Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B
This journal is © The Royal Society of Chemistry 2014

Electronic Supporting Information

Light-triggered CO release from nanoporous non-wovens

Carmen Bohlender,[a] Steve Gläser,[a] Moritz Klein[c], Jürgen Weisser,[b] Susanne Thein,[b] Ute Neugebauer[c,f], Jürgen Popp[c,e,f], Ralf Wyrwa,[b] Alexander Schiller*[ade]

[a] Friedrich Schiller University Jena, Institute for Inorganic and Analytical Chemistry (IAAC), Humboldtstr. 8, 07743 Jena, Germany. Fax: +49 3641 948 102; Tel: +49 3641 948 113; E-mail: alexander.schiller@uni-jena.de

[b] INNOVENT e.V., Biomaterials Department, Pruessingstr. 27 B, 07745 Jena, Germany

[c] Leibniz Institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena

[d] Friedrich Schiller University Jena, Jena Center for Soft Matter (JCSM), Humboldtstr. 10, 07743 Jena, Germany

[e] Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schiller University Jena, Max Wien Platz 1, 07743 Jena, Germany

[f] Center for Sepsis Control and Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena

1. Supplementary Methods and References
2. Supplementary Figures
3. Supplementary Tables
4. Video of CO release at LSM: CO release.mpg

1. Supplementary Methods and References

Differential scanning calorimetry. DSC was performed on dry non-woven mats in a Pyris 1 apparatus (PerkinElmer). The samples were subjected to a heating scan from 25 °C up to 200 °C at a rate of 10 °C/min. After cooling down to 25 °C at a rate of 10 °C/min, another heating run was performed from that temperature up to 200 °C at 10 °C/min.

BET surface measurements. The BET measurements were carried out by Quantachrome GmbH, Odelzhausen. Dinitrogen and Krypton adsorption/desorption measurements were performed on a Quantchrome Autosorb-3 instrument at 77.4 K. Prior to the measurements, the samples were dried under vacuum (20 mTorr) at room temperature (23-26 °C) for 40 h. N_2-BET measurements were not successful due to the very low surface area. Kr-BET resulted in reasonable values. In addition, mercury porosimetry was carried by Quantachrome GmbH on a POREMASTER 60-GT.

Analysis of the Manganese content of CORMA-1-PLA fibers. Samples of about 1 mg of CORMA-1-PLA were accurately weighed into a glass vial and dissolved in 1 ml of 65% ultrapure nitric acid. The acidic solutions were ultrasonicated at 60°C for 4 hours to completely decompose the polylactide matrix. After cooling to room temperature a given aliquote was taken from each sample and filled up with MilliQ water to reach a final nitric acid concentration of 4%. The quantity of manganese in the aqueous solutions was determined by ICP-MS (XSeriesII from Thermo Fisher Scientific, Germany).

Leaching of CORM-1 from CORMA-1-PLA fibres. To determine the loss of CORM-1 from the non-woven samples of about 1 mg of CORMA-1-PLA were weighed into a glass vial and filled up with MilliQ water. The samples were gently shaken for 3 hours or 3 days, respectively, the non-wovens were removed, MilliQ water and 65% ultrapure nitric acid was added to obtain a final nitric acid concentration of 4%. For another leaching experiment, samples were irradiated at 365 nm for 1 hour (in 2 ml MilliQ water) before storing the non-wovens in a fresh aqueous solution for 3 hours or 3 days, respectively. The sample preparation after leaching was the same as before. The quantity of manganese in the aqueous solutions was determined by ICP-MS.

CO release profile at 365 nm. CO release from CORMA in air was monitored with a portable CO detector from Draeger Safety Austria GmbH, Vienna (Draeger Pac7000). For qualitative checkup with 440-480 nm illumination a CORMA-1-PLA20 sample was directly placed on the detector filter of the CO sensor and covered with the glare protection of the lamp. At the sample the light intensity was 400 mW/cm^2 (determined with a UV-Power meter, VIS sensor, Dr. Hönle AG, Germany). The CO signal was recorded every 10 seconds. To confirm the solely light induced CO release at this wavelength, a sample of CORMA-1-PLA20 was placed in a small beaker (~210 ml) 1.2 cm above a dental LED array placed outside and the CO detector was arranged above the CORMA sample. The beaker was covered with a watch glass. The sample was repeatedly illuminated for 10 seconds followed by a 6 minute waiting time to allow equilibration of the gas and the CO signal was recorded every 10 seconds.

Kinetic experiments at 365 and 480 nm were undertaken in a desiccator setup. CORMA-1-PLA20 samples were attached to a cuvette wall and placed 3.5 cm from a HD-LED module (365 or 480 nm) consisting of 5 vertically aligned LED lamps (INNOTAS Elektronik GmbH, Germany). This setup was placed within a desiccator that was sealed with a rubber septum. The septum was pierced to enable threading the lamp wires through and connect them with the control device outside the desiccator. This setup allows easy lamp handling from the outside (switch on/off, intensity adjustment) even during the experiment if required. To compare CO release rates at 365 and 480 nm both lamps were adjusted to 10 mW/cm^2 at the given distance.

Gas IR. The non-woven was placed on a glass window which is attached to a home-made IR gas cuvette via a sample holding slot. The IR gas cuvette, consisting of a long tube with the sample, was integrated in the probe chamber of the instrument. Irradiation was performed at 365 nm (1 mW/cm^2). Single IR spectra were recorded every 8 s to 20 s with a DTGS detector. The chosen spectral resolution was 1 cm^{-1}. The recorded IR spectra were evaluated with the program R [1,2], in which a linear baseline correction and the integration of the CO vibration band are implemented. For each spectrum, the area of the integrated CO vibrational band was then converted into the total CO amount by using a calibration curve. To average repeated measurement the non-equidistance time points were interpolated.

[1]: R Development Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

[2]: Claudia Beleites and Valter Sergo: hyperSpec: a package to handle hyperspectral data sets in R, in preparation, R package version 0.98-20120713. http://hyperspec.r-forge.r-project.org

2. Supplementary Figures

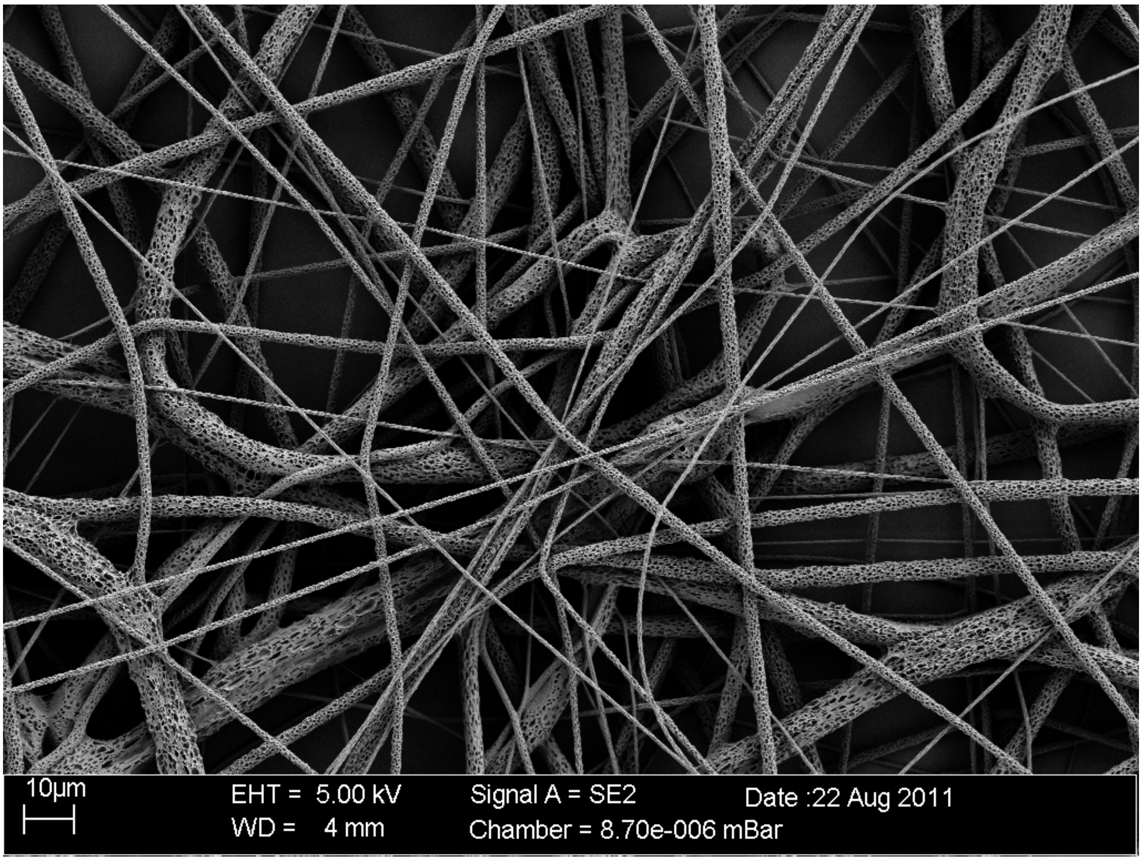

Figure S1. SEM image of CORMA-1-PLA10. Details (~ x 10 magnification) are given in Figure 2 b in the main manuscript.

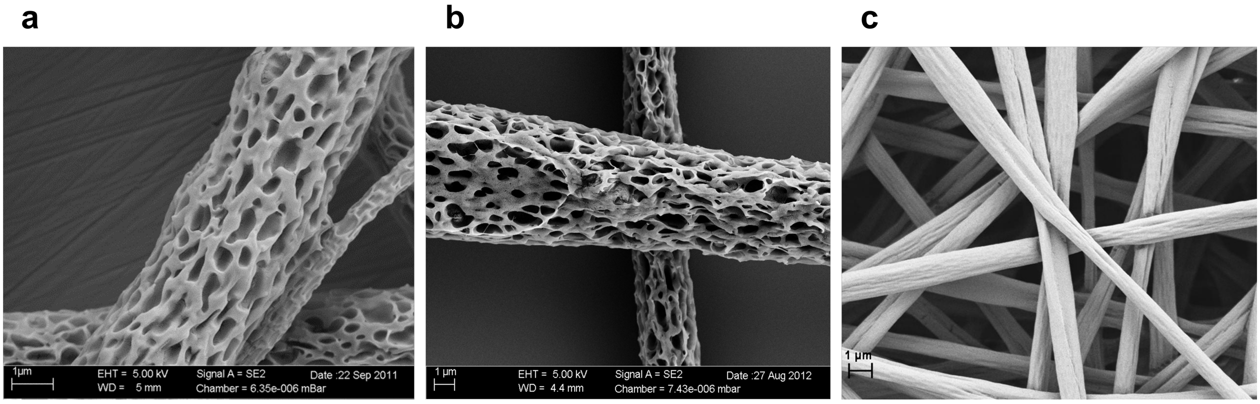

Figure S2. SEM images of a reproduced batch of CORMA-1-PLA10 (a) and CORMA-1-PLA20 (b). Electrospun PLA fibers without incorporated CORM-1 show a substantially smooth morphology (c). When CORMA-1-PLA20 is spun in the dark (Fig 2d), the fiber surface appears with nearly no porosity compared to samples that were prepared without light protection (a,b).

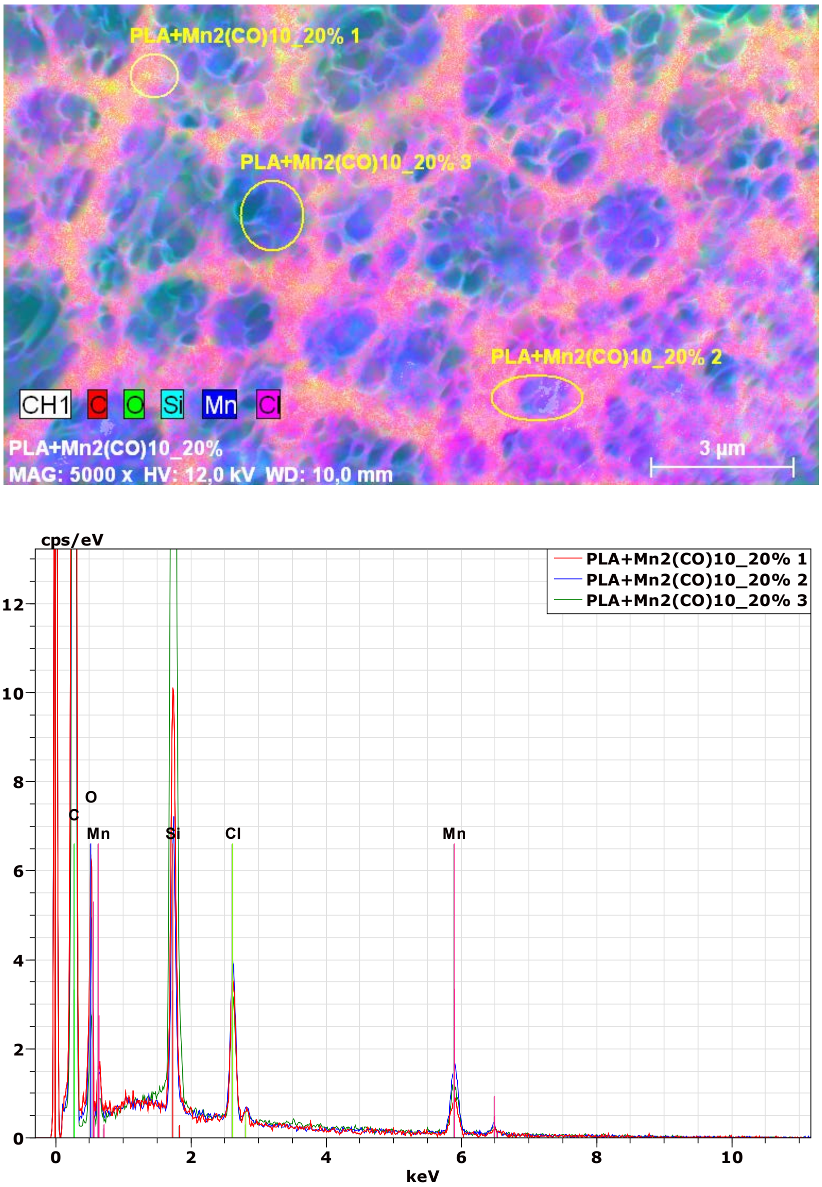

Figure S3. EDX spectra (bottom) from marked areas of the SEM-EDX mapping (top). Please note that chlorine can be found from the electrospinning solvent $CHCl_3$.

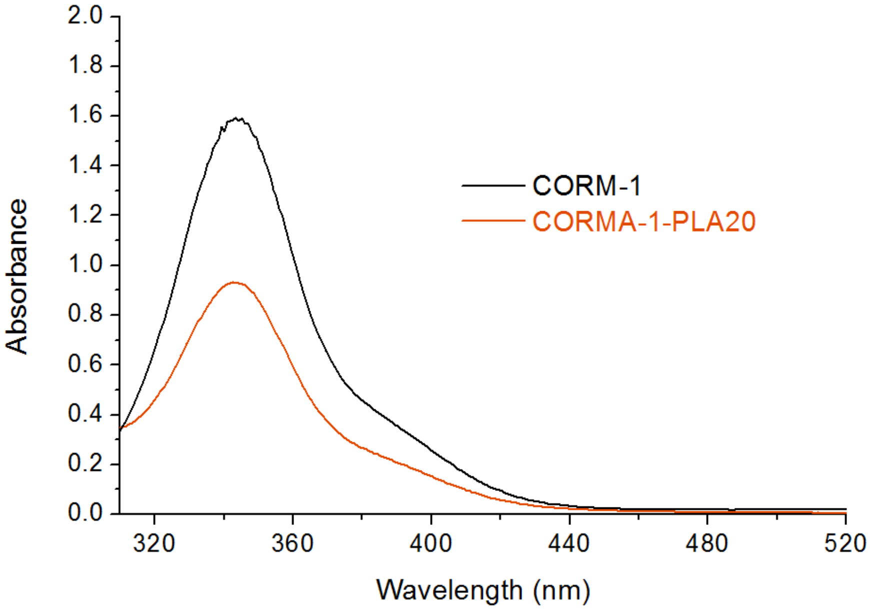

Figure S4. UV-Vis spectra of CORMA-1-PLA20 (brown line) and CORM-1 (black line) in deaerated chloroform. Both samples contain the same initial amount of CORM-1.

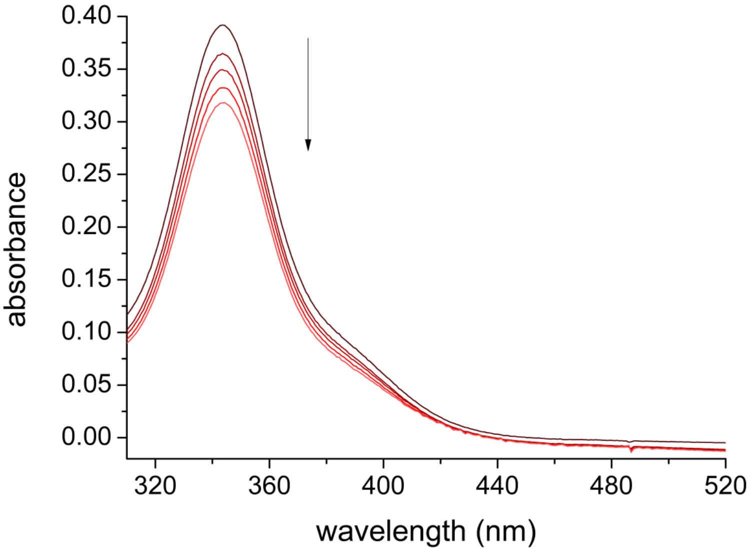

Figure S5. UV-Vis spectra of CORM-1 in non-deaerated chloroform in the dark. Spectra were taken every 30 minutes and show the degradation of CORM-1 in the presence of oxygen.

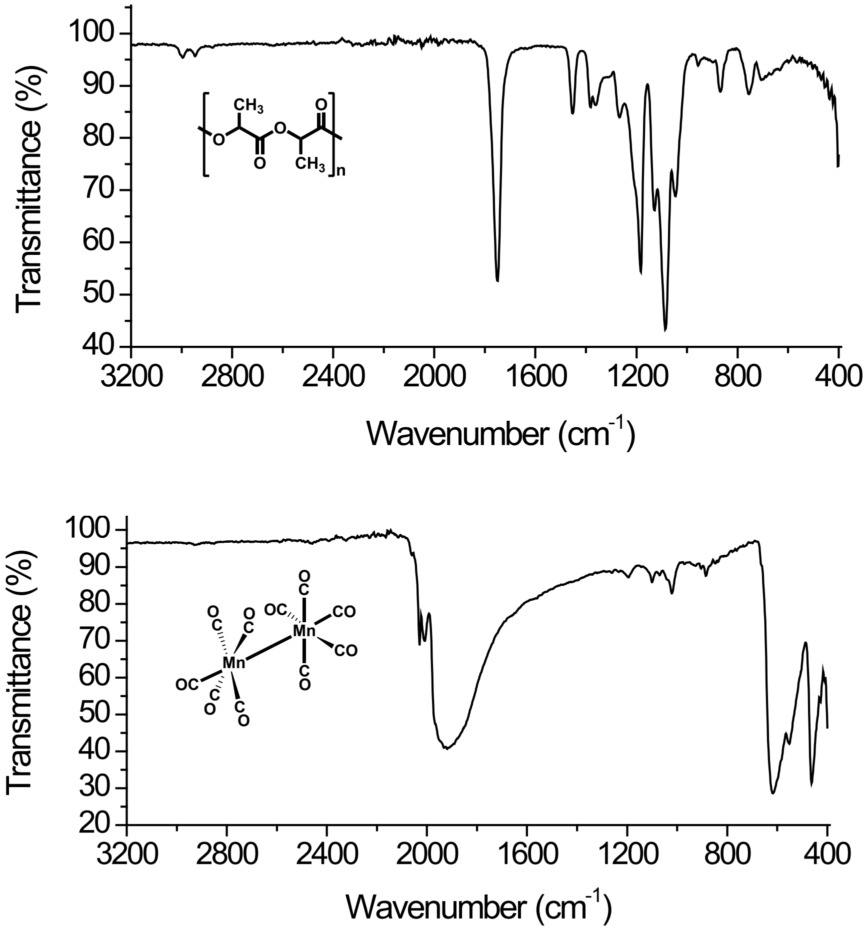

Figure S6. ATR-IR spectra of PLA (top) and CORM-1 (bottom).

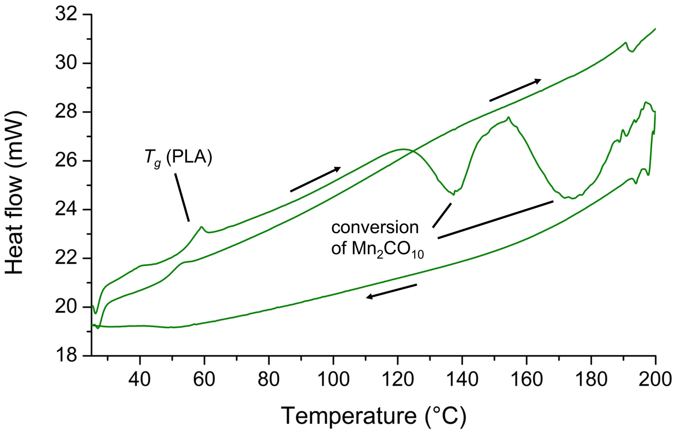

Figure S7. DSC thermogram with two heating cycles of CORMA-1-PLA20. The glass transition of PLA is observed at 59°C and is consistent with the data from the supplier. Two exothermic conversions of CORM-1 are observed at 137 and 174°C leading to a degradation of CORM-1.

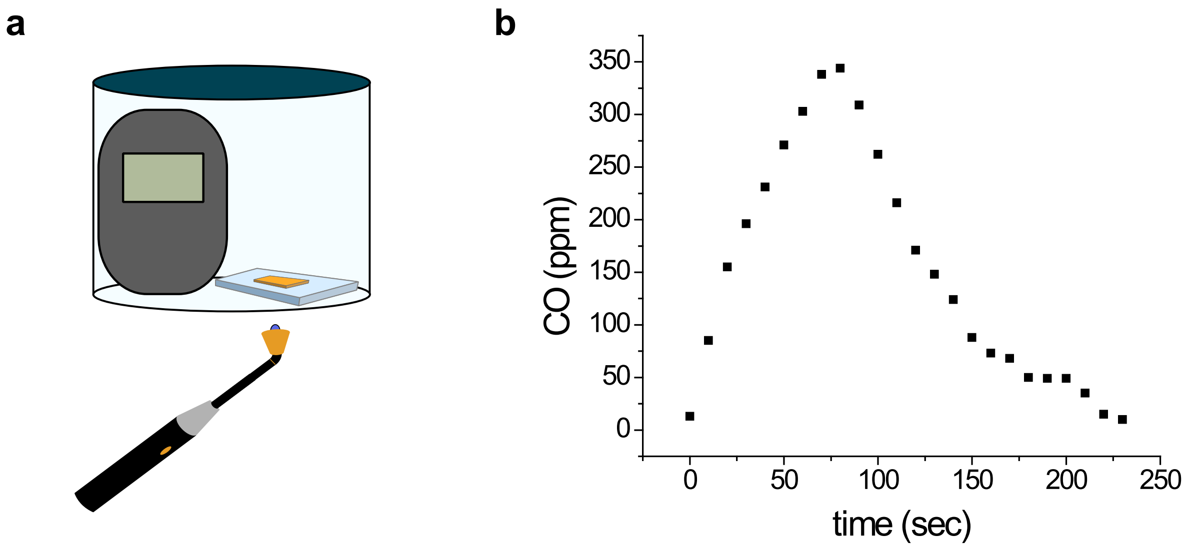

Figure S8. Experimental setup for the determination of released CO gas with the DraegerPac 7000. Sample and sensor are stored within a sealed beaker, while the light source (Translux Power Blue, 440-480 nm) handling is performed from outside (a). Placing and illumination (Translux Power Blue, 440-480 nm) of a sample directly on the sensor allows a quick and safe check on the CO release ability (b). Loss of the CO concentration after 75 sec occurs after opening the beaker.

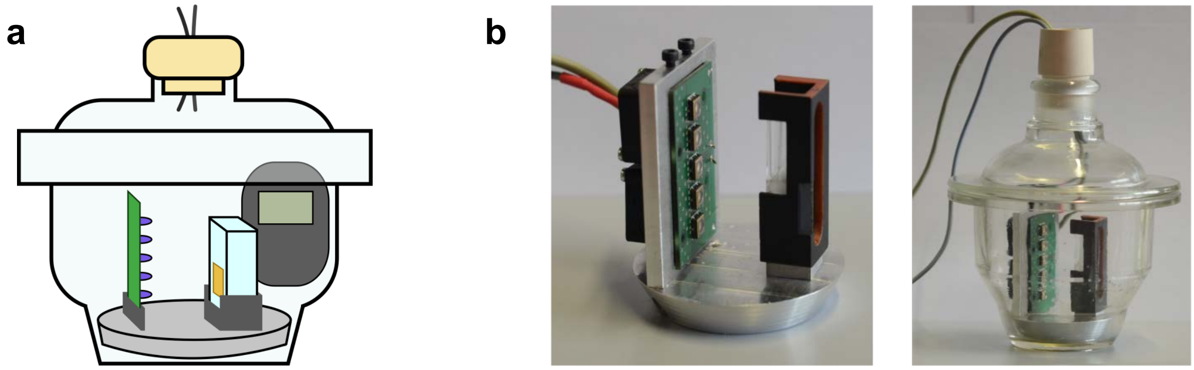

Figure S9. Experimental setup for the determination of released CO gas with the DraegerPac 7000. Sample and sensor are stored within a sealed desiccator together with a light source (LED lamps, 365 or 480 nm) that can be adjusted from outside (a and b).

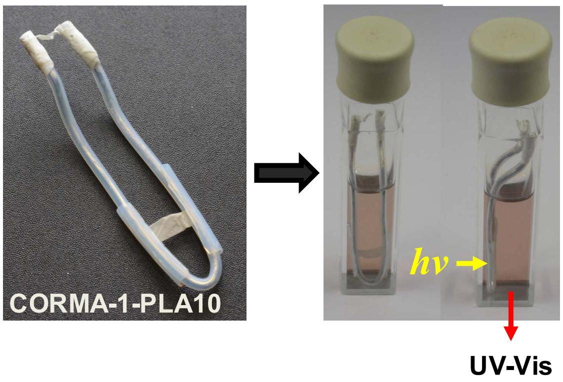

Figure S10. Experimental setup for the heterogeneous myoglobin assay experiments. The fixed non-woven can be illuminated from one side of the fluorescence cuvette, while UV-Vis spectra can be taken orthogonal to the sample.

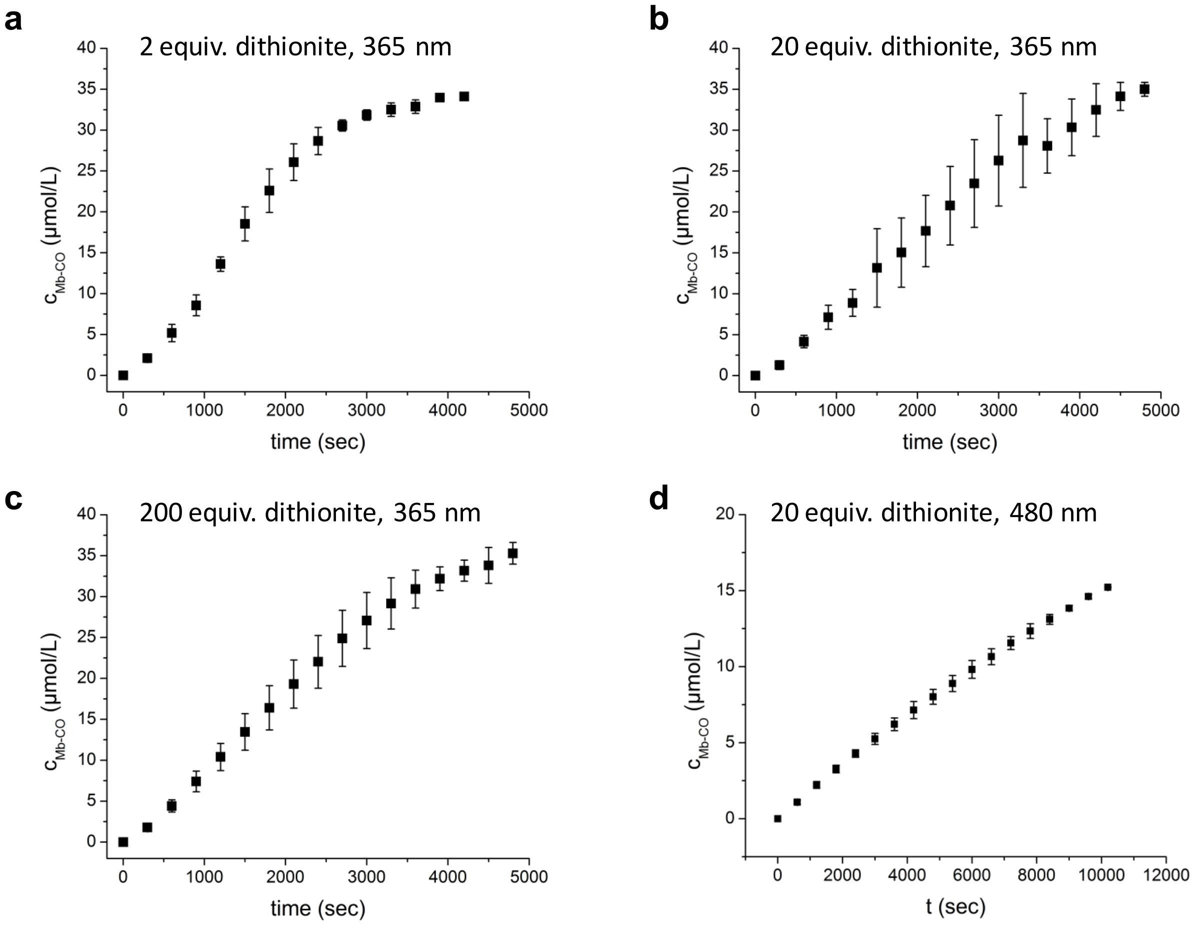

Figure S11. Myoglobin assay experiments (concentration of Mb-CO versus time) using different concentrations of sodium dithionite (2, 20 and 200 equivalents) at 365 nm (a-c). Illumination at 480 nm was performed in the presence of 20 equivalents (c).

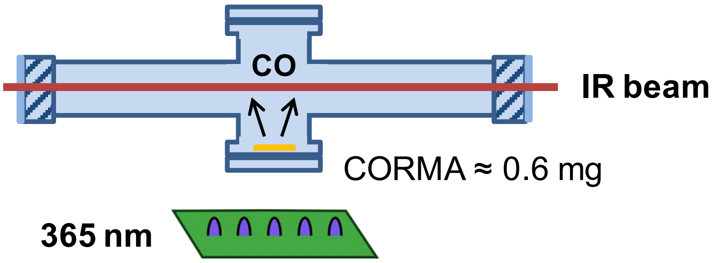

Figure S12. Experimental setup for the gas IR experiments. The non-woven is stored within the gas chamber and illuminated from outside. Released CO is diffusing into the chamber and detected by the IR spectrometer.

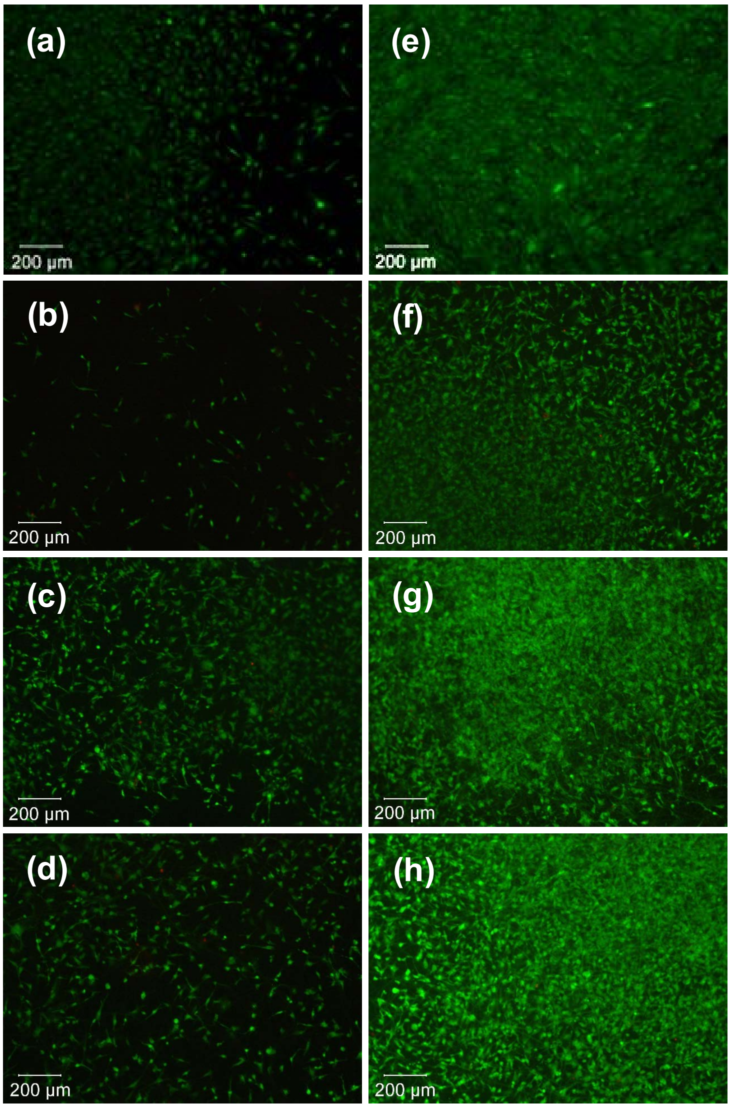

Figure S13. Cytotoxicity experiment with 3T3 mouse fibroblasts cells after 1 day (left) and 4 days (right): (a) and (e) glass control; (b) and (f) CORMA-1-PLA1; (c) and (g) CORMA-1-PLA10; (d) and (h) CORMA-1-PLA20. Green: living cells; red: dead cells.

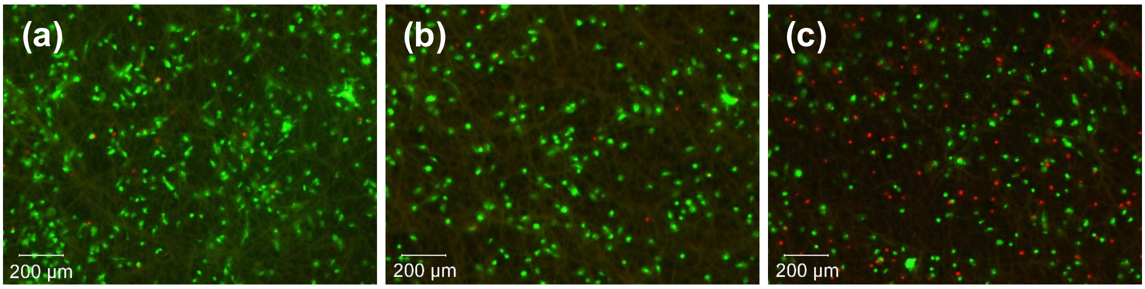

Figure S14. Phototoxicity experiment with 3T3 mouse fibroblasts cells on CORMA-1-PLA20. Illumination was performed at 365 nm and pictures were taken after 0 min (a), 15 min (b) and 60 min (c). Green: living cells; red: dead cells.

3. Supplementary Tables

Table S1. Kr-BET and Hg porosity measurements of a control PLA non-woven without CORM-1 and CORMA-1-PLA10 and CORMA-1-PLA20.

	Kr-BET surface area [m²/g]	**Abs. surface** [m^2]	**Specific pore volume** [cc/g]	**Pore diameter** [µm]
PLA control	2.96	0.11	4.191	2.01
CORMA-1-PLA10	2.38	0.04	7.908	7.14
CORMA-1-PLA20	0.94	0.03	4.791	9.99

Our attempt was to corroborate the SEM images of CORMA-1-PLA1, CORMA-1-PLA10 and CORMA-1-PLA20 with an increase in surface area. However, first Kr-BET measurements revealed only typical surfaces areas of about 3 m^2/g with a fiber diameter of ~ 1 mm for all fleeces tested (J. H. Wendorff, S. Agarwal and A. Greiner, *Electrospinning: Materials, Processing, and Applications*, Wiley-VCH, Weinheim, 2012). It seems to be notoriously difficult to measure high surface areas with porous electrospun non-wovens. Further, due to the low density several batches of non-wovens (3-5!) have to be used in a single BET experiment. Therefore, we refrained from discussing the comparison betweeen BET and SEM measurements in detail.

Table S2. Leaching experiments with CORMA-1-PLA10 and CORMA-1-PLA20 samples (exp. 1A-12A). The influence of irradiation on leaching (exp. 13A-16B) was tested as well as the manganese content in unleached CORMA samples (exp. 23-31)

During the experiments we also checked the degree of wetting of the materials after the agitation. In most of the experiments the PLA fibers remained dry after soaking, the water had not penetrated between the fibers. However, in some cases when a sample was cut from the edge of the initial non-woven, where the material is thinner and the fibers less tight, a wetting of the samples was observed. In such cases the loss of CORM-1 is slightly higher, but never more than 0.8%. Also a higher volume of water during agitation did not raise the amount of lost CORM-1.

Exp.	sample weight (mg)	weight of Mn (µg)	estimated conc. (µg/l)	estimated leaching portion (%.)	measured conc. (µg/l)	Stand. dev. (µg/l)	calculated leaching portion (%.)	calculated leaching portion irradiation (%.)	leaching volume	leaching duration	comment
1A	0,99	27,92	28,00	0,50	26,1000	0,2000	0,47		2ml	3h	weight ratio ~ 2000
2A	1,09	30,74	31,00	0,50	27,1300	0,0600	0,44				
3A	0,87	24,54	49,00	1,00	35,0000	0,4000	0,71			3d	
4A	1,00	28,21	56,00	1,00	27,6000	0,3000	0,49				
5A	1,13	31,87	15,00	0,50	12,3000	0,1000	0,41		8ml	3h	weight ratio ~ 8000
6A	0,97	27,36	14,00	0,50	11,5000	0,2000	0,41				
7A	1,06	29,90	30,00	1,00	21,7600	0,0700	0,73			3d	
8A	1,13	31,87	32,00	1,00	20,0900	0,0700	0,63				
9A	0,91	51,33	51,00	0,50	7,9400	0,0600	0,08		2ml	3h	weight ratio ~ 2000
10A	1,13	63,74	64,00	0,50	8,7000	0,1000	0,07				
11A	0,96	54,15	108,00	1,00	24,9000	0,3000	0,23			3d	
12A	1,02	57,54	115,00	1,00	28,1000	0,2000	0,24				
13A	1,00	28,21	28,00	0,50	70,4000	0,2000		1,26	2ml	3h	60 min irrad.
13B	1,00	28,21	28,00	0,50	38,6000	0,6000	0,69				
14A	0,94	26,51	27,00	0,50	54,2000	0,5000		1,00			60 min irrad.
14B	0,94	26,51	27,00	0,50	39,7000	0,2000	0,74				
15A	0,97	27,36	27,00	0,50	79,3000	0,1000		1,47		3d	60 min irrad.
15B	0,97	27,36	55,00	1,00	44,0300	0,1000	0,80				
16A	1,02	28,77	29,00	0,50	137,4000	0,0000		2,37			60 min irrad.
16B	1,02	28,77	58,00	1,00	43,3000	0,4000	0,75				
23	0,63	17,77	1186,98	10,00	941	8	7,93				determ. of Mn content in unleached non-woven
24	0,71	20,03	1337,71	10,00	1097	43	8,20				
25	0,67	18,90	1262,35	10,00	1034	41	8,19				
26	0,59	16,64	1111,62	10,00	835,05	0,07	7,51				
2	0,59	16,64	1111,62	10,00	890,1	0,4	8,01				
28	0,55	15,51	1036,26	10,00	785	7	7,58				
29	0,62	34,97	2336,29	20,00	1722	16	14,74				
30	0,72	40,62	2713,11	20,00	2045	19	15,07				
31	0,64	36,10	2411,65	20,00	1747	22	14,49				
32	0,4-0,46	0,00	0,00		0,5450	0,0010					pure PLA blank