Elastic CNT-polyurethane nanocomposite: Synthesis, performance and assessment of fragments released during use

Online Supporting Information

Methods of lifecycle simulation testing

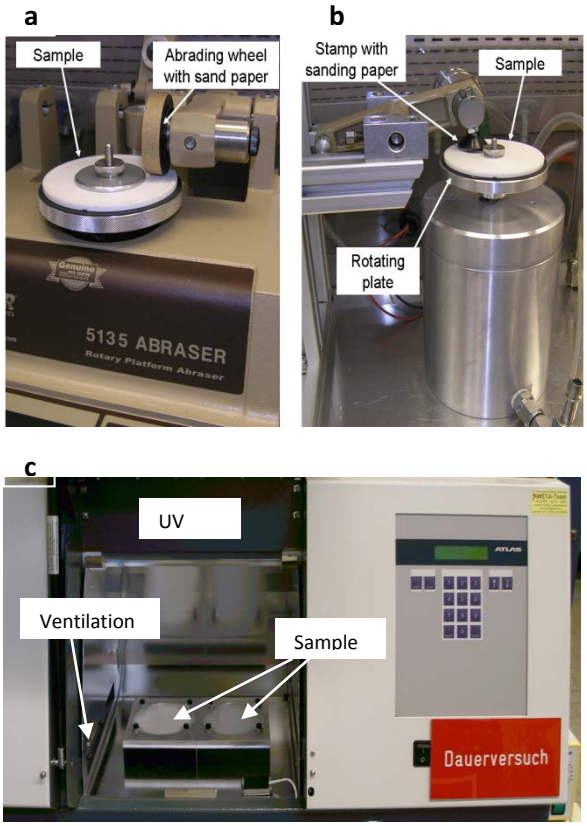

Fig. SI_1 Setups used to quantify release by three degradation scenarios. a) Taber Abraser™ with 0.29 m/s and 1kg load; b) Sanding paper with 6.5 m/s relative velocity and 0.25 kg load. The atmosphere is sampled with a hood surrounding the stamp. The electrical motor is encapsulated, because otherwise the aerosol is dominated by the wear from the graphite brush transducers; c) Weathering by UV irradiation in the Suntest XLS+ (in line with ISO 3892-2:2006). The samples are cooled by air ventilation of the metallic sample holders. UV-transparent glass covers ensure that any debris developed is not blown off.[1]

Method to quantify the content of smaller-than-150nm-fragments

The detection equipment employed, known as analytical ultracentrifugation (AUC) [2, 3] is especially suited to quantify traces of colloids within a heterogeneous mixture and is well established for CNT dispersions [4]. AUC quantifies the amount and the diameter of dispersed nanofillers and composite fragments independently of each other (0.5 – 10,000 nm diameter). [2] Here we use interference optics (Beckman model 'XLI proteome lab') and the raw data is fitted by the free-ware software SedFit. The mass concentrations read directly from the interference fringe shift without further conversion. To define the detection limit in the sub-100-nm region, we measured a water blank with interference-AUC and obtained the curve shown in Fig. SI_2a, blue line

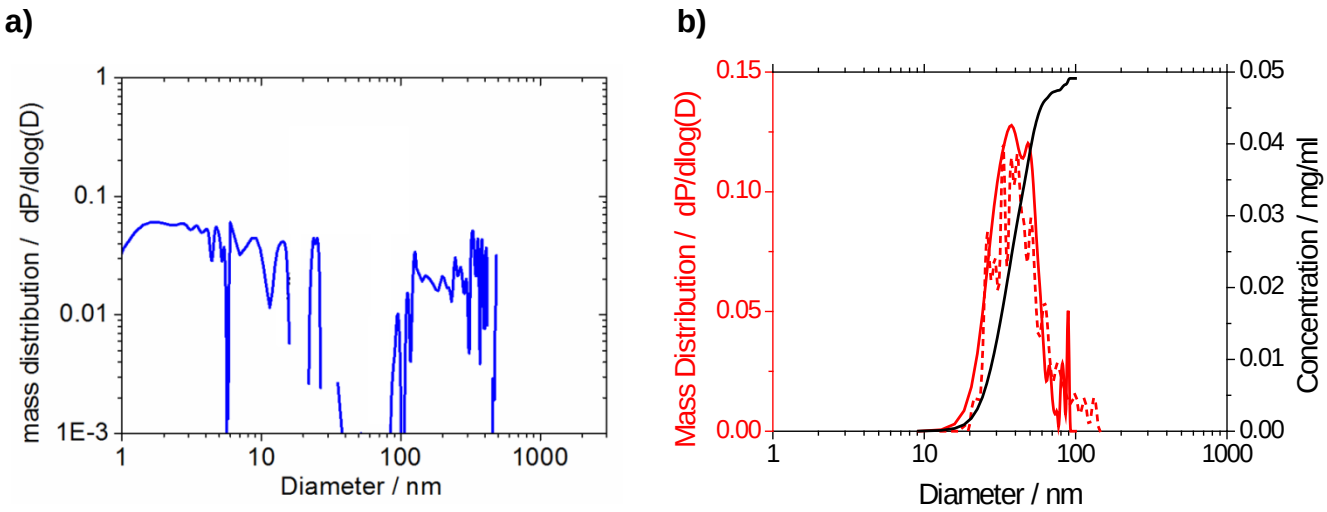

Fig. SI_2 Validation of the quantification of free CNTs in suspended sanding fragments. a) Water as negative control (blue solid line); b) Positive control: NC7000 suspended at 0.05 mg/ml in a 1 g/l SDS solution, measured after the same de-agglomeration protocol (red line: differential distribution, black line: cumulative distribution with absolute concentration from the interference detector, right-hand Y axis). The red dashed size distribution was measured after further addition of a 200-fold excess of TPU abrasion particles to the same CNT-suspension.

as negative control. Assuming a typical refractive index increment dn/dc = 0.2 cm^3/g, the integrated area under this curves gives a concentration of 0.05 mg/ml = 50 ppm. This noise level defines the detection limit. Note that the interference optics is strictly linear with the concentration, and no saturation occurs, no Mie correction is required.

The de-agglomeration protocol used probe ultrasonication (UP200S from Hielscher GmbH, used at 75W, 2 min., 24kHz, probe tip of 2 mm thickness, immersed 10 mm) with 1mg/ml SDS as dispersant. The sample volume was 5 ml in a 10 ml beaker.

We verify with our specific CNTs (Nanocyl NC7000) that the medium of suspension is effective in dispersing these CNTs, suspended at 0.05 mg/ml by probe ultrasonication. The interference centrifuge finds a characteristic signal at 20 nm – 100 nm (Figure SI_2b, solid red line). This first positive control confirms a good individualization of the CNTs by the above protocol. A second positive control verifies that the presence of larger particles does not interfere with the quantitative detection of CNTs. We prepared by the above protocol a suspension that contains both 10.00 mg/ml TPU abrasion powder (i.e. the CNT-free reference) and 0.05 mg/ml of CNTs and re-measured the particle size distribution (dashed red line in Figure SI_2b). The presence of the larger particles has slightly increased the noise level, but clearly the characteristic signal of the CNTs is recovered. This result was expected due to the fractionating measurement principle of AUC and confirms that by our protocol we can detect freely released CNTs in sanding powders. The integration of the interference signal (Fig. SI_2b, black line) retrieves the correct concentration with only 6% systematic error for the pure CNT suspension and no more than 20%

systematic error for CNT traces in the presence of large sanding fragments. This proves quantitative, size-selective identification down to the detection limit. The measured systematic error margins of 20% exceed the scatter in replicate measurements that is on the order of 5%. The combined error is indicated in Table 1. The hydrodynamic diameter is – as expected for tubes – closer to the cross-section than to the length as measured by TEM on a pure CNT reference.

The content of small fragments in the size range that would be compatible with free nanofillers c_{fragment} is set into relation to the total CNT content $c_{\text{filler\_total}}$ = 3% to derive the lower limit percentage x of nanofillers that remain embedded:

$$x = 1 - c_{\text{fragment}} / c_{\text{filler\_total}}$$

This estimation is a lower limit since the measured c_{fragment} does not necessarily represent only free nanofillers, but may also include matrix fragments within the same size range.

Results from mechanical treatments (Taber abraser and sanding)

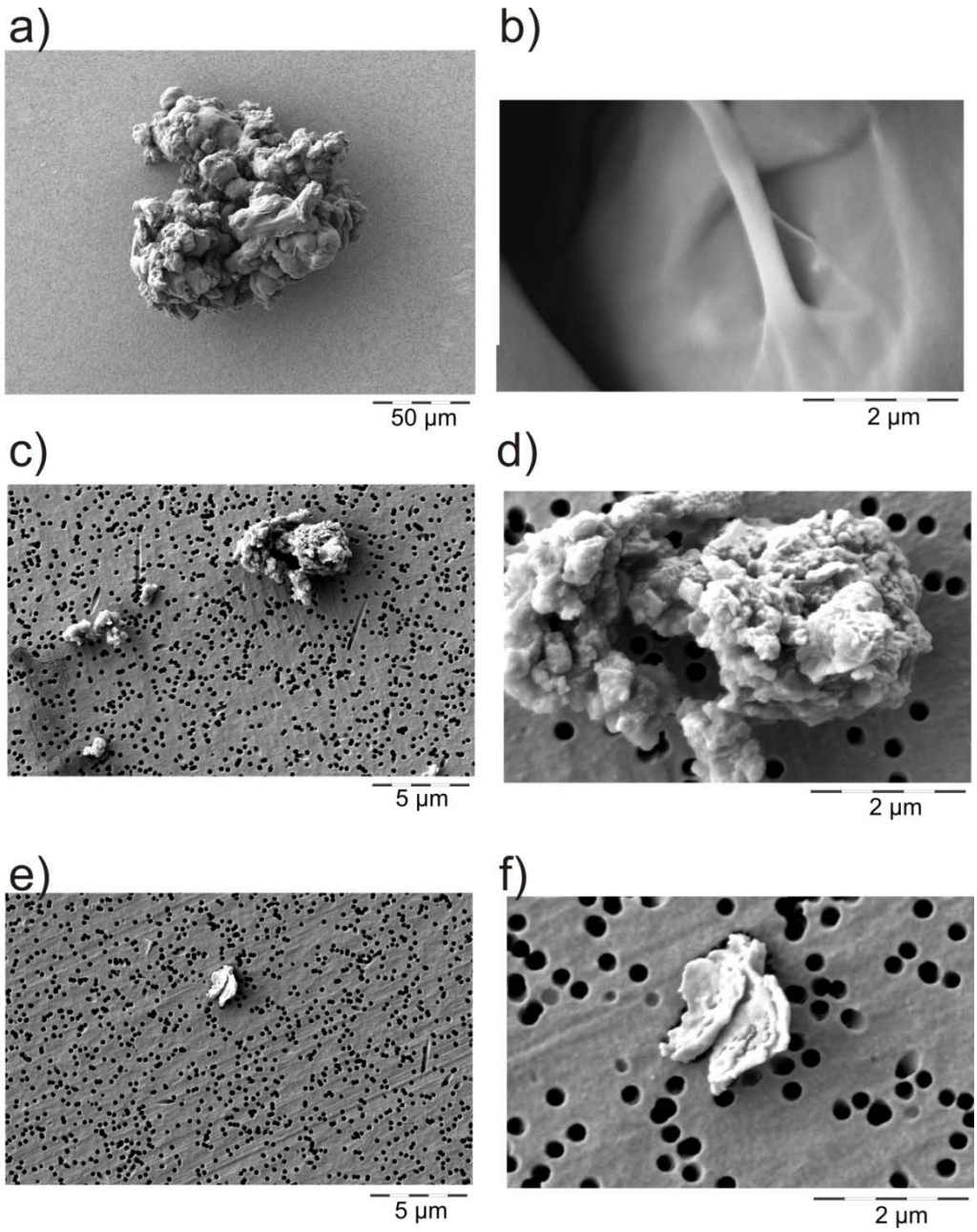

Fig. SI_3 Airborne structures released during sanding of the TPU+CNT nanocomposite, collected on gold filter and imaged by SEM. The filter surfaces were searched for 1.5 days, but no free fibrous structures with the dimensions of NC7000 CNTs were detected. The scanned area in a) is shown in higher magnification in b), and likewise for c) – d) and for e) – f). The EDXS elemental identification is compatible with polyurethane.

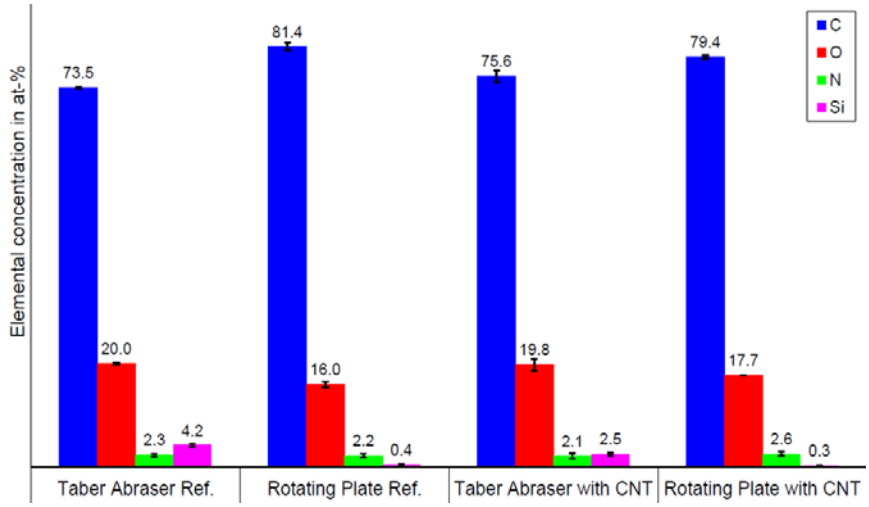

Fig. SI_4 Surface chemistry of the powders collected after mechanical treatment by Taber abraser or by sanding. XPS quantification of elements, showing some contamination by SiO_2 from the abrasive material of the Taber, but no significant difference of e.g. C with or without CNTs in the sample. Error bars represent the standard deviation of n=3 spots measured on the sample, as described in the methods section.

Results from weathering (with rain cycle)

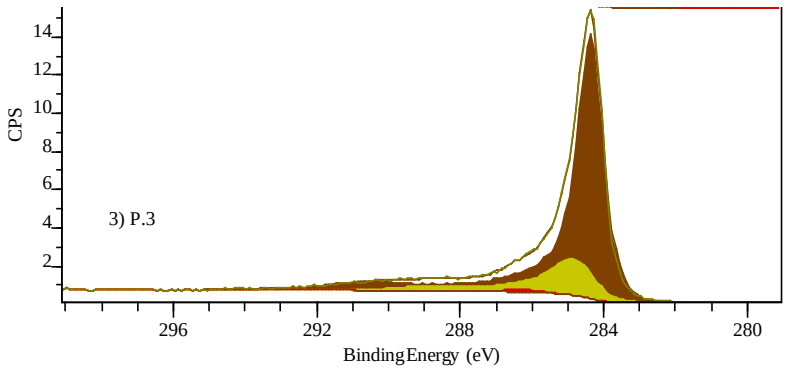

Fig. SI_5 Detailed identification of Carbon chemistry by XPS C(1s) spectral analysis after 1243h weathering with UV + rain. Yellow area: TPU reference; brown area: TPU+CNT after weathering; dark olive line: positive control CNT. The nanocomposite surface after wet weathering is dominated by the signal of pure CNTs that make up for 72% of the surface with 3% curve fitting uncertainty.

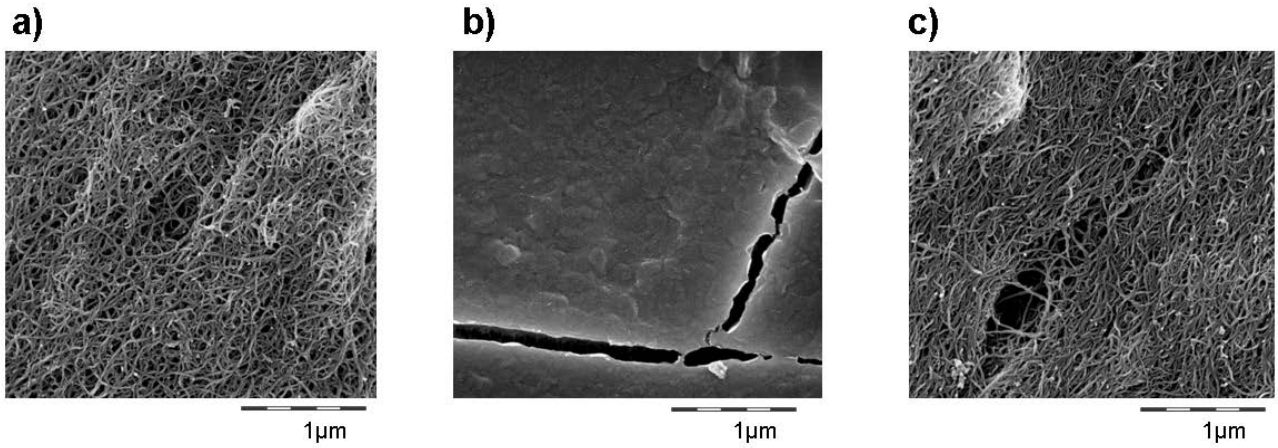

Fig. SI_6 Surface morphology (SEM) after 1243h (9 months equivalent) humidity + UV weathering. a) TPU+CNT, extrusion sample; b) TPU, injection molding sample; c) TPU+CNT, injection-molding sample

In-Vitro testing: Interaction of several nanomaterials with the LDH reagent, confirmatory cytotoxicity results

In order to exclude interferences between LDH and the tested materials, LDH standard was added to test preparations of ZnO, CNT, TPU and TPU + CNT. Analogous controls with the same test preparations were performed for the WST-1 assay and for the BCA assay.

Materials:

TPU	stock solution: 20 mg/mL, 0.5% BSA ;	final dilution 20 000 µg/mL
TPU + CNT	stock solution: 20 mg/mL, 0.5% BSA;	final dilution 20 000 µg/mL
CNT (NC7000)	stock solution: 3 mg/mL, 5% BSA;	final dilution 1 000 µg/mL
ZnO (NM-111)	stock solution: 10 mg/mL, 5% BSA;	final dilution 1 000 µg/mL

LDH Standard from Roche (550 U/mg@25°C), 100 mg/mL; dilution to 0.1 U/mL: 150 µL LDH stock plus 14.85 mL DMEM medium

Substance preparation:

First, a stock dispersion (3, 10 or 20 mg/mL) was prepared. Therefore the test substance was weight into a glass flask and covered with the respective volume of medium (DMEM/F-12 incl. 1% Penicillin/Streptomycin). 1 part of a 5% or 0.5% BSA solution (w/v) was added to 9 parts stock dispersion. To homogenize the solution it was shaken manually and sonicated three times with a probe sonicator for 2.5 min at 200 Watt (power: 20-30%; cycle: 100%). During sonication the flask was cooled with ice water in order not to increase the temperature of the dispersion. The final diluted dispersions were prepared in the required concentrations out of the stock dispersion. The dispersion was stirred for 24 h at 700 rpm at room temperature on a magnetic stirrer.

LDH Assay:
Four different approaches were assessed on a 96 well plate.

1. 50 µL Substance solution
   + 50 µL LDH Reagent

2. 50 µL Substance solution after centrifugation for 5 min. at 300 g
   + 50 µL LDH Reagent

3. 500 µL Substance solution were added to two frozen PCLuS and shaken for around 20 min, than centrifuged for 5 min. at 300 g and
   50 µL supernatant (substance solution) was mixed with
   + 50 µL LDH Reagent

4. 25 µL substance solution (after centrifugation for 5 min. at 300 g)
   + 25 µL of LDH Standard (0.1U/mL)
   + 50 µL LDH Reagent

After adding the LDH Reagent the plate was incubated for 20 min at RT in the dark and measured afterwards at 490 nm and 600 nm (reference wavelength).
No differences to the medium were detectable; all particles could be centrifuged easily. There are hence no indications of interferences between the particles and the read-out principle.

BCA assay:

50 µL of the substance solution was added to 500 µL 1% Triton solution --> duplicates
incubation for 1 hour at cell culture conditions
25 µL were pipetted into a well of a 96 well plate --> duplicates
200 µL of BCA reagent was added
incubation for 0,5 hour at cell culture conditions
measurement at 570 nm

To investigate interferences, the entire BCA assay was performed on suspensions containing the test substances, but without PCLuS, and had the following results:

	OD
0.5% BSA	0.20
5%BSA	0.18
TPU	0.20
TPU+CNT	0.19
CNT	0.25
NM110	0.22

We find that the optical density (OD) resulting in the presence of the TPU and TPU+CNT materials is identical to the results from the pure buffer with BSA content either 0.5% or 5%. A slight deviation (20% higher OD) occurs only for the suspension of the ‘comparison material’ of pure CNTs due to its UVVIS absorbance (black color of the suspension). A comparable black color is not observed in the lysed PCLuS of the CNT in vitro experiment, due to the lower tissue-bound CNT content. We conclude that no relevant interferences occur for the BCA assay in the TPU and TPU+CNT in vitro experiments.

WST assay:

50 µL of the substance solution was added to 250 µL WST solution (1:10 with Medium) in a 24 well plate --> duplicates
incubation for 1 hour at cell culture conditions
100 µL were pipette in a well of a 96 well plate --> duplicates
measurement at 450 nm (reference 690 nm)

To investigate interferences, the entire WST-1 assay was performed on the same suspensions as above without PCLuS, and had the following results:

	OD
0,5% BSA	0.046
5%BSA	0.043
TPU	0.042
TPU+CNT	0.045
CNT	0.037
NM110	0.050

Again, the results in the presence of the TPU and TPU+CNT materials are identical to the results from the pure buffer with BSA content either 0.5% or 5%. Again, a slight deviation (14% lower OD) occurs for the suspension of pure CNTs. With regard to the 20-fold higher OD level in the PCLuS experiment, where the TPU has OD of 0.85, the slight difference in the interference control of the 'comparison material' is regarded as not significant. The WST-1 assay successfully mitigates the known issues with the MTT assay.[5] We conclude that no relevant interferences occur for the WST-1 assay.

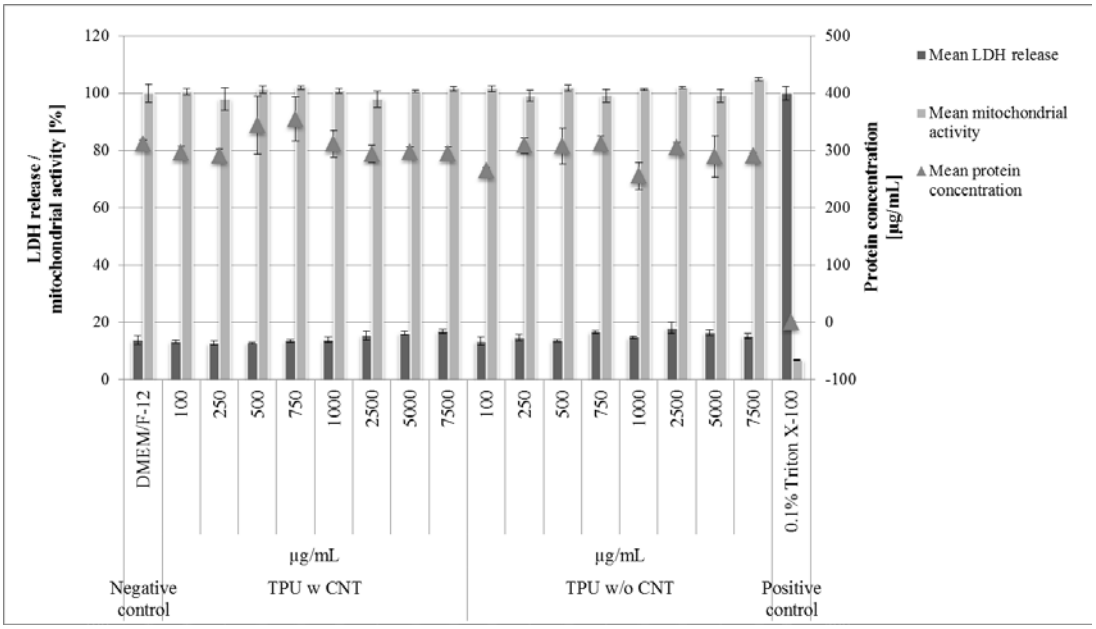

Fig. SI_7 Mean cytotoxicity (LDH release plotted in black bars and mitochondrial activity plotted in grey bars) and protein content (triangles) of PCLuS treated with positive control (Triton X-100) and TPU with or without CNT (mean ± MIN/MAX of 2 replicates). The mitochondrial activity (WST-1) was calculated relative to the negative control (=100%). The cell membrane integrity (LDH release) was calculated relative to the Triton X-100 positive control (= 100%). Protein content was calculated from standard curve included in the experiment.

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