Electronic Supplementary Material (ESI) for Chemical Communications

Introduction of disulfide bridges within silica nanoparticles to control their intra-cellular degradation

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Electronic Supplementary Informations

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ESI-1 : Detailed experimental protocols

Synthesis of x%SS particles:

x%SS particles were synthesised based on the Stöber process. Briefly, tetraethyl orthosilicate (TEOS 98 wt%, Aldrich) was added to a stirring solution of ammonium hydroxyde solution (30%, CarloErba Reagents) in ethanol directly followed by the dropwise addition of Bis(triethoxysilylpropyl)disulfide (BTSPD 98 wt%, ABCR) and finally the addition of a solution of fluorescein-grafted aminopropyl triethoxysilane (APTES, Merck; FITC isomer 1 95%, Alpha Aesar). All solutions had the same composition except for the concentration of BTSPD: 0.16 mol/L TEOS, 0.34 mol/L ammonium hydroxide, 0.09 mmol/L final FITC concentration, 6 mM final APTES concentration. BTSPD concentrations for 10%SS, 20%SS, 30%SS and 40%SS were respectively 6.7, 13.4, 20.2 and 26.9 mmol/L. The solution was stirred for 48h at room temperature before purification of the nanoparticles by dialysis (Spectra/Por, diameter 25 mm, pore diameter 4.2–5.0 nm from Carl Roth) with 200 mL particle suspension being dialysed 5 times under magnetic stirring against 2 L of ultrapure water (Milli-Q) with a minimum time of 4 h.

Solid-state NMR

Solid-state NMR spectra were performed on a Bruker AVANCE III 300 WB spectrometer at B_0 = 7.04 T with $\nu_0(^1H)$ = 300 MHz, $\nu_0(^{13}C)$ = 75.51 MHz, $\nu_0(^{29}Si)$ = 59.66 MHz using a 7 mm probe for 1D and a 4 mm probe for 2D HETCOR experiments. Cross-polarization coupled to magic angle spinning (CP-MAS) was performed on dried powders finely grounded and packed into ZrO_2 rotors. The spinning rate was 5 kHz for 1D and 14 kHz for 2D experiments. 1D CP-MAS experiments were carried out using ramp up polarization transfer conditions, 90° pulse, recycle delays of 1 s, contact times of 1 ms and 3 ms for respectively

{^{1}H}-^{13}C CP-MAS and {^{1}H}-^{29}Si CP-MAS experiments, recording 4.096 scans. 2D HETCOR-MAS experiments were carried out using tangent ramp polarization transfer conditions, recycle delays of 1 s, spectral width of 14 kHz, contact times of 1 ms and 3 ms for respectively {^{1}H}-^{13}C HETCOR and {^{1}H}-^{29}Si HETCOR experiments, recording 32 slices (f1 dimension) and 1.680 scans for {^{1}H}-^{13}C HETCOR and 6.800 scans for {^{1}H}-^{29}Si HETCOR experiments. For both 1D and 2D experiments, a line broadening of 30 Hz was applied for {^{1}H}-^{13}C and 50 Hz for {^{1}H}-^{29}Si experiments using an exponential apodization function on the f2 dimension. For 2D experiments, a cosine apodization function was applied on the f1 dimension.

Refocused {^{1}H}-^{29}Si INEPT sequence (Insensitive Nuclei Enhanced by Polarization Transfer) was carried out using a 4 mm probe heated at 353 K, spinning at 8 kHz, with a recycling delay of 2 s, recording 72.240 scans and using 10 ms for both evolution and refocalisation delays. A line broadening of 10 Hz was applied.

Dissolution and reduction by DTT

Dissolution of x%SS particles was assessed at pH 7.4 in Tris-HCl buffer (50 mM with 10 mM KCl) and at pH 4.5 in acetate buffer (50 mM with 10 mM KCl) with the silicomolybdate method as described in [13].

Reduction of the disulfide by dithiothreitol (DTT) was monitored by UV-visible spectroscopy through the absorbance at 290 nm of the cyclic compound resulting from the oxidation of DTT. x%SS particles were suspended in Tris-HCl buffer pH 7.4 at 0.6 mg/mL and dioxygen was removed by flowing nitrogen in the solution for 5 minutes and thus avoid oxidation of DTT by oxygen. Subsequently, DTT solution in phosphate buffer (0.2 M) was added to obtain

a final concentration of 1.3 mM and the solution was kept at 37˚C in oxygen-free atmosphere. At determined times, 1 mL solution was removed, centrifuged 15 minutes at 6,000 rpm and absorbance at 290 nm was recorded.

Cells and treatments with nanoparticles

Normal human dermal fibroblasts (from Promocell) were grown in Dulbecco's Modified Eagle Culture Medium (DMEM, Gibco BRL) supplemented with fetal Calf Serum (10%, from Gibco BRL), penicillin (100 units mL^{-1}), streptomycin (100 µg mL^{-1}, from Gibco BRL) and fungizone (0.25 µg mL^{-1}, from Gibco BRL). The culture flasks (75 cm^2) were kept at 37 °C in 95% humidity and 5% CO_2 atmosphere. At confluence, fibroblasts were removed from cultured flasks by treatment with 0.1% trypsin and 0.02% EDTA. Cells were rinced and resuspended in the supplemented DMEM media. Fibroblasts were used at passage 7-8 for the experiments.

Uptake of silica nanoparticles was determined by fluorescence spectroscopy and TEM. The cells were seeded at a density of 30,000 cells per well in 24-well plate with round glass coverslips at the bottom of the wells. The cells were kept 24h with culture medium at 37 °C in 95% humidity and 5% CO_2 atmosphere previous to incubation with 0.6 mg mL^{-1} of x%SS nanoparticles for different times (from 4 hours to 14 days) with replacement of the supernatant by fresh medium on day 7.

Nanoparticle internalization

For fluorescence microscopy, coverslips with the cells were removed at determined intervals of time, washed three times with PBS 1X, and fixed with 4% paraformaldehyde (1 h, 4 °C). Staining of the membranes with wheat germ agglutinin Alexa Fluor 555 conjugate (Invitrogen) and the endosomes with Blue/Yellow Lysosensor (Invitrogen) were used to

investigate the internalization and location of the nanoparticles within the cells. Briefly, the coverslips were incubated 15 min at room temperature with Lysosensor, rinsed with HBSS 1X and then incubated for 10 min with the other marker before observation under a fluorescent microscope (Axio 100, Carl Zeiss).

For TEM, cells were fixed at determined intervals of time using 3.63% glutaraldehyde in 0.05 M sodium cacodylate buffer with 0.3 M saccharose for 1 h at 4 °C. Following this fixation step, samples were washed three times before post-fixing with 2% osmium tetraoxide for 1 h at 4 °C. After the washing steps, the cells were detached from the culture flasks and centrifuged. The pellets were dehydrated with an ascending ethanol series ending with propylene oxide and embedded in araldite. Ultra thin sections were prepared with an Ultracut ultramicrotome (Reichert, France). Slides were analyzed with a FEI Tecnai electron microscope operating at 120 kV. Images were obtained for at least 10 cells for each sample.

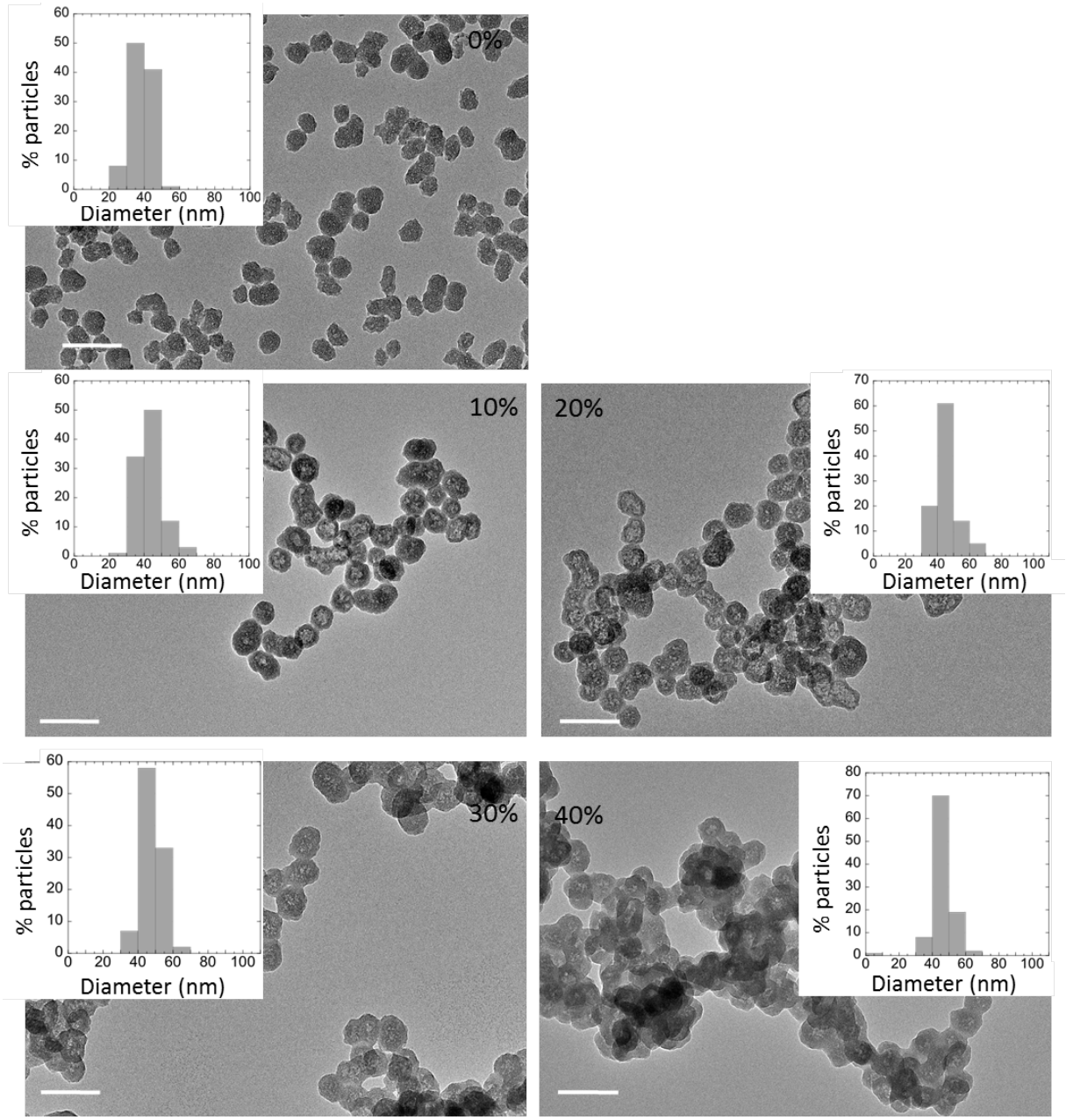
Exocytosis and intracellular dissolution

To assess the possible exocytosis of internalized particles, cells were exposed to a suspension of 0.6 mg mL^{-1} nanoparticles for 7 days in culture medium as described for the uptake experiments. Culture medium was then removed and cells rinsed before adding fresh medium. The fluorescence intensity of medium was analyzed both directly and after centrifugation in a Nanosep® 3kD centrifugal device to remove particles and have access to the proportion of released FITC over time. As a reference, a suspension of 0.6 mg/mL nanoparticles in culture medium was kept at 37 °C under mechanical stirring. All experiments were performed in triplicate.

Toxicological assay

Cellular activity of cells in 24-well plate was evaluated with Alamar Blue assay (n = 6). Cells were rinced with medium and incubated, at 37 °C in a humidified 5% CO_2 air atmosphere, for 4 h with a 10% solution of alamar blue in phenol red-free culture medium. Absorbance of the medium at 570 nm and 600 nm was recorded with a UV–visible spectrometer and cellular activity was calculated. Incubation of the particles with Alamar Blue gave negligible absorbance values.

ESI-2 : TEM images of x%SS nanoparticles (scale bar = 100nm) and corresponding size distribution



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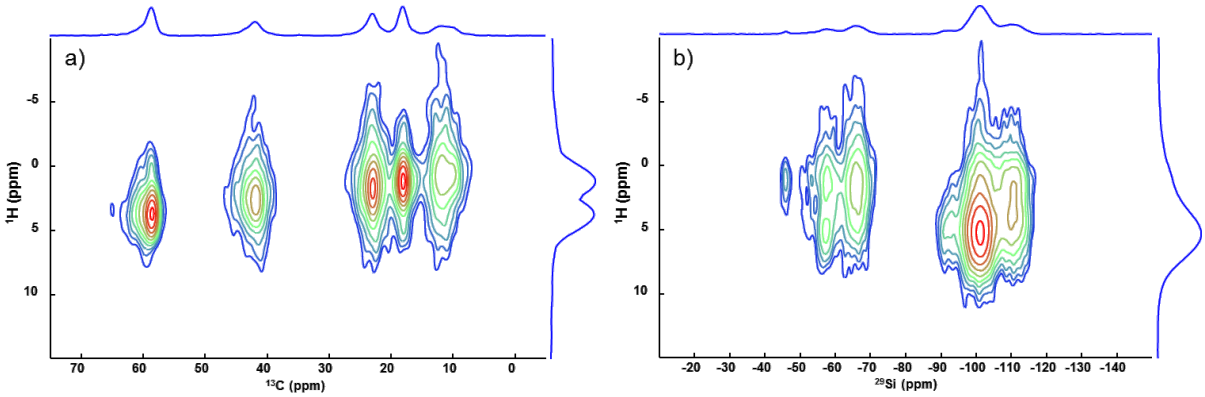

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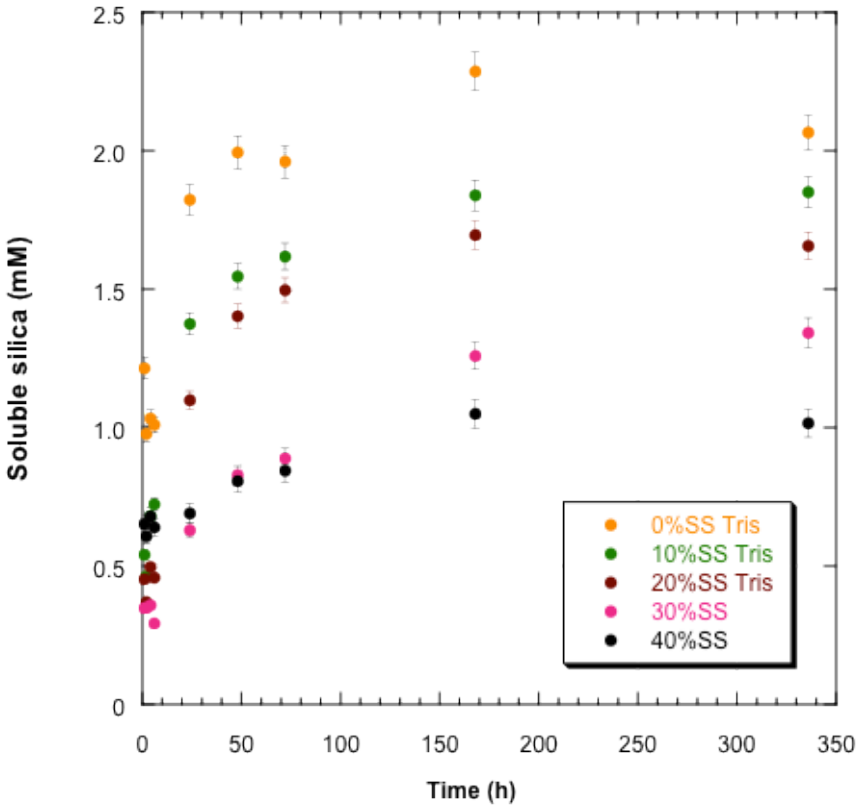

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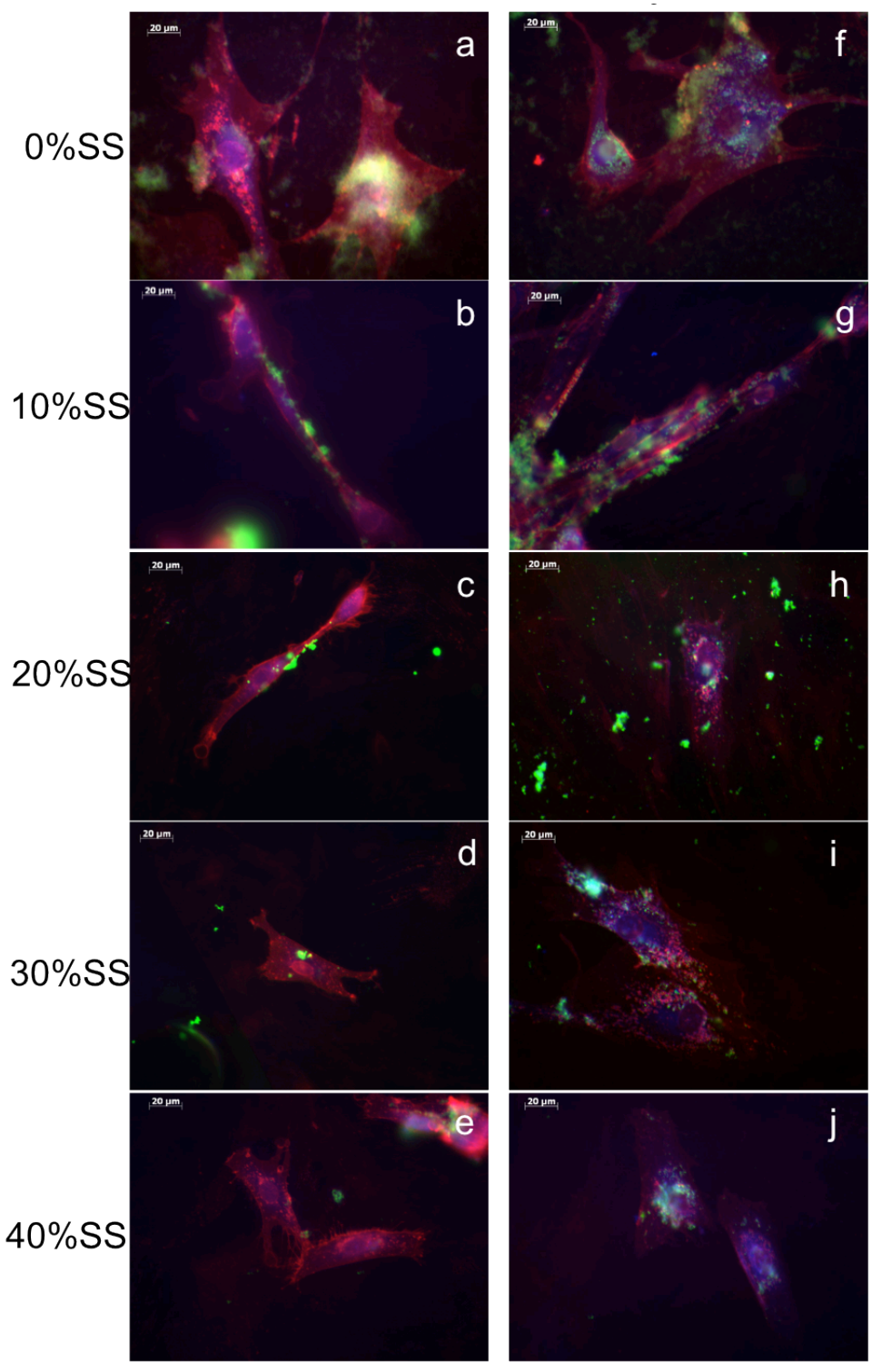

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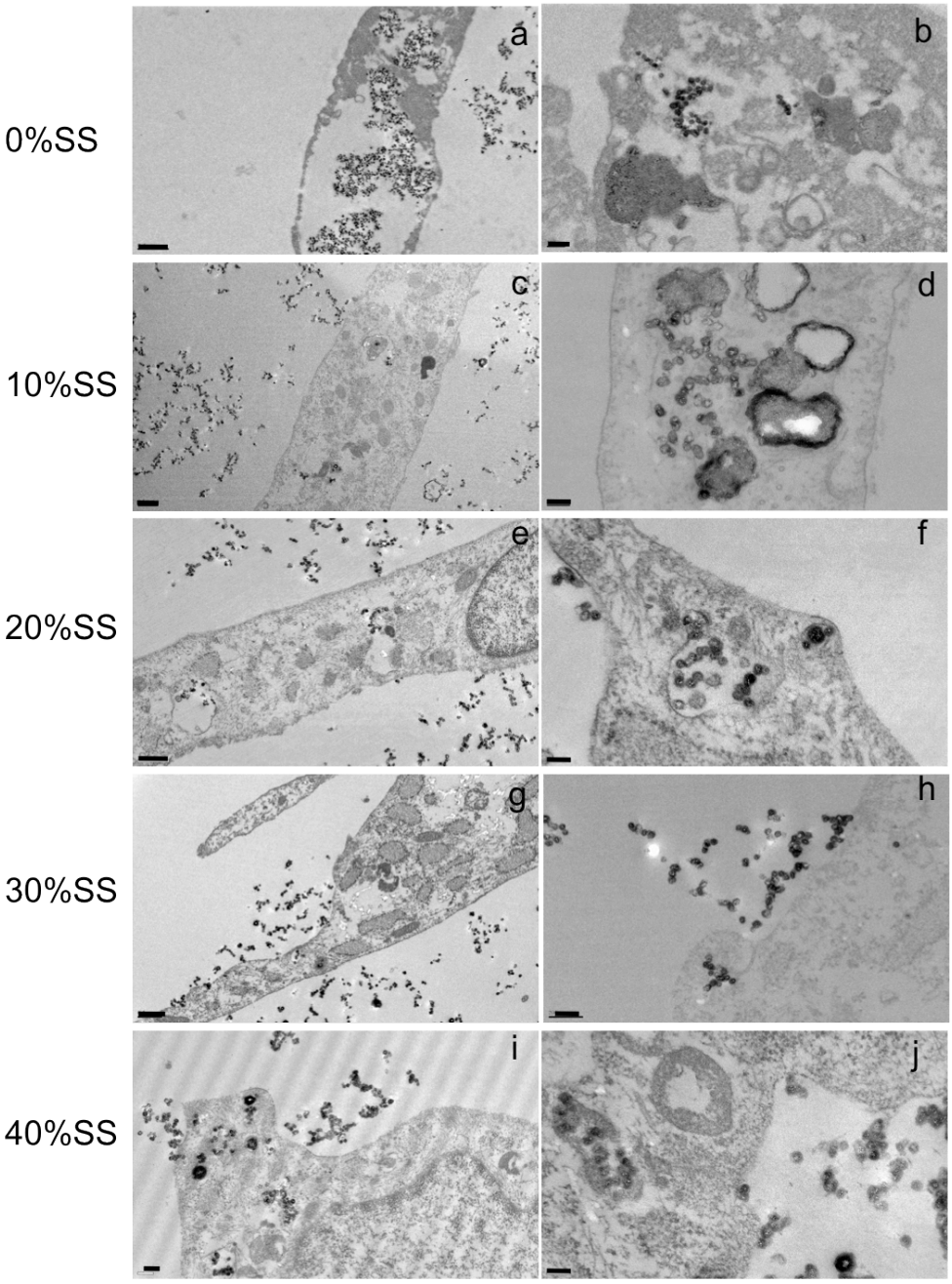

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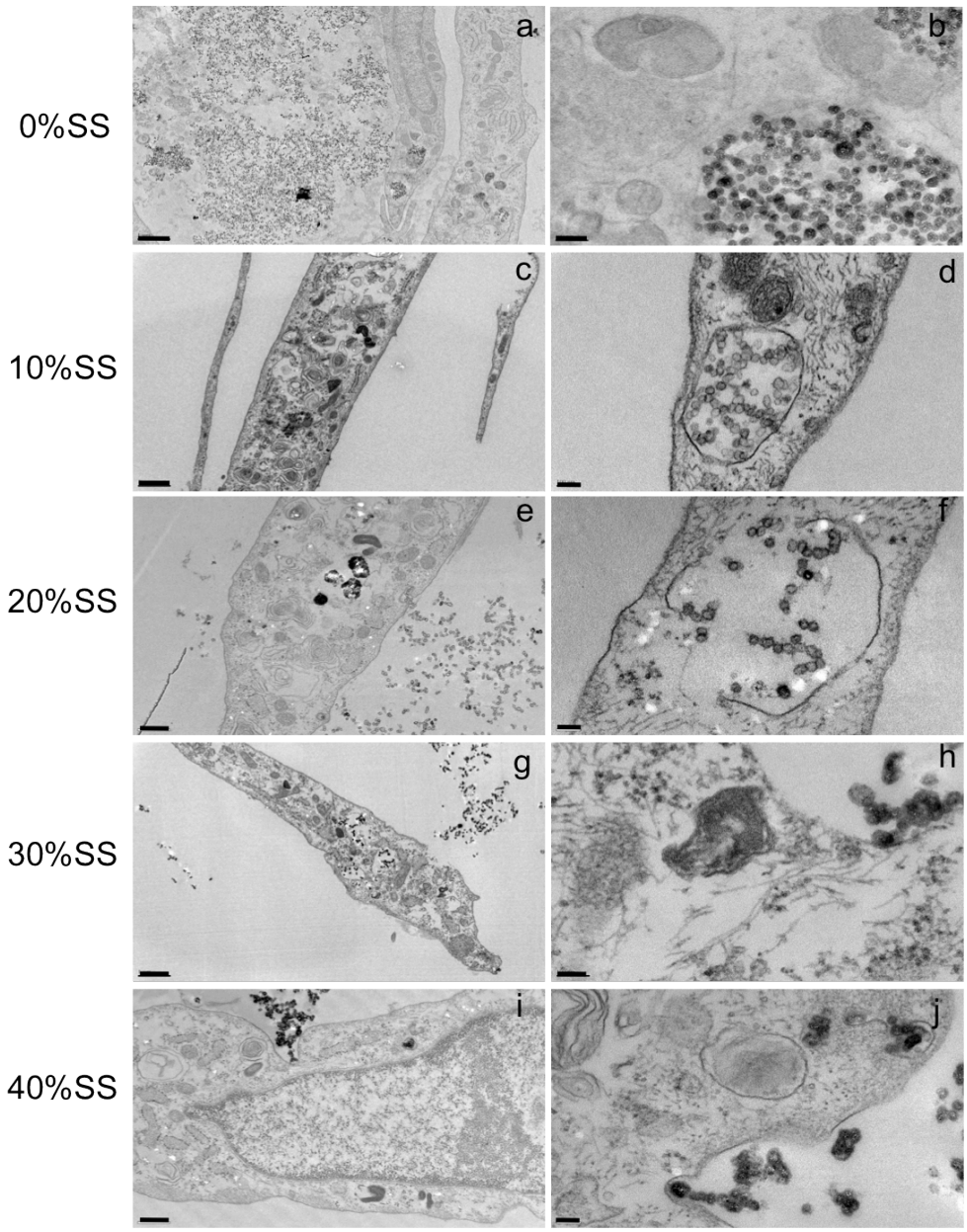

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