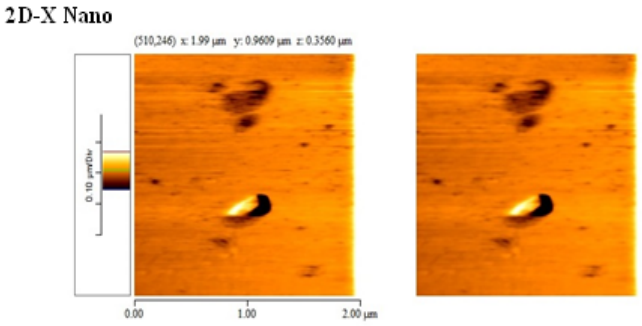
S-1$_b$: Results of the same concentration (5µg/ml) of the different nano-formulated contrast agent and gadobutrol for a period of 24 hrs on MCF-7 cell lines. S-1$_a$: Results of the same concentration (5µg/ml) of the different nano-formulated contrast agent and gadobutrol for a period of 24 hrs on HEK-293 cell lines.

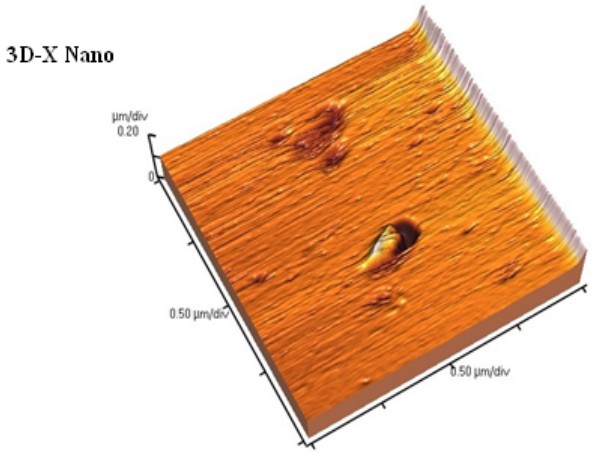


AFM Imaging

For morphology investigations of the proposed nano-formulations X and 2X Atomic Force Microscopy were employed and two and three dimensional images were obtained as demonstrated in S-2_a and S-2_b.

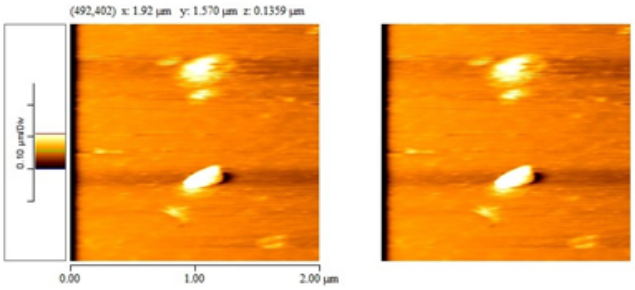
S-2_a: 2D and 3D AFM images of nanoformulation X.



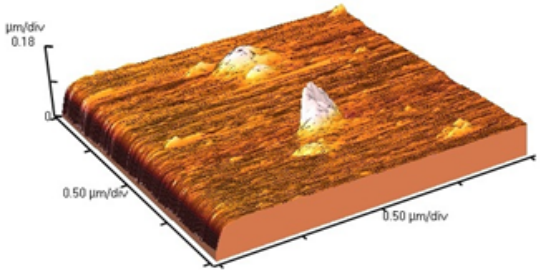


S-2_b: 2D and 3D AFM images of nanoformulation 2X.

2D-2X Nano



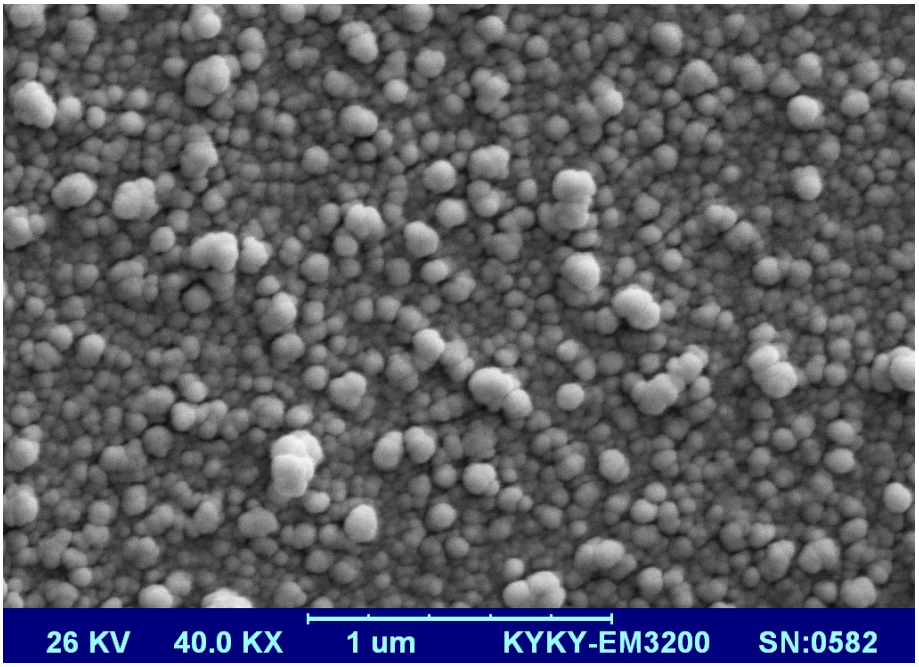
3D-2X Nano



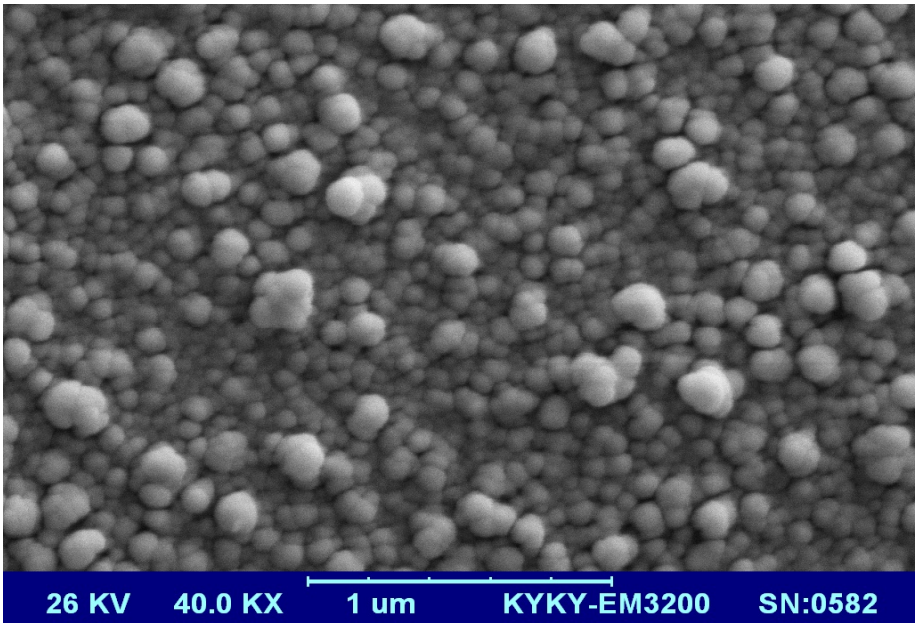
SEM Imaging

For more morphology investigations of the proposed nano-formulations X and 2X sizes, Single Electron Microscopy were used and images were obtained as demonstrated in S-3_a and S-3_b. Before preparation of imaging samples the formulations were rigorously sonicated to avoid any unwanted nanoparticle's aggregations. According to the results sizes obtained at a lower ranged (<200 nm) than observed by AFM or Zetasizer's data.

S-3_a:SEM image of X nano-formulation



S-3_b: SEM image of 2X nano-formulation


26 KV
40.0 KX
1 um
KYKY-EM3200
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