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Cellular accumulation of PEGylated and non-PEGylated nanoparticles was investigated *in vitro* by fluorescence or by radioactivity quantification in human prostate cancer cell lines. The fluorescence intensities of the PEGylated and non-PEGylated particles prior *in vitro* studies were nearly identical which is why differences in fluorescence intensities in the cell experiments can be directly related to differences in cellular binding and uptake of the MSNs.

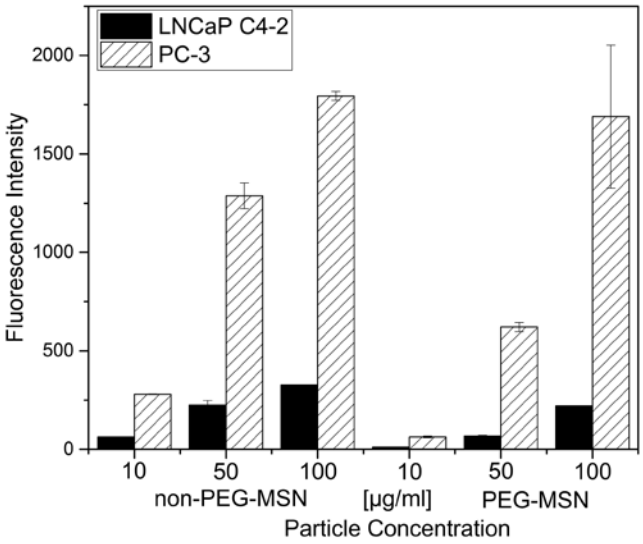

Figure S1. Evaluation of particle accumulation by flow cytometry in prostate carcinoma cell line PC-3 and LNCaP C4-2. The mean of the fluorescence intensities measured is given for the respective particle concentration including standard deviation. PEG-MSN and non-PEG-MSN were applied to equal amounts of final concentrations of 10 µg/ml, 50 µg/ml and 100 µg/ml. Fluorescence intensity was observed 24 h post application for 25 000 cells.

The results of flow cytometry analysis after 24 h of incubation are presented in Figure S1. The PEGylated MSNs were taken up at a lower degree than the non-PEGylated MSNs and more particles were internalized at increasing particle concentration in all cases, in agreement with previous studies.[37, 38] However, significantly higher fluorescence intensity was detected

for PC-3 cells in comparison to LNCaP C4-2 cells under the identical experimental conditions. For non-PEGylated MSNs up to a 5.7-fold (50 µg) higher accumulation in PC-3 as compared to in LNCaP C4-2 cells was observed, and for PEGylated MSNs an up to 9.2-fold (50 µg) difference observed, highlighting a cell line-dependency of unspecific nanoparticle uptake.

In addition to fluorescence-based analyses, radioactivity measurements were also performed, as the detection sensitivity for the ^{89}Zr-labeled MSNs allow quantitative measurements to be performed at much lower particle concentration levels, reflecting the magnitude of concentration of the *in vivo* studies. Cellular uptake determined using radio-labeled MSNs is presented in Figure S2. Concordantly to fluorescence-measurements, significant differences in cellular uptake depending on the surface modification of MSNs were observed also for the radiolabeled MSN. Very low levels of radioactivity uptake were detected for PEGylated MSNs in all cases independent of particle concentration, while for non-PEGylated particles a significantly higher uptake was observed. Significant cell line specific differences between LNCaP C4-2 und PC-3 as those observed in the FACS measurements were, however, not observed at these low particle concentrations. Here the results slightly alternate between PEGylated and non-PEGylated particles, as for PEGylated MSNs there is minor particle concentration-dependent increase in the uptake for PC-3 cells, while for non-PEGylated particles the results for both cell lines are within the same standard deviation. Thus, at low particle concentration levels, the effects of cell line specific variations as observed for fluorescence labeled MSNs, seem to be balanced.

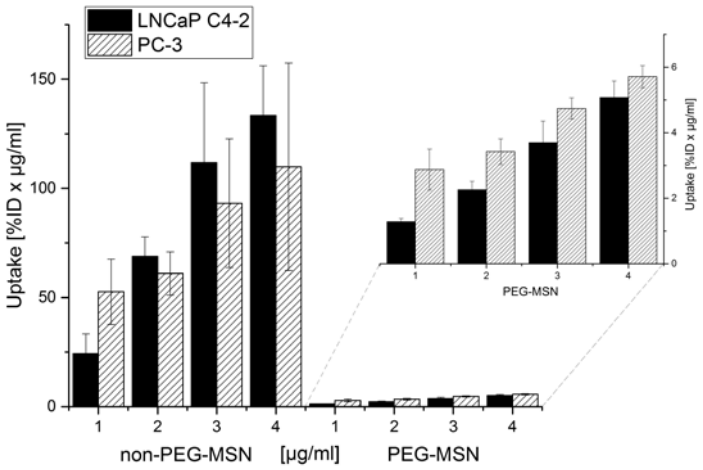

Figure S2. Comparative specific uptake of radiolabeled MSN in cell lines LNCaP C4-2 and PC-3. Following incubation at particle concentrations of 1 µg/ml, 2 µg/ml, 3 µg/ml and 4 µg/ml per well for 24 hours, activity was detected using an automated gamma counter. The uptake was determined as percent of the applied dose times the respective particle concentration. The data were adjusted to get comparability to the FACS results.

In a further set of experiments, fluorescence microscopy was performed with identical experimental settings to allow for an as direct as possible comparison of the images. See Figure S3. The total measured fluorescence intensities after washing were 3.44 (PC-3), and 0.02 (LNCaP C4-2) for PEGylated MSNs and 4.76 (PC-3), 1.62 (LNCaP C4-2) for non-PEGylated particles, thus being in qualitative agreement with the more quantitative FACS results. Higher fluorescence intensities and therefore accumulation of fluorescent MSNs was observed in PC-3 cells in comparison to in LNCaP C4-2 cells. Furthermore, stronger fluorescence was observed in cells incubated with non-PEGylated MSNs as compared to in corresponding systems using PEG-MSN. Additionally, Z-Stack studies with 0.58 µm layer distance confirmed the intracellular location of both non-PEG-MSNs and PEG-MSNs.

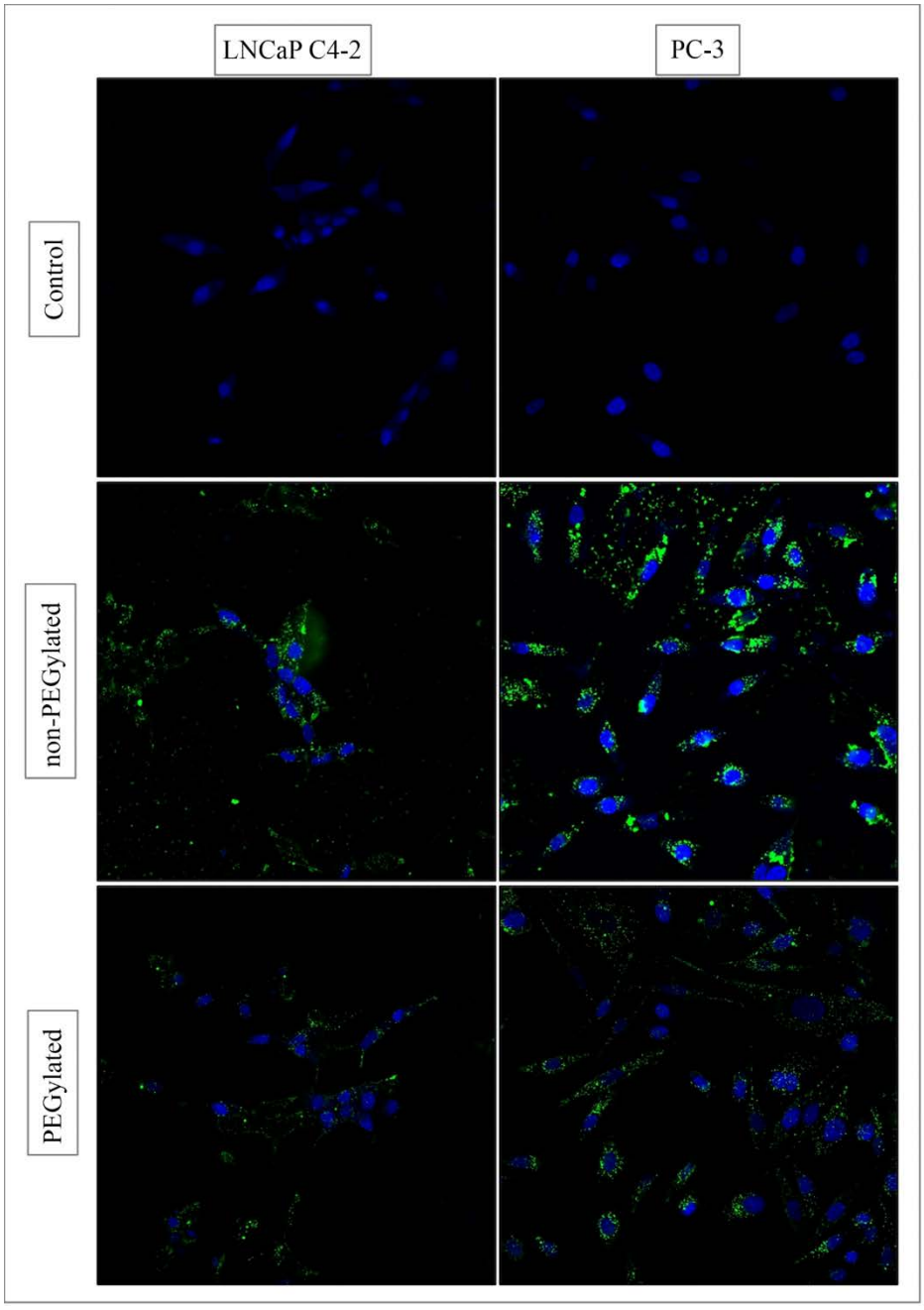

Figure S3. Confocal laser-scanning microscopy images of LNCaP C4-2 and PC-3 cells incubated with 50 µg/mL of AlexaFluor488-labeled, PEGylated and non-PEGylated nanoparticles for 24 h. The cell nucleus is stained with DAPI (blue). The green-fluorescent MSN are localized inside the cells in intracellular compartments.