**Supplementary information**

# Release of graphene-related materials from epoxy-based composites: characterization, quantification and hazard assessment in vitro

Woranan Netkueakul[1,2,#], Daria Korejwo[3,4,#], Tobias Hammer[1,2], Savvina Chortarea[3], Patrick Rupper [5], Oliver Braun[6,7], Michel Calame[5,6,8], Barbara Rothen-Rutishauser[4], Tina Buerki-Thurnherr[3], Peter Wick[3,‡], Jing Wang[1,2,‡]

[1] Institute of Environmental Engineering, ETH Zurich, 8093, Zurich, Switzerland

[2] Laboratory for Advanced Analytical Technologies, Empa, Swiss Federal Laboratories for Materials Science and Technology, 8600, Dübendorf, Switzerland

[3] Particles-Biology Interactions Lab, Empa, Swiss Federal Laboratories for Materials Science and Technology, 9014, St. Gallen, Switzerland

[4] Adolphe Merkle Institute, University of Fribourg, 1700 Fribourg, Switzerland

[5] Laboratory for Advanced Fibers, Empa, Swiss Federal Laboratories for Materials Science and Technology, 9014, St. Gallen, Switzerland

[6] Transport at Nanoscale Interfaces Laboratory, Empa, Swiss Federal Laboratories for Materials Science and Technology, 8600, Dübendorf, Switzerland

[7] University of Basel, Department of Physics, Basel, 4056, Switzerland

[8] University of Basel, Swiss Nanoscience Institute, Basel, 4056, Switzerland

[#] shared first author contribution

[‡] shared corresponding author contribution

**Abrasion process and particle collection**

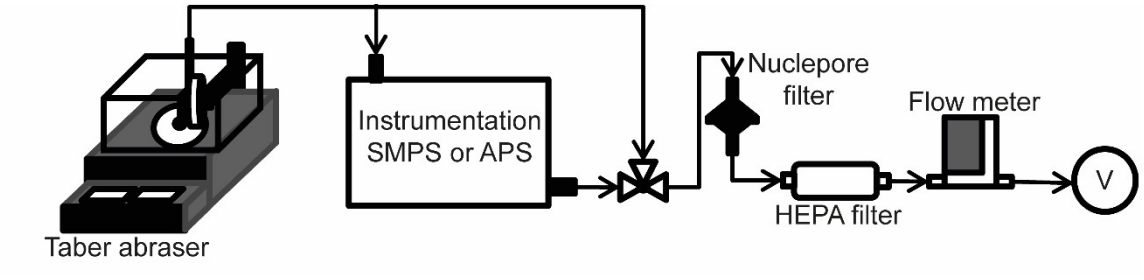



**Figure S1:** Schematic representation of experimental setup for particle size distribution measurement.

**Influence of the rectangular probe on particle sampling**

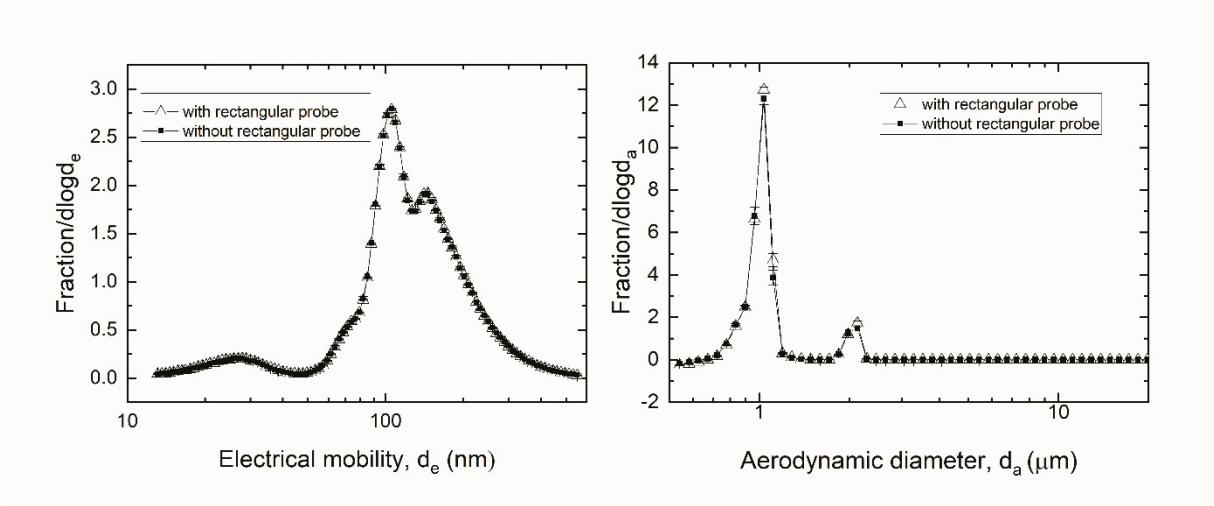
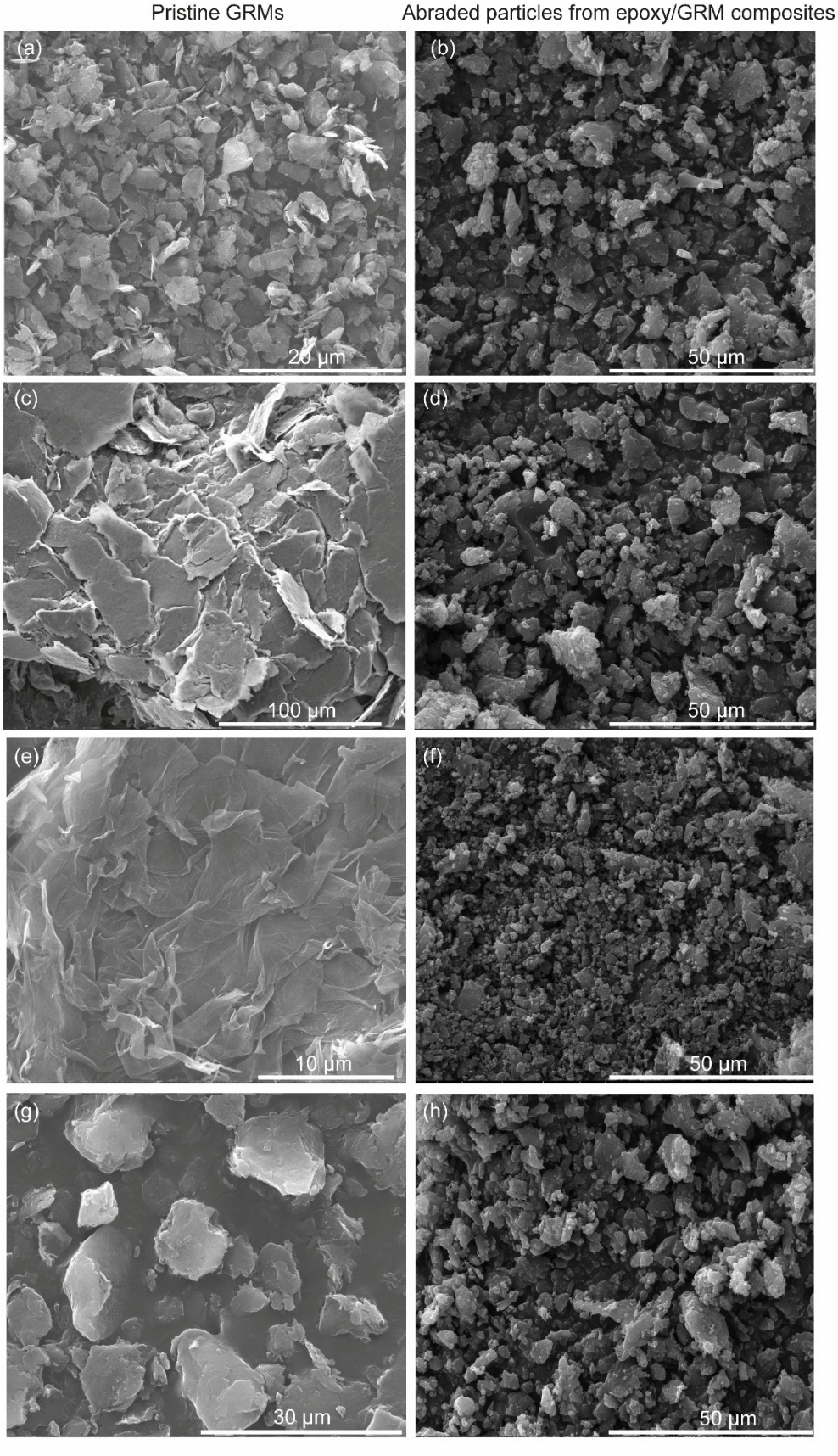



**Figure S2:** Size distribution of aerosolized PSL particles sampled in presence or absence of a rectangular probe. (a) Samples of aerosolized 105 nm PSL particles measured by SMPS from three independent measurements. (b) Samples of 1 μm and 2 μm PSL particles analyzed by APS from five independent measurements. Data represent mean +/- SD.

33 **Characterization of pristine GRMs and abraded particles**




34

**Figure S3:** SEM of pristine GRMs and abraded particles from epoxy/GRM composites. Images of pristine and abraded particles from samples of (a,b) GNP-1; (c,d) GNP-2, (e,f) GO-1 and (g,h) rGO.

From SEM images (Figure S3), in abraded particles from epoxy/GRM composites, GRMs cannot be distinguished from epoxy matrix. Therefore, it is not possible to observe the structural transformation of GRMs from SEM images. We performed Raman spectroscopy, which can be ones of the methods used to explain structural transformation of the GRMs in the composites. As shown in Figure 3b, by comparing the Raman spectrum of the pristine GNP-2 to that of the GNP-2 in the abraded composite, the I(D)/I(G) ratio appeared to have different intensity (spectra B and C). This suggested that GNP might be transformed during the fabrication or abrasion process resulting in defected structure. In addition, the effect of manufacturing process on the sizes of GRMs were studied by analysis of optical microscopic images of GRMs in epoxy matrix, which is demonstrated below.

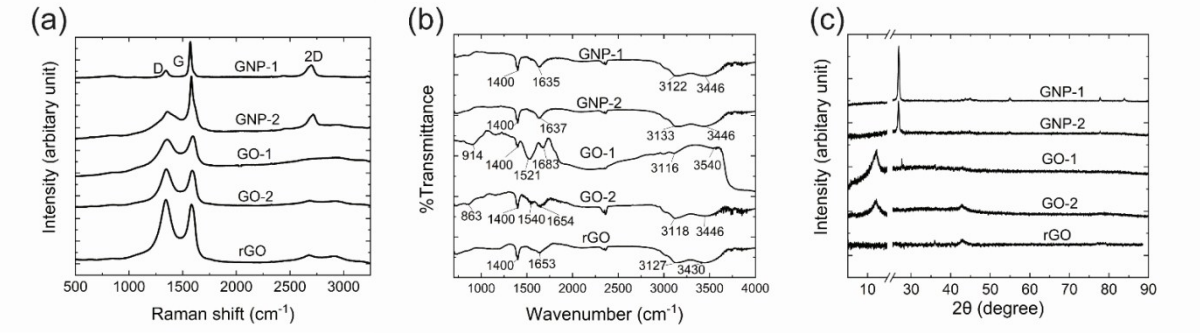



**Figure S4:** Characterization of pristine GRMs. (a) Raman spectra (b) FTIR spectra and (c) XRD patterns of pristine GRMs.

**Table S1** Hydrodynamic size of pristine GRMs

| | **$Z_{ave}$ diameter (nm)/ PdI** | | | | |
|---|---|---|---|---|---|
| **GRMs** / **Dispersant** | GNP-1 | GNP-2 | GO-1 | GO-2 | rGO |
| **Water** | NA | NA | 765 ± 17.1/ 0.42 ± 0.03 | 255 ± 1.68/ 0.18 ± 0.01 | 243 ± 8.13/ 0.45 ± 0.07 |
| **Complete RPMI-1640 medium** | NA | NA | 940 ± 87.7/ 0.55 ± 0.04 | 275 ± 16.1/ 0.24 ± 0.04 | 95.4 ± 24.0/ 0.62 ± 0.14 |

NA: When polydisperse index (PdI) is larger than 0.7, the data is not valid and the results are marked as NA (Not Applicable). The DLS measurement is not suitable for these particles because GNP-1 and GNP-2are polydisperse and tend to agglomerate.

**Table S2** Hydrodynamic size of abraded particles from epoxy/GRM composites

| | **$Z_{ave}$ diameter (nm)/ PdI** | | | | | |
|---|---|---|---|---|---|---|
| **Abraded particles** / **Dispersant** | Neat epoxy | Epoxy/GNP-1 | Epoxy/GNP-2 | Epoxy/GO-1 | Epoxy/GO-2 | Epoxy/rGO |
| **Water** | 1230 ± 260/ 0.696 ± 0.180 | 1822 ± 139/ 0.559 ± 0.122 | NA | NA | NA | NA |
| **Complete RPMI-1640 medium** | NA | NA | NA | NA | NA | NA |

NA: When polydisperse index (PdI) is larger than 0.7, the data is not valid and the results are marked as NA (Not Applicable). The DLS measurement is not suitable for most of these particles because they are polydisperse and tend to agglomerate.

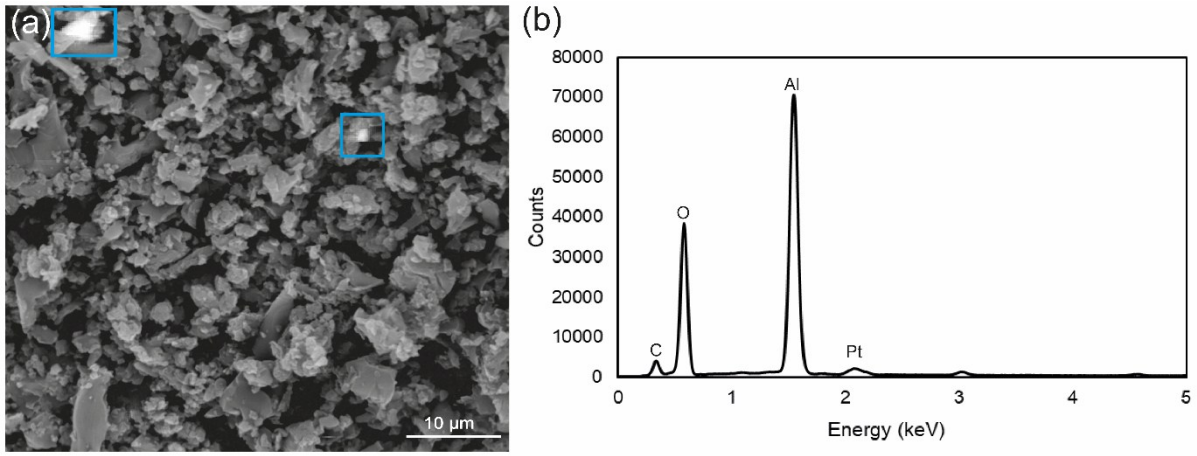



**Figure S5:** SEM-EDX analysis of abraded particles from neat epoxy. (a) Representative SEM image of abraded particles from neat epoxy. Aluminum appears as white spots, which were marked in blue boxes.(b) EDX spectrum from point analysis of the white spot marked by a rectangle in (a).

**Table S3** Summary of the analyzed parameters from the fitted particle size distributions

| Sample | Parameter | Mode 1 | Mode 2 | Mode 3 | Mode 4 |
|---|---|---|---|---|---|
| Neat epoxy | $d_e$ or $d_a$ [(a)] | 0.333 ± 0.0073 | 1.087 ± 0.0826 | 2.271 ± 0.0565 | - |
| | CMD | 0.461 ± 0.011 | 1.274 ± 0.1220 | 2.516 ± 0.0548 | - |
| | $\sigma_g$ | 1.76 ± 0.01 | 1.48 ± 0.04 | 1.38 ± 0.01 | - |
| | F | | 0.26 ± 0.01 | 0.74 ± 0.01 | - |
| E/GNP-1 | $d_e$ or $d_a$ [(a)] | 0.302 ± 0.0123 | 1.148 ± 0.0760 | 2.306 ± 0.0791 | - |
| | CMD | 0.445 ± 0.011 | 1.372 ± 0.1248 | 2.605 ± 0.0710 | - |
| | $\sigma_g$ | 1.86 ± 0.03 | 1.52 ± 0.05 | 1.39 ± 0.01 | - |
| | F | | 0.22 ± 0.01 | 0.78 ± 0.01 | - |
| E/GNP-2 | $d_e$ or $d_a$ [(a)] | 0.314 ± 0.0102 | 1.075 ± 0.0834 | 2.300 ± 0.0601 | - |
| | CMD | 0.480 ± 0.0212 | 1.259 ± 0.1323 | 2.574 ± 0.0416 | - |
| | $\sigma_g$ | 1.81 ± 0.03 | 1.48 ± 0.05 | 1.40 ± 0.02 | - |
| | F | | 0.21 ± 0.01 | 0.79 ± 0.01 | - |
| E/GO-1 | $d_e$ or $d_a$ [(a)] | 0.338 ± 0.0159 | 0.632 ± 0.0072 | 0.832 ± 0.0159 | 2.075 ± 0.0249 |
| | CMD | 0.465 ± 0.0176 | 0.637 ± 0.0066 | 0.974 ± 0.0027 | 2.286 ± 0.0362 |
| | $\sigma_g$ | 1.76 ± 0.02 | 1.09 ± 0.00 | 1.49 ± 0.04 | 1.36 ± 0.01 |
| | F | | 0.01 ± 0.00 | 0.40 ± 0.03 | 0.59 ± 0.03 |
| E/GO-2 | $d_e$ or $d_a$ [(a)] | 0.327 ± 0.0143 | 0.632 ± 0.0072 | 0.847 ± 0.0078 | 2.027 ± 0.0273 |
| | CMD | 0.460 ± 0.0257 | 0.636 ± 0.0020 | 0.959 ± 0.0013 | 2.303 ± 0.0412 |
| | $\sigma_g$ | 1.77 ± 0.02 | 1.09 ± 0.00 | 1.42 ± 0.01 | 1.43 ± 0.01 |
| | F | | 0.01 ± 0.00 | 0.28 ± 0.02 | 0.71 ± 0.02 |
| E/rGO | $d_e$ or $d_a$ [(a)] | 0.341 ± 0.0036 | 0.582 ± 0.0014 | 0.891 ± 0.0164 | 1.902 ± 0.0713 |
| | CMD | 0.479 ± 0.0142 | 0.595 ± 0.0048 | 1.008 ± 0.0426 | 2.123 ± 0.0573 |
| | $\sigma_g$ | 1.79 ± 0.03 | 1.15 ± 0.01 | 1.42 ± 0.05 | 1.39 ± 0.02 |
| | F | | 0.02 ± 0.01 | 0.33 ± 0.04 | 0.65 ± 0.03 |

[a] Mode 1 was obtained from SMPS, whose particle size corresponded to electrical mobility $d_e$, while mode 2 – 4 were obtained from APS, whose particle size corresponded to aerodynamic diameter $d_a$.

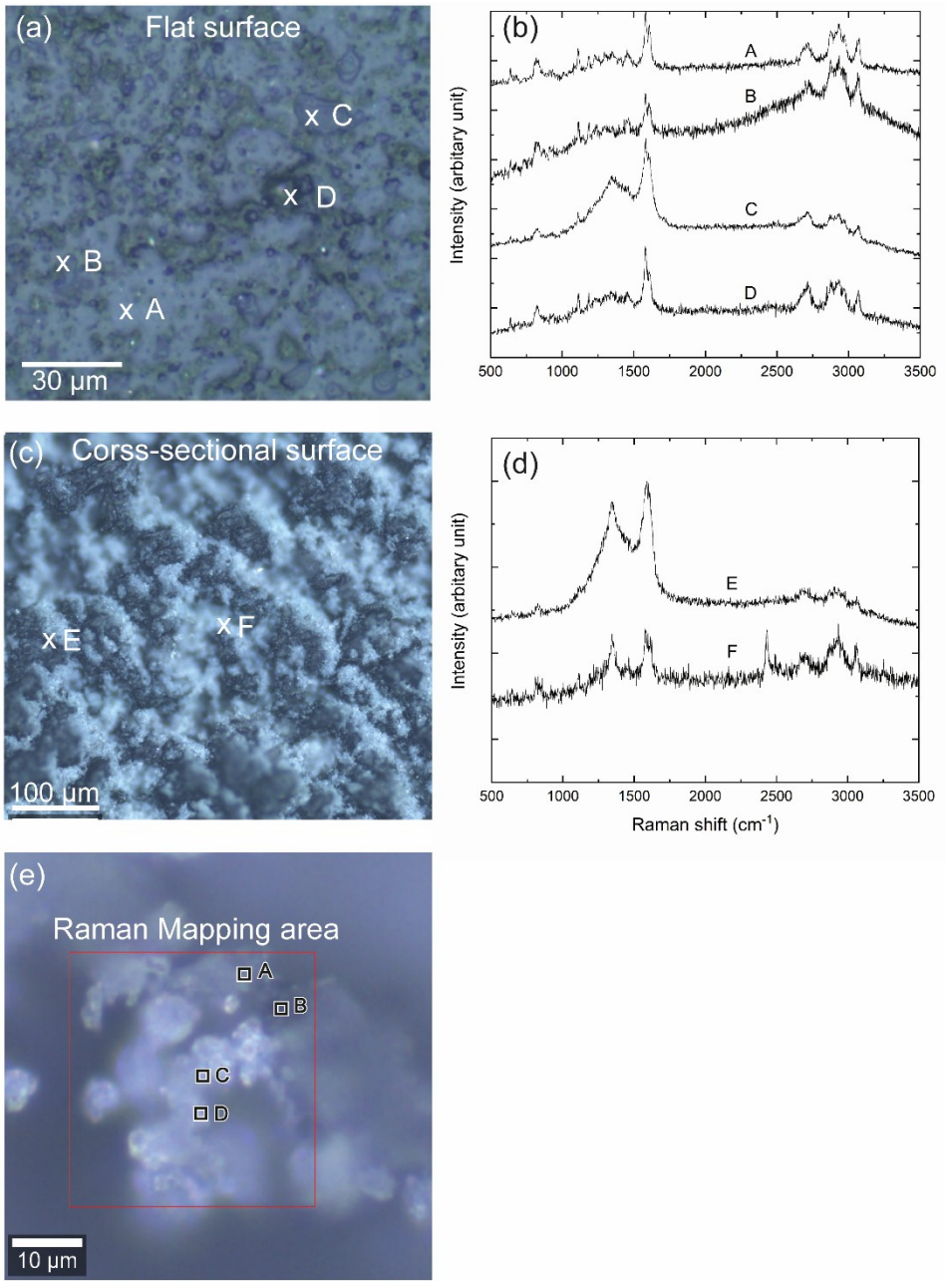



**Figure S6:** Spots of Raman spectroscopy analysis of (a) flat surface and (c) cross-sectional surface of E/GNP-2 composites and the corresponding spectra on (b) flat surface and (d) cross-sectional surface. (e) Optical image of the abraded particles from E/GNP-2 with Raman mapping area marked by red square and four points (A-D) where the individual spectrum was shown in Figure 3.

**Effect of manufacturing process on sizes of GRMs**

We analyzed the transformation of GRM size using images from SEM and optical microscopy. ImageJ software was employed to detect the particles and obtain the projected area of the detected particles. SEM images of pristine GNP-2 (Figure S7) were analyzed by manually drawing the particle edge and the projected area of the identified particles were calculated using ImageJ. Optical microscopic images of GRMs in epoxy matrix were analyzed using ImageJ to detect GRM particles and then to calculate the corresponding particles' projected area. Since the observed GRM particles have irregular shape, particle size can be referred to projected area equivalent diameter, which is the diameter of a spherical particle having the same projected area as the considered particle.

Since the processing of epoxy/GRM nanocomposites involved high speed mixing, three-roll milling, addition of hardener and curing, we showed the GRM sizes in the epoxy matrix after two important steps before curing i.e. high speed mixing and addition of hardener after three-roll milling. Optical images of GNP-2 particles in epoxy matrix after high speed mixing and after three-roll milling were displayed in Figure 8a-c and Figure 8d-f, respectively. Figure S9 and Figure S10 show GNP-1 and GO-1 in epoxy matrix after three-roll milling, respectively.

Histograms of the particle sizes of GNP-2 after each step, and GNP-1 and GO-1 after three-roll milling were summarized in Figure S11. At least three images were analyzed to plot a histogram.

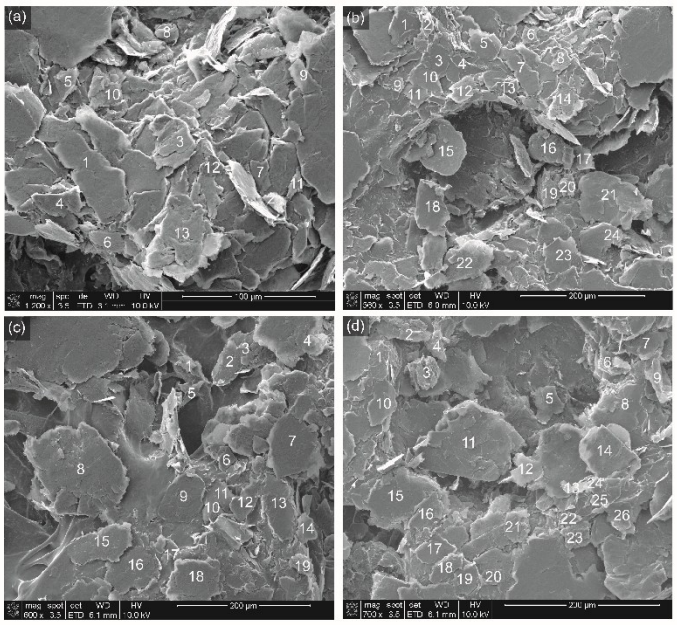
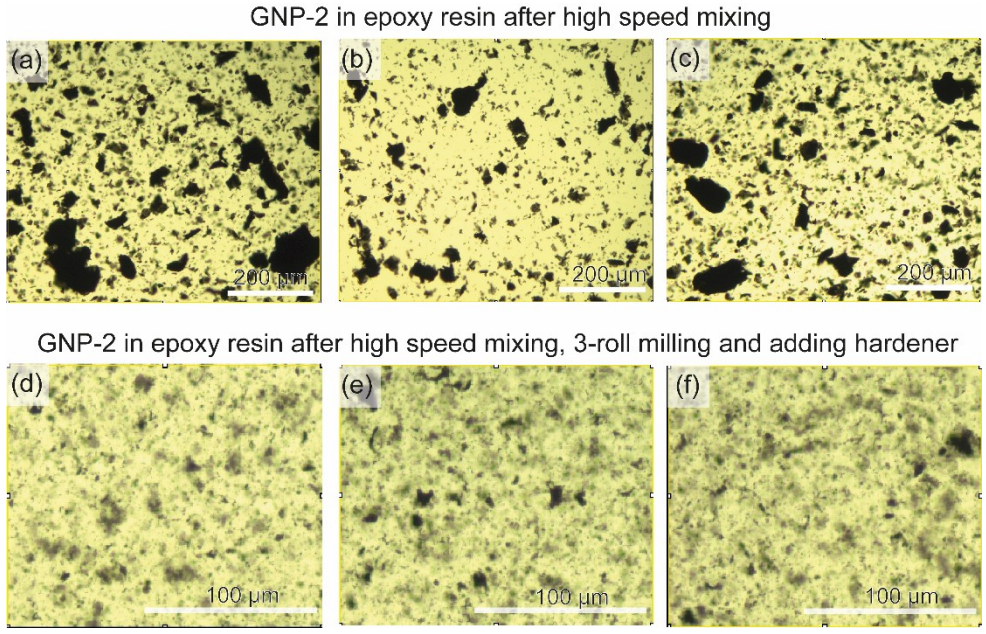


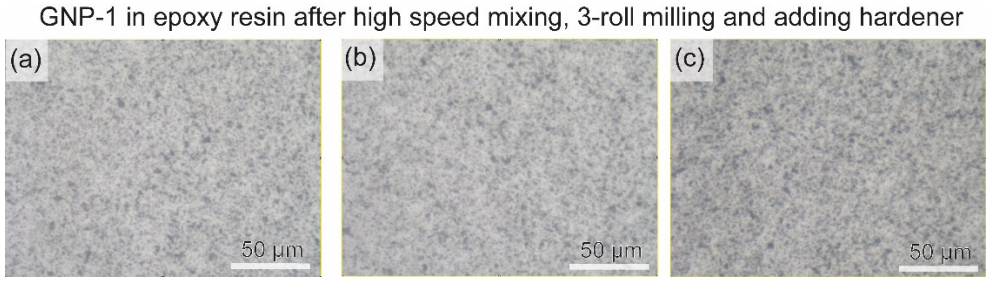

**Figure S7:** SEM images of pristine GNP-2. Number labelling indicates the particles whose edge could be identified and thus their corresponding projected area could be analyzed by ImageJ.




96

97 **Figure S8:** Optical micrographs of GNP-2 in epoxy resin matrix (a)- (c) after high speed mixing and (d)

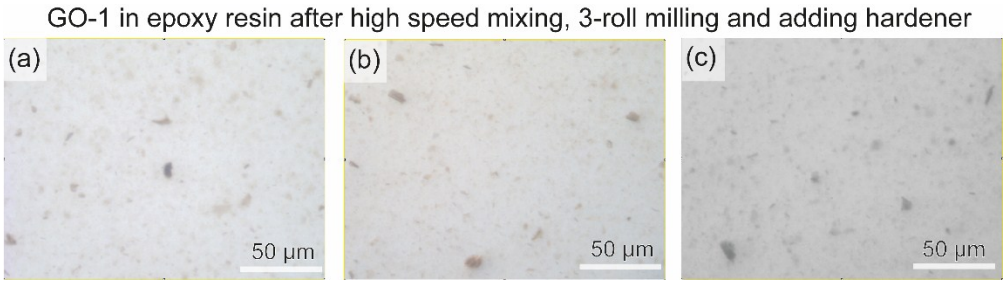
98 – (f) after high seed mixing, three-roll milling and adding hardener




99

100 **Figure S9:** Optical micrographs of GNP-1 in epoxy resin matrix after high speed mixing followed by

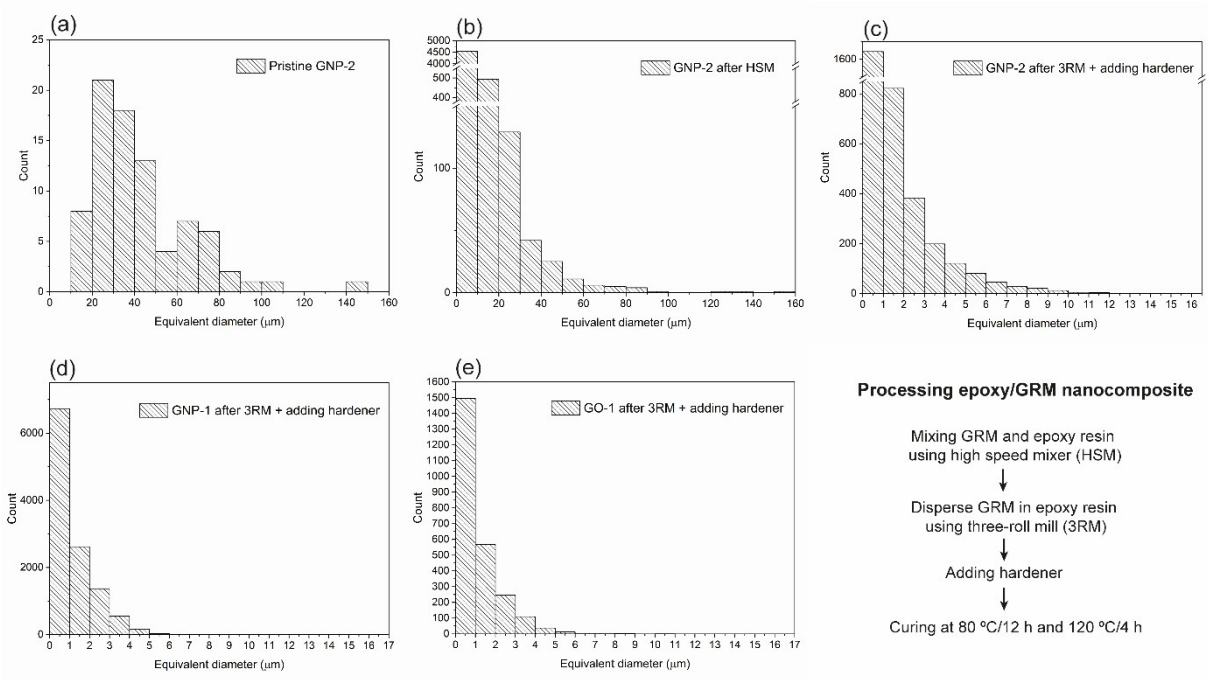
101 three-roll milling and adding hardener




102

103 **Figure S10:** Optical micrographs of GO-1 in epoxy resin matrix after high speed mixing followed by

104 three-roll milling and adding hardener




105

106 **Figure S11:** Histograms of GRM size before and during processing of epoxy/GRM nanocomposite.
107 Histogram of (a) pristine GNP-2, (b) GNP-2 in epoxy resin matrix after high speed mixing (HSM), and
108 (c) after three-roll milling (3RM) and adding hardener. Histogram of (d) GNP-1 and (e) GO-1 after
109 three-roll milling and adding hardener.

110

111

**Exclusion of interferences of GRMs and abraded neat epoxy particles in the MTS and DCF assays**

MTS interference was performed regarding the ability of GRMs to reduce MTS without cellular contribution. The treatment of interference control samples and the assay procedure were identical to the protocol described in materials and methods. GRMs and neat epoxy show a certain intrinsic absorbance, which was corrected by subtracting the background, but are not reactive, i.e. do not process MTS (Figure S7a) when comparing the absorbance to an untreated control (-) including cells. To determine the influence of GRMs and neat epoxy quenching an existing fluorescent signal, particles were incubated with the fluorescent dye DCF as described in the material and methods. Figure S7b shows the relative fluorescence in percentage to the control samples with no GRMs as 100%, whereas GRM containing samples show the impact on the intensity of the fluorescence.

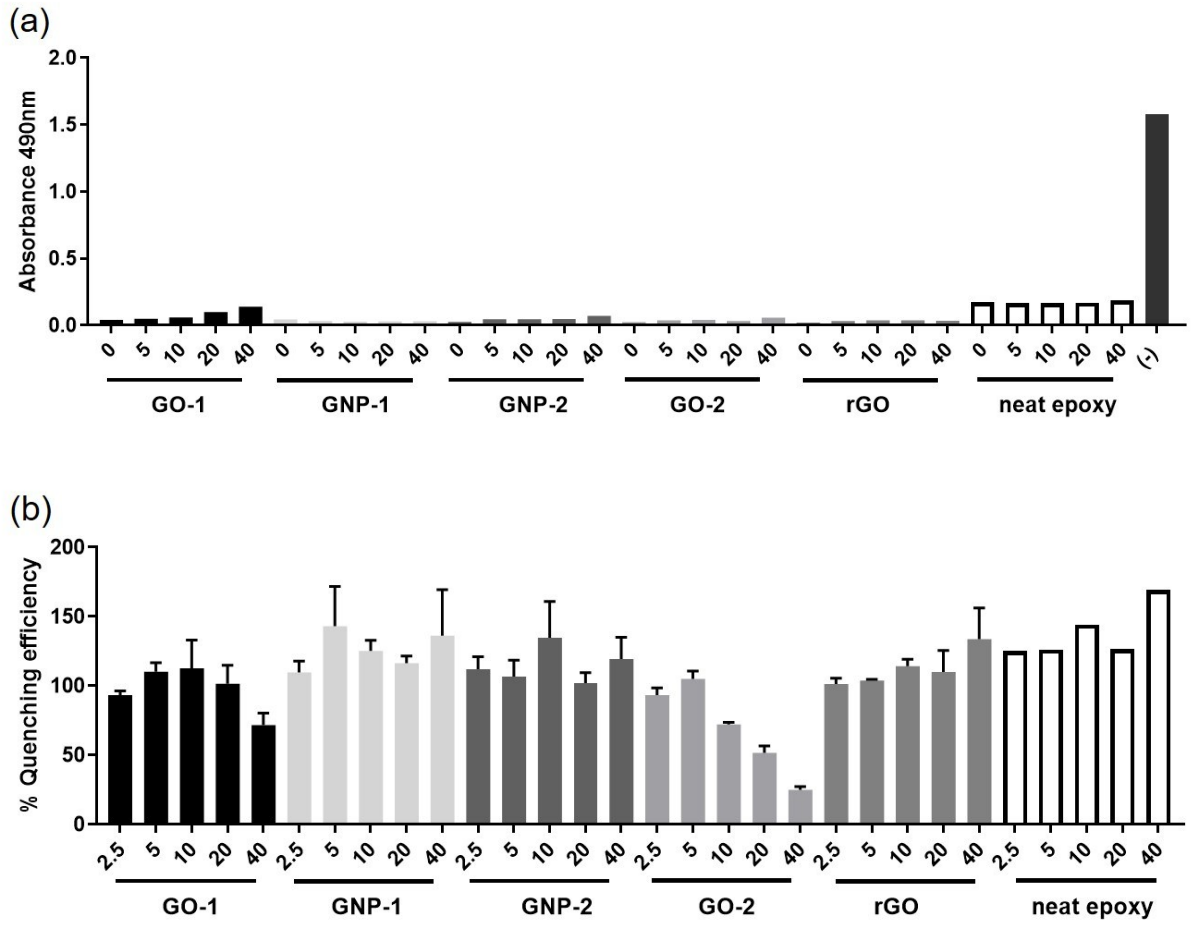



**Figure S12:** Interference assessment of the pristine GRMs and neat epoxy with the MTS and DCF assay. (a)Pristine GRMs and abraded neat epoxy particles did not interfere with the MTS measurements. (-) is a representative absorbance value of an untreated control measurement with

THP-1 cells. Data represent a single experiment (b) Abraded neat epoxy particles did not quench, but GRMs quench an existing DCF signal. % Quenching efficiency displays relative fluorescent values (to untreated control). Mean values and corresponding standard deviations from three independent experiments are shown for pristine GRMs while one only one experiment was performed for abraded neat epoxy particles.

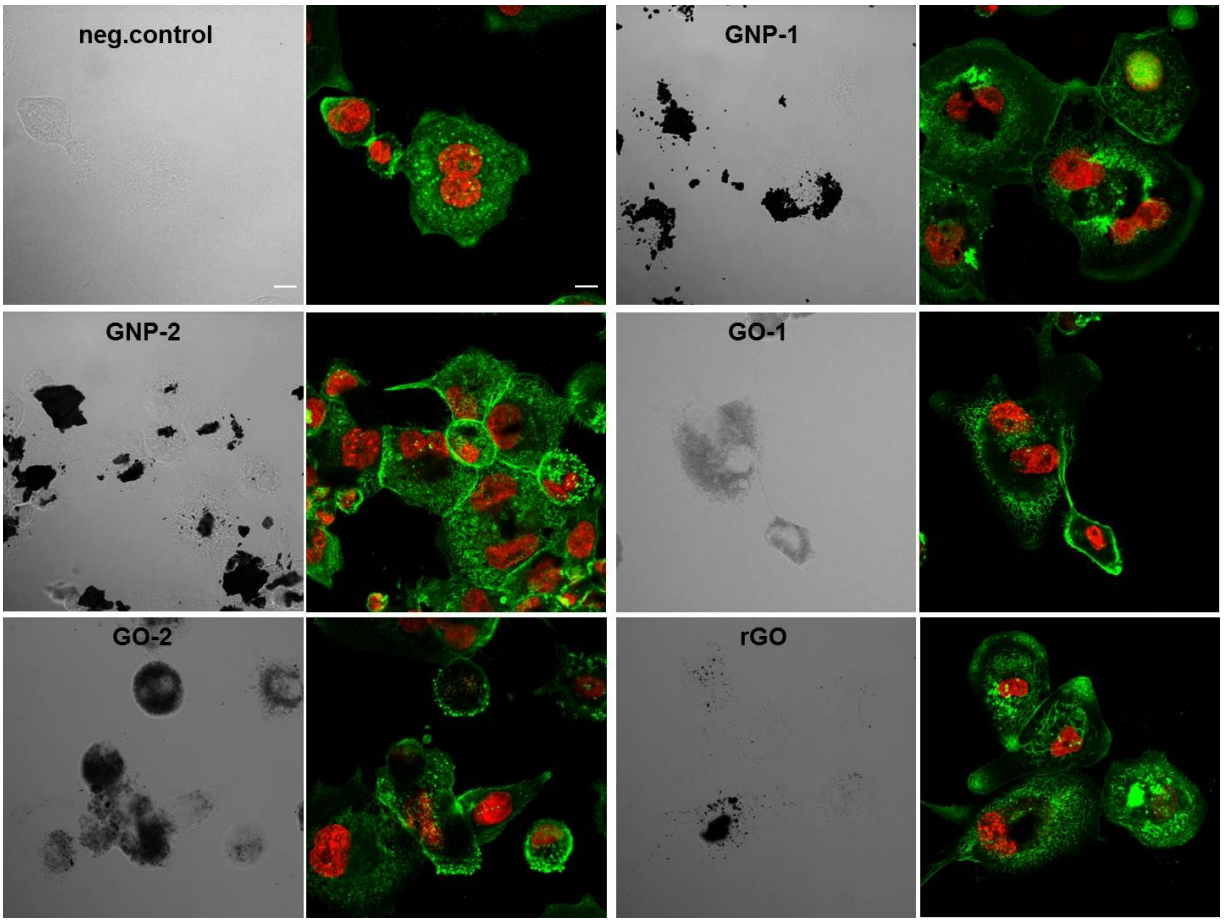



**Figure S13:** Light microscopy and confocal microscopy images of THP-1 macrophages exposed to 40µg/mL GRM for 48h, Actin cytoskeleton (green), Nuclei (red) and GRMs (black), scale bar =10µm

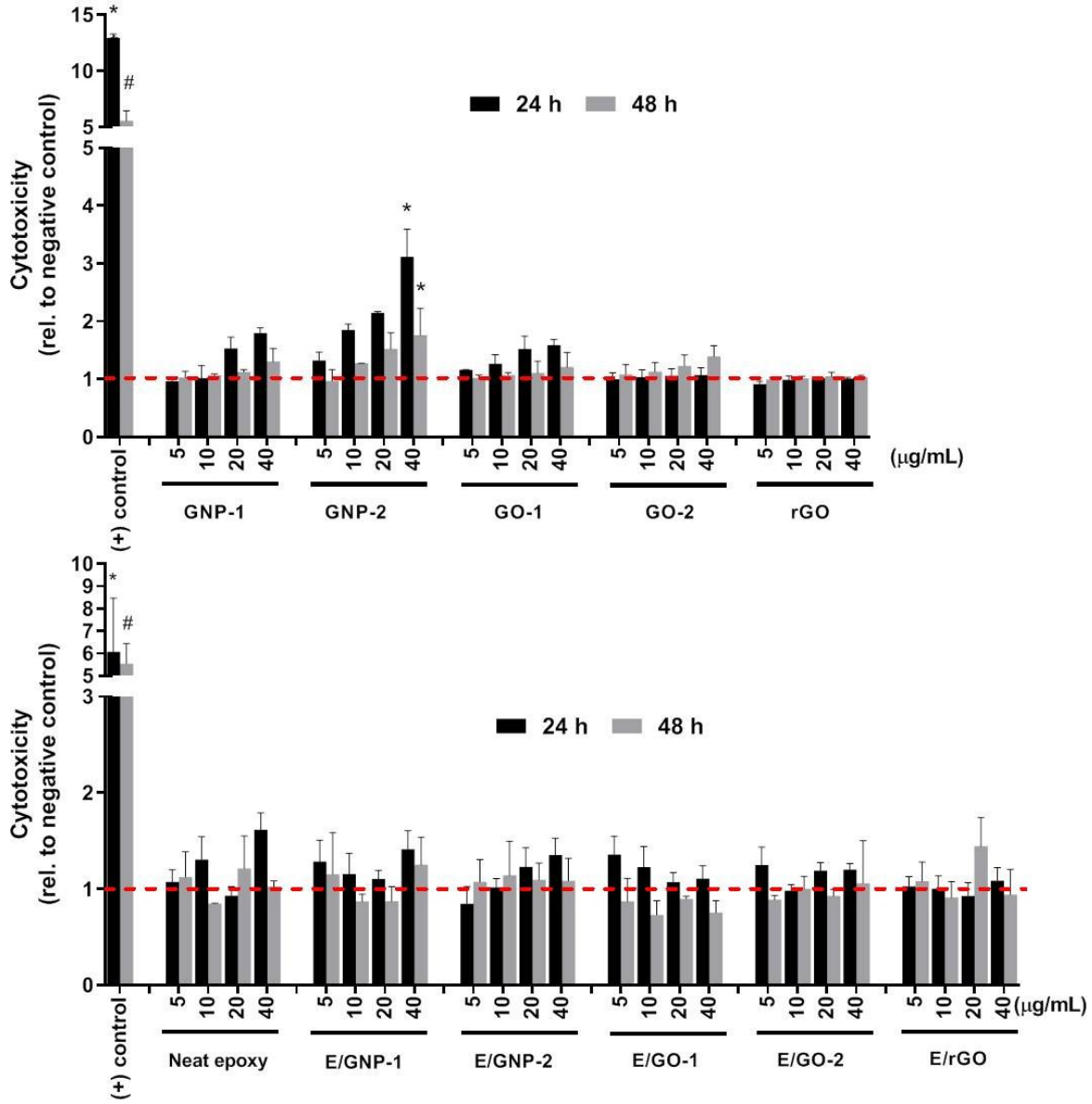



**Figure S14:** LDH release from THP-1 macrophages after exposure to GRMs and E/GRMs for 24 h and 48 h. Data shown represent the mean ± StEM of at least three independent experiments**.** The * symbol represents $p<0.05$ as compared to negative control (untreated cells). Incubation of cells for 1h to 0.2% Triton-X served as positive control (+)