ARTICLE

Designing Functionalized Nanodiamonds with Hyaluronic Acid-Phospholipid Conjugates for Enhanced Cancer Cell Targeting and Fluorescence Imaging Capabilities

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Nanomedicine aims to develop smart approaches for treating cancer and other diseases to improve patient survival and quality of life. Novel nanoparticles as nanodiamonds (NDs) represent promising candidates to overcome current limitations. In this study, NDs were functionalized with a 200 kDa hyaluronic acid-phospholipid conjugate (HA/DMPE) enhancing the stability of the nanoparticles in water-based solutions as well as selectivity for cancer cells overexpressing the specific HA cluster determinant 44 (CD44) receptors. These nanoparticles were characterized by Diffuse Reflectance Fourier-Transform Infrared spectroscopy, Raman spectroscopy, and photoluminescence spectroscopy confirming the efficacy of the functionalization process. Scanning Electron Microscope was employed to evaluate the size distribution of the dry particles, while Dynamic Light Scattering and Zeta Potential measurements were utilized to evaluate NDs behavior in a water-based medium. Furthermore, NDs biocompatibility and uptake mediated by CD44 receptors in three different models of human adenocarcinoma cells were assessed by performing cytofluorimetric assay and confocal microscopy. HA-functionalized nanodiamonds demonstrated the advantage of active targeting in the presence of cancer cells expressing CD44 on the surface, suggesting higher drug delivery to tumors over non-tumor tissues. Even CD44-poorly expressing cancers could be targeted by NDs, thanks to their good passive diffusion within cancer cells.

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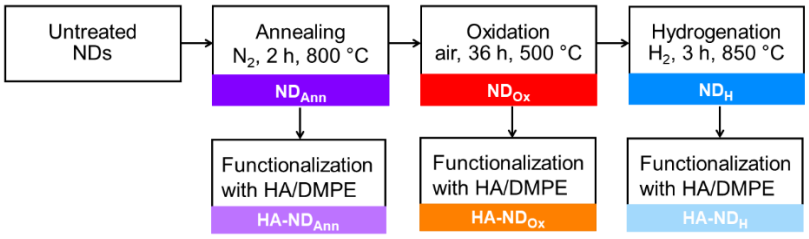

Figure S1 Summary scheme for the thermal treatments and functionalization procedures with HA carried out on the NDs. All the NDs under exam were modified via thermal treatments, as shown in the first row of white-background rectangles. Subsequently, the samples were functionalized with HA by non-covalent attachment of the HA/DMPE conjugate, as displayed in the second row of white-background rectangles. The names given to the samples after the different processing steps are reported inside the coloured rectangles.

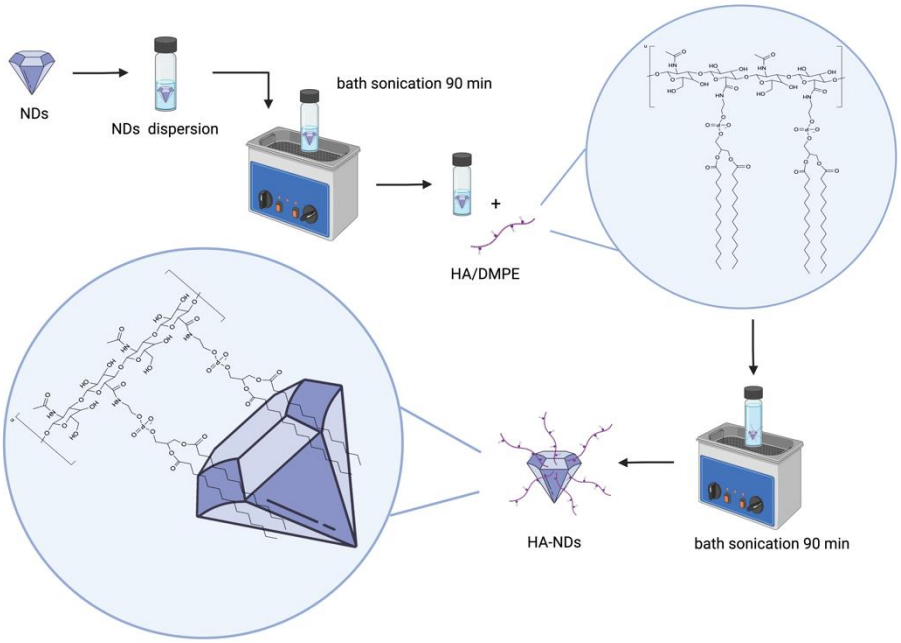

Figure S2 Schematic representation of the functionalization method of NDs with HA/DMPE conjugate (created with BioRender.com).

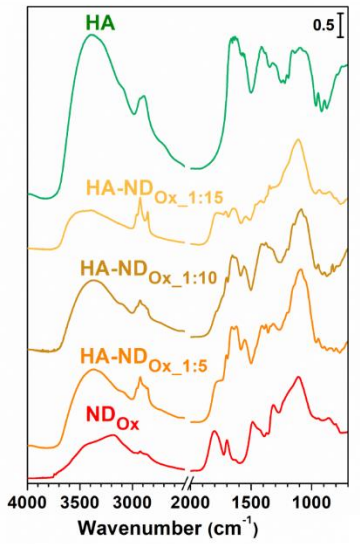

Figure S3 Comparison between the DRIFT spectra of the ND_{OX} and the ones collected from HA-ND_{Ox} for different HA/DMPE:NDs ratios, *i.e.*, 1:5 (HA-ND_{Ox}_1:5), 1:10 (HA-ND_{Ox}_1:10) and 1:15 (HA-ND_{Ox}_1:15). The spectrum labeled as "HA-ND_{Ox}_1:5" is the one indicated as "HA-ND_{Ox}" in the main text.

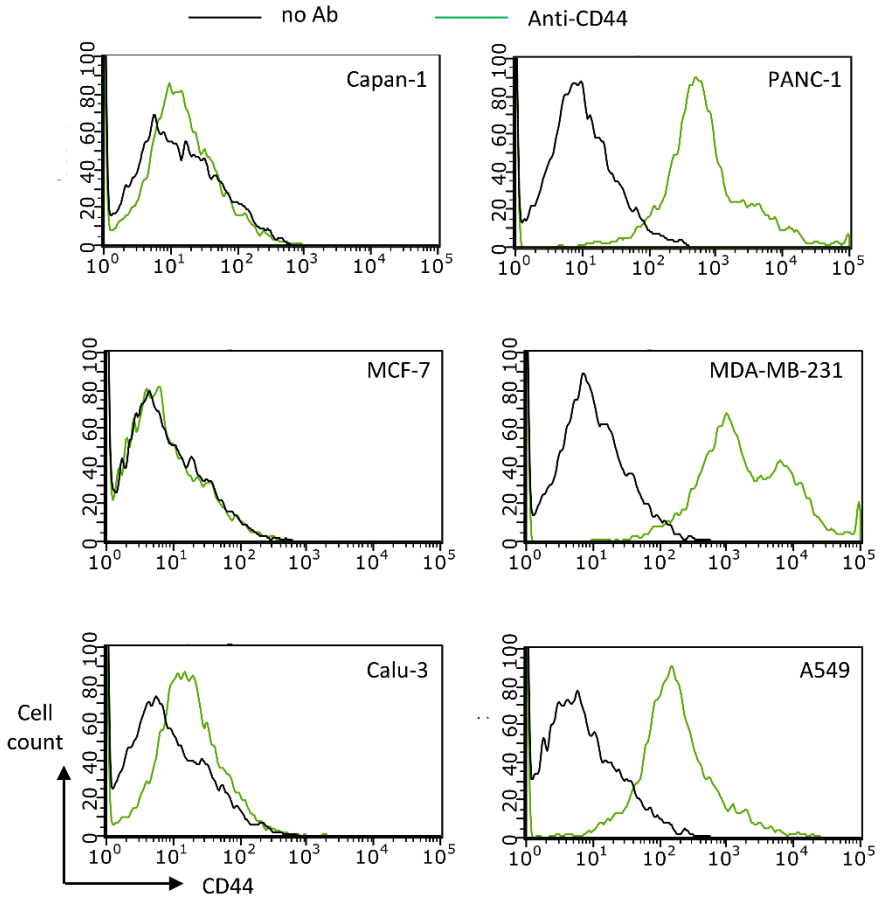

Figure S4 Flow cytometry analysis of surface CD44 in human pancreatic adenocarcinoma Capan-1 and PANC-1 cells, human breast cancer MCF-7 and MDA-MB-231 cells, non-small cell lung cancer Calu-3 and A549 cells. Black line: unstained cells. Green line: cell stained with anti-CD44 antibody. The histograms are representatives of 1 out of 3 experiments.

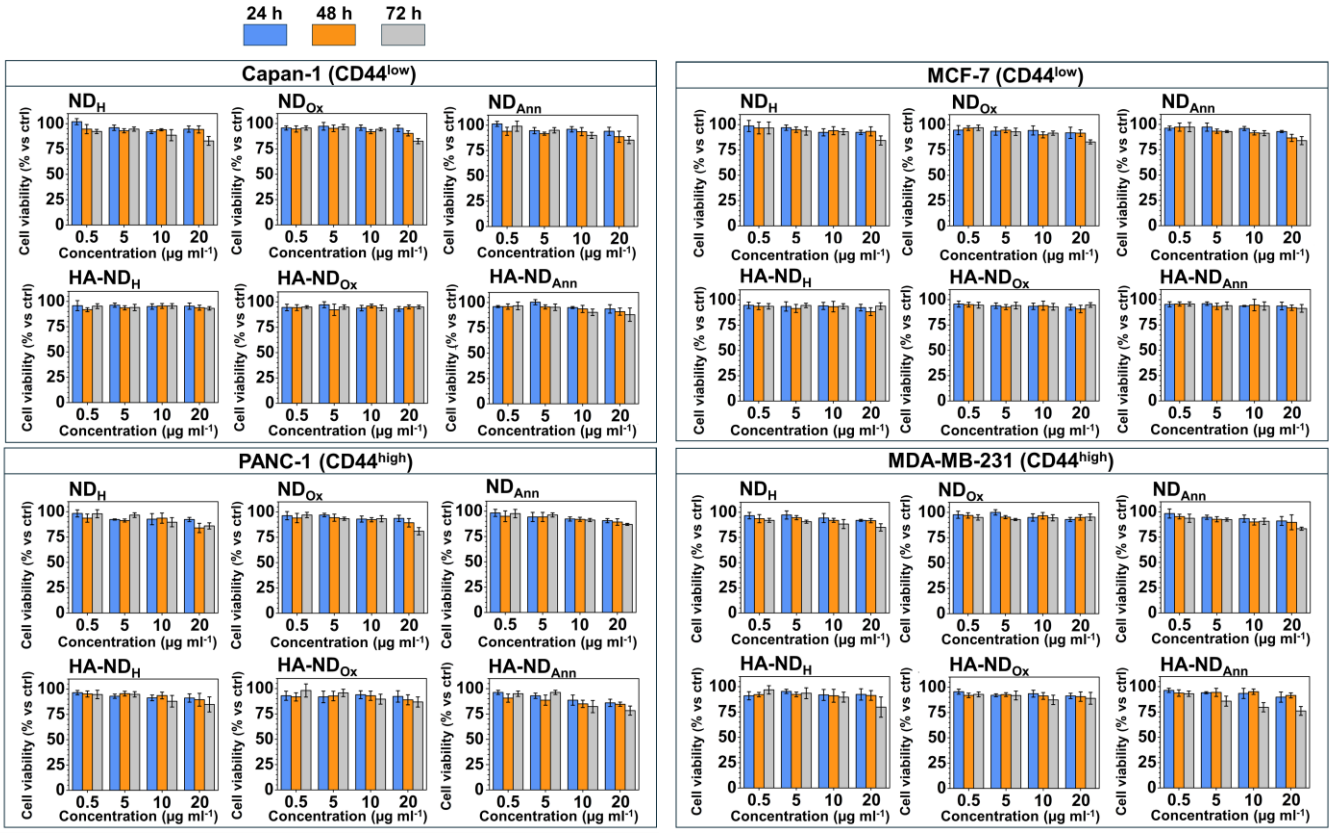

Figure S5 Cell viability at 24, 48 and 72 h in human pancreatic adenocarcinoma Capan-1 and PANC-1 cells, human breast cancer MCF-7 and MDA-MB-231 cells, incubated with ND_{Ann}, ND_{H}, ND_{Ox}, HA-ND_{Ann}, HA-ND_{H} and HA-ND_{Ox} at the indicated concentration, by a chemiluminescence-based assay, in triplicates (*n*=3 independent experiments). Data are means + SD

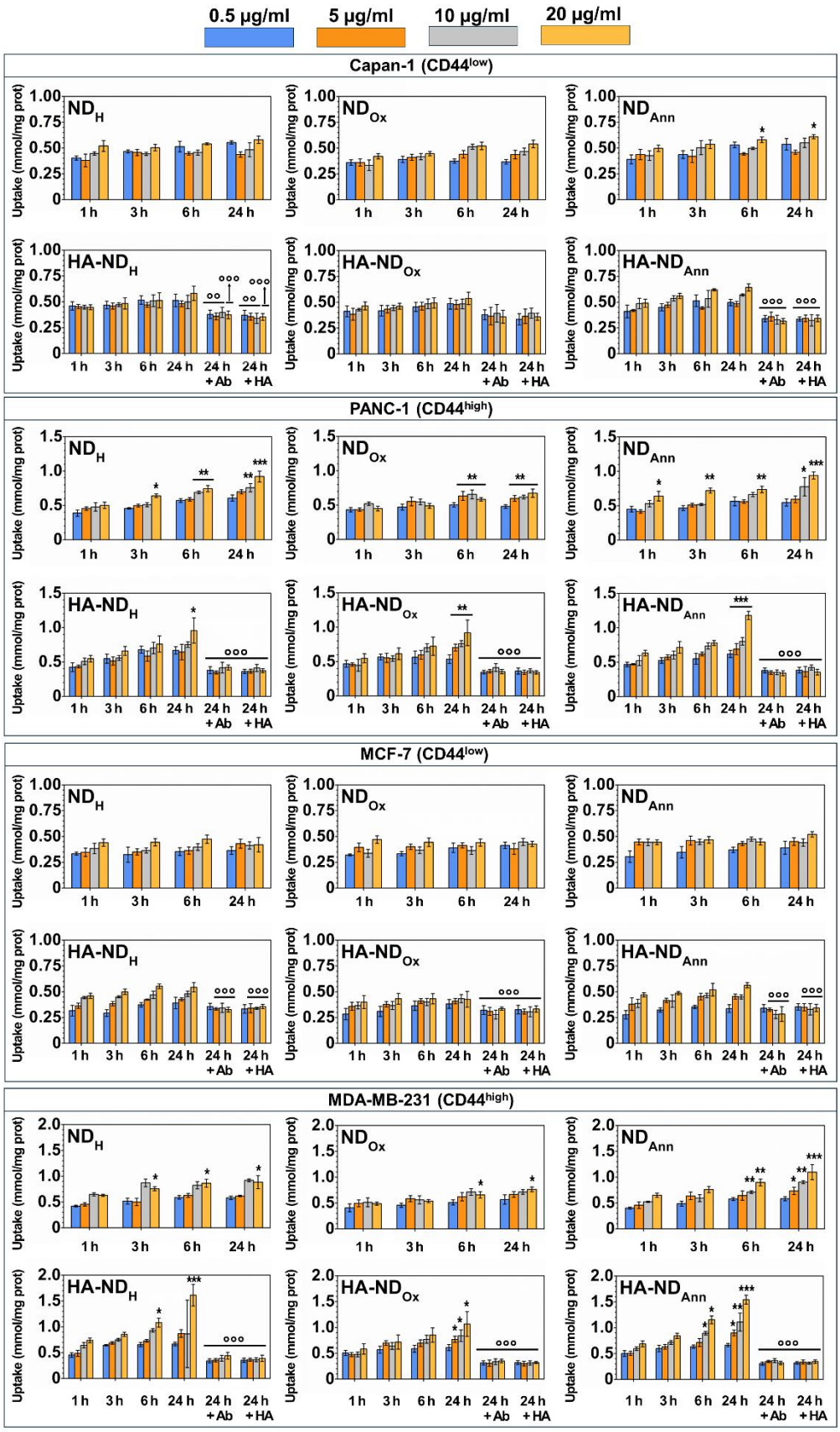

Figure S6 Uptake of ND_{Ann}, ND_H, ND_{Ox}, HA-ND_{Ann}, HA-ND_H and HA-ND_{Ox} at the indicated concentrations after 1,3,6, and 24 h in non-small cell cancer human pancreatic adenocarcinoma Capan-1 and PANC-1 cells, human breast cancer MCF-7 and MDA-MB-231 cells, by a fluorimetry-based assay, in triplicates (*n*=3 independent experiments). When indicated an anti-CD44 neutralizing antibody (Ab, diluted 1/100) or 100 µM hyaluronic acid (HA) were co-incubated. Data are means + SD. *p<0.05, **p<0.01, ***p<0.001: ND-fluorescence vs cell autofluorescence; °°p<0.01, °°°p<0.001: ND-fluorescence in cells treated with Ab/HA vs ND-fluorescence in cells without Ab/HA

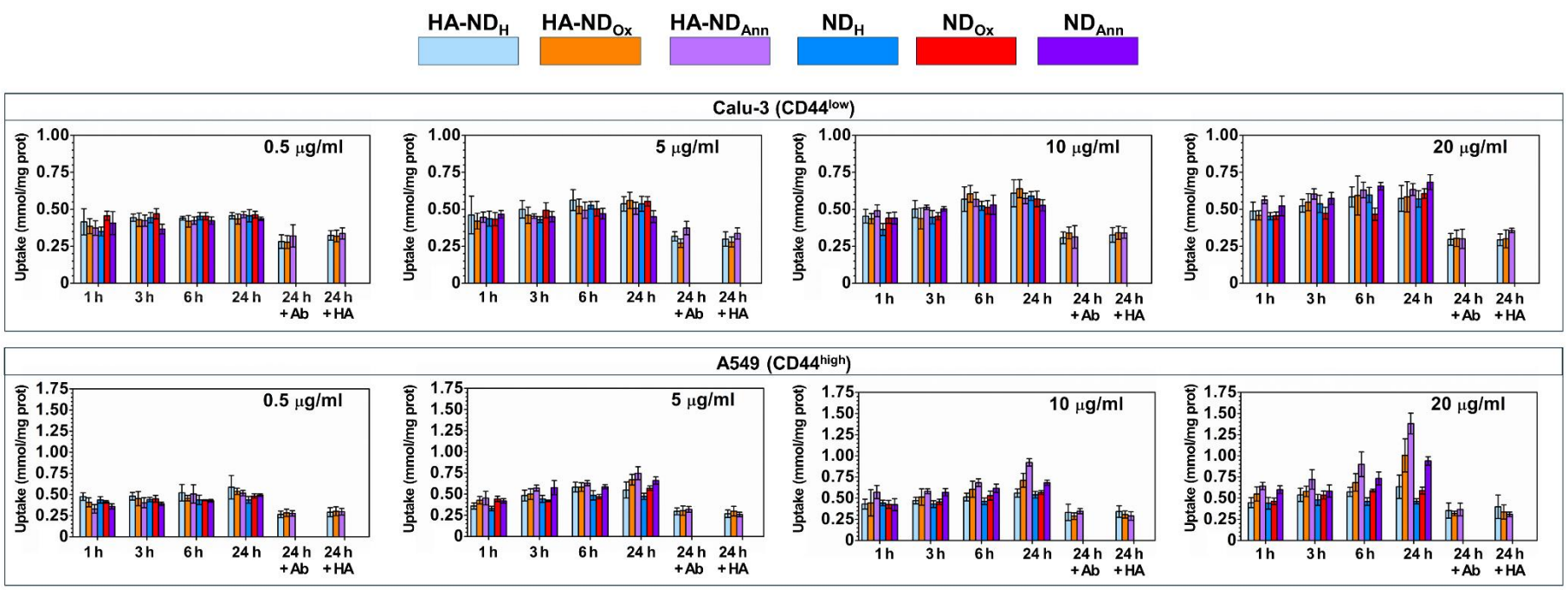

Figure S7: alternative representation of figure 6 of the main text. Each of the eight graphs refers to a single NDs concentration (0.5 μg ml^{-1}, 5 μg ml^{-1}, 10 μg ml^{-1} and 20 μg ml^{-1}) and compares the different uptake of ND$_{Ann}$, ND$_H$, ND$_{Ox}$, HA-ND$_{Ann}$, HA-ND$_H$ and HA-ND$_{Ox}$ after 1 h, 3 h, 6 h, and 24 h in Calu-3 cells and A549 cells.