Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

For

CO-induced Apoptotic Death of Colorectal Cancer Cells by a Luminescent PhotoCORM Grafted on Biocompatible Carboxymethyl Chitosan

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Materials and Instrumentation

All the reagents were of commercial grade and were used without further purification. 4-pyridinecarboxaldehye was purchased from Aldrich Chemical Co., and 1,10-phenanthroline (phen) was purchased from Ark Pharm, Inc. Carboxymethyl chitosan was procured from Santa Cruz Biotechnology. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and CellEvent™ Caspase 3/7-detection agent were purchased from Life Technologies. The solvents were purified according to standard procedures.[1] The precursor complexes $[ReBr(CO)_3(phen)]$, $[Re(CF_3SO_3)(CO)_3(phen)]$ and $[Re(CO)_3(MeCN)(phen)](CF_3SO_3)$ were synthesized according to procedures reported by us in an earlier account.[2]
The ^1H NMR spectrum was recorded at 298K on a Varian Unity Inova 500 MHz instrument. A PerkinElmer Spectrum-One FT-IR spectrometer was utilized to monitor all the IR spectra of the complex and the composite material. UV/Vis spectra were recorded with a Cary 50 UV/Vis spectrophotometer. Fluorescence spectra were recorded with a Cary Eclipse spectrometer. Microanalyses (C, H, N) were preformed using a PerkinElmer 2400 Series II elemental analyzer. The elemental analysis for Re metal was performed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). For this study the sample (ReCMC) was fused with sodium peroxide over a Bunsen burner. The fused sample was then dissolved in water. The resulting solution was subsequently acidified and subjected to ICP-AES study. HRSTEM and EDS analysis were performed on a FEI Titan microscope at the National Center for Electron Microscopy, Lawrence Berkeley National Laboratories. For HRSTEM the ReCMC micro polymer was dispersed in ethanol and drop cast on to a lacey carbon coated 400 mesh copper grid. The FEI Titan was operated at 300 kV. The STEM probe had a convergence semiangle (α) of 10 mrad and a beam current of 20 nA. STEM image was acquired using a Fischione high angle annular dark-field (HAADF) detector with an inner semiangle (β) of 24 mrad. The Bruker Esprit EDS analysis package software was used for the EDS mapping (calibrated against mineral standards for quantitative accuracy). The absorbance reading of MTT assays were done using a Molecular Devices VersaMax tunable microplate reader.

Synthesis of $[Re(CO)_3(phen)(pyAl)](CF_3SO_3)$ (1)

To a refluxing solution of $[Re(MeCN)(CO)_3(phen)]CF_3SO_3$ (192 mg, 0.30 mmol) in 50 mL of chloroform, 4-pyridinecarboxaldehyde (pyAl) (321mg, 3.0 mmol) was added in a drop wise manner. The color of the solution was initially bright yellow. The reaction mixture was then heated to reflux under stirring condition for 20 h. During this period, the color of the solution turned in to more intense yellow. After this time the reaction mixture was cooled down to ambient temperature and subsequently the solvent was reduced to ~5 mL under vacuum and subjected to silica gel column chromatography. The silica gel column was prepared with toluene and after administration of the crude reaction mixture to the column; initially ~30 mL of toluene was passed through the column to remove the excess of pyAl. Next a yellow band was eluted using a 50:50 mixture of

toluene : acetonitrile. Evaporation of the solvent from this eluent resulted a yellow paste. This residue was then triturated with diethyl ether (~5 mL) for five times to obtain 198 mg of $[Re(CO)_3(phen)(pyAl)](CF_3SO_3)$ as a yellow solid (Yield 94%). Anal. Calcd (%) for $C_{22}H_{13}N_3O_6SF_3Re$ (706.63): C 37.42; H 1.89; N 5.98; Found: C 37.39, H 1.85, N 5.95. IR (KBr disk, cm^{-1}): 2032(s), 1933(s), 1900(s), 1716(m), 1432(w), 1276(s), 1229(m), 1150(m), 1033(m), 856(w), 1636(m), UV/Vis (MeCN), λ_{max} (ε, M^{-1} cm^{-1}): 275 (24,000), 360 (3,000). ^1H NMR data (CD_3CN): δ 9.64 (s, 1H), 9.39 (d, 2H)(5Hz), 8.62 (d, 2H)(10 Hz), 8.30 (d, 2H)(5Hz), 7.94 (s, 2H), 7.90 (m, 2H), 7.32 (d, 2H)(5Hz).

Synthesis of ReCMC

100 mg of carboxymethyl chitosan (CMC) was taken in a glass vial and placed inside a vacuum oven for 1 h at 110° C to remove any moisture. Then the CMC was suspended in ~ 5mL of dry methanol (MeOH) and 200 mg of complex **1** was added. This mixture was then heated to reflux under N_2 for 3 days. After this time the reaction mixture was cooled down to ambient temperature and then filtered. The residue was then thoroughly washed with dry acetonitrile (MeCN) and finally dried under vacuum to collect the ReCMC (90 mg).

Single-Crystal X-ray Crystallography

Single crystals for complex **1** were grown by layering hexanes over its dichloromethane solution. One suitable crystal was selected and fixed on top of MiTiGen micromount using Paratone N-Oil and transferred to the diffractometer. Data were collected on a Bruker APEX II single-crystal X-ray diffractometer with graphite monochromated Mo-Kα- radiation (λ = 0.71073 Å) using ω-scan technique in the range of $3 \leq 2\theta \leq 50°$. Multi-scan absorption corrections [3] were applied to the data set using SADABS. The structure was generated using *SHELXT* (intrinsic phasing)[4] and subsequently refined by full-matrix least squares procedure on F^2 with *SHELXL*.[5] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in calculated positions on the C atoms to which they are bonded, with C-H = 0.93 Å and $U_{iso}(H) = 1.2U_{eq}(C)$. Calculations and molecular graphics were preformed using *SHELXTL* 2014[5] and *Olex2* programs.[6] Crystal data and structure refinement parameters are listed in Table S1.

CCDC 1524183 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request.cif.

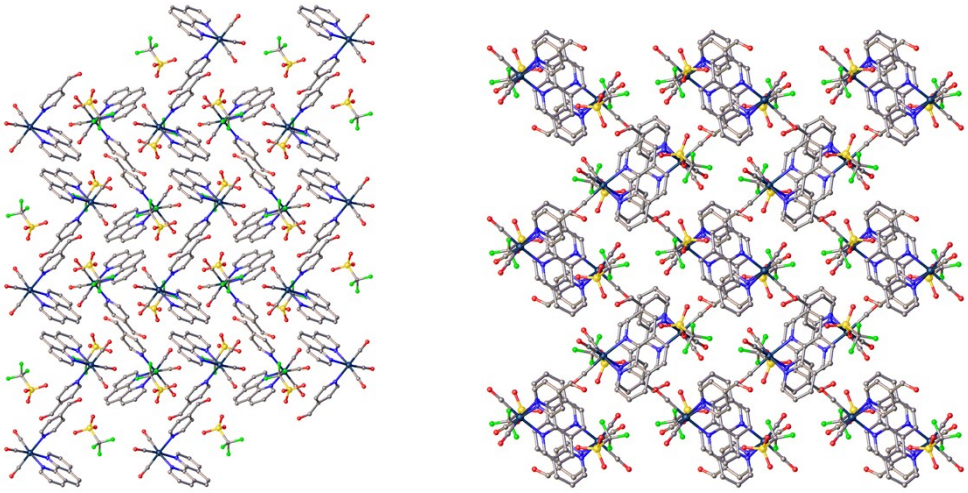

Fig. S1. Packing pattern of **1** along a axis (left panel) and c axis (right panel)

Table S1. Crystal data and structure refinement parameters for **1**

	1
Empirical formula	$C_{22}H_{13}F_3N_3O_7ReS$
Formula weight	706.61
T(K)	298
λ (Å)	0.71073
Crystal system	Monoclinic
Space group	*P*21/*n*
a (Å)	12.5447(19)
b (Å)	12.1776(18)
c (Å)	15.348(2)
α (°)	90
β □°□	92.870(2)
γ□□°□	90
V (Å^3)	2341.6(6)
Z	4
D_{calc} (Mg m^{-3})	2.004
Absorption Coeff (mm^{-1})	5.352
No. of unique reflections	3853
Goodness-of-fit[a] on F^2	0.990
R_1[b]	0.0290
wR_2[c]	0.0632
largest diff peak and hole	0.938, -0.386

[a]GOF = $[\Sigma[\omega(F_o^2-F_c^2)^2]/(N_o-N_v)]^{1/2}$ (N_o = number of observations, N_v = number of variables).
[b]$R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$. [c]$wR_2 = [(\Sigma\omega(F_o^2-F_c^2)^2/\Sigma|F_o|^2)]^{1/2}$

Cell Culture and Imaging

The human colon cancer (HT-29, ATCC® no. HTB-38™), human breast cancer (MDA-MB-231, HTB-26™) and human embryonic kidney (HEK-293, ATCC® no. CRL-1573™) cells were purchased from American Type Culture Collection (ATCC), cultured in McCoy's 5A (HT-29) and Dulbecco's modified Eagle's medium (MDA-MB-231 and HEK-293) both supplemented with 10% fetal bovine serum (FBS). For cellular internalization experiments the cells were plated on to 35 mm imaging dishes (purchased from ibidi), and allowed to grow overnight. After this time, the old media were aspirated and fresh appropriate media (devoid of phenol red and FBS) was employed to prepare the treatment solutions. The cells were then treated with 300 μg/mL of ReCMC and allowed to incubate at 37°C with 5% CO_2(g) for 1 h. Next, the cells were washed three times with PBS carefully under dark conditions. Finally, the cells were imaged in fresh DMEM and/or McCoy's 5A media (free of phenol red and FBS) with the aid of a Leica SP5 confocal microscope (PL Apo 40X/1.25 n.a.; Oil DIC objective) using 405 nm illumination at 30% power. The gain and offset were set to adjust the brightest pixels below saturation, and there were only a few zero-intensity pixels. The detection gate was set to 550-650 nm. The zoom and the frame size were set to give the pixels that were approximately 115 nm (just below the Nyquist criterion). Images were collected at 340 nm intervals for Z-stacks. The scan speed was set to 100 Hz, and a 2X line averaging was used. Differential interference contrast (DIC) images were collected in parallel. *Application Suite Advanced Fluorescence* software was used to acquire the initial images. The bright-field images were acquired in DIC or phase-contrast mode. The images were further processed with Fiji-ImageJ, version 1.52c, and ImageJ (micromanager), version 1.49h, software.

Cellular imaging experiments for caspase activation

In this experiment, the HT-29 cells were plated on to 35 mm imaging dishes (purchased from ibidi), and allowed to grow overnight. After this time the old McCoy's 5A medium was aspirated and fresh McCoy's 5A medium (devoid of phenol red and FBS) was employed to make the treatment solution. The cells were then treated with 300 μg/mL of ReCMC and allowed to incubate for 1 h at 37°C and 5% CO_2(g). Next, the cells were carefully washed with PBS. Then fresh PBS was added to the dishes and exposed to hand-held UV light (power, 5mW/cm^2) for 10 and 30 min. Then the PBS was aspirated carefully from the dishes and 2 mL of McCoy's 5A medium (devoid of phenol red and FBS) was added along with 5 μM of CellEvent Caspase-3/7 Detection agent and the cells were further incubated for 1 h at 37°C. Next, the images were acquired on a Leica SP5 confocal microscopy using a 20X/0.7 HCX PL Apo objective. The pixel size was set to 190 nm. ReCMC sample was excited with a 405 nm laser at 50% and emission was collected from 497-597 nm, a polarized light and images were collected in parallel. In a separate (sequential) scan the 488 nm laser was used at 3.75% (overall) to excite caspase-3/7 detection agent and emission was collected from 496-540 nm. Detector gain was set to maximize signal strength while avoiding pixel saturation. The scan speed was set to

200 Hz, 2x line averaging was used and the pinhole was set to 1 Airy unit. Multiple fields of view were collected at each time-point, 2, 10 min and 30 min.

MTT assay

Human colon cancer (HT-29) and human embryonic kidney (HEK-293) cells were plated in four independent 96-well plates (20,000 cells/well) and incubated for 24 h prior to the experiments. First 6 mg of ReCMC was suspended in 4 mL of PBS and sonicated for 15 min. A stock solution was then prepared by further adding 6 mL of appropriate media (as mentioned in above sections). 5mL of this stock solution was added further to 5 mL of media to make a treatment solution consisting of 300μg/mL of ReCMC. Then 5 mL of this solution was again added to 5 mL of media to make another treatment solution consisting of 150μg/mL of ReCMC material. Cells were then treated with these two solutions (300μg/mL and 150 μg/mL of ReCMC) and also with 300 μg/mL of CMC as control. Then all the plates were incubated for 2 h at 37°C and 5% CO_2(g) for the internalization of the composite material. All the plates were kept under completely dark conditions during this process. Next, two plates were kept completely under dark conditions for incubation (1 h) and other two plates (with HT-29 and 293 cells) were taken out and washed once with PBS and subsequently PBS was added to each of these plates (one containing HT-29 and the other HEK-293 cells). Light was administered from a hand-held UV lamp (power, 5mW/cm^2) at a distance of 1.5 cm for 20 min. After this time all the plates (light and dark conditions) were taken, and the media/PBS was aspirated and fresh media (with FBS) was added, and the cells were allowed to recover for 3 h. Finally, MTT was added according to the manufacturer's protocol (Life Technologies), and the viability of the cells was assessed.

Additional Figures:

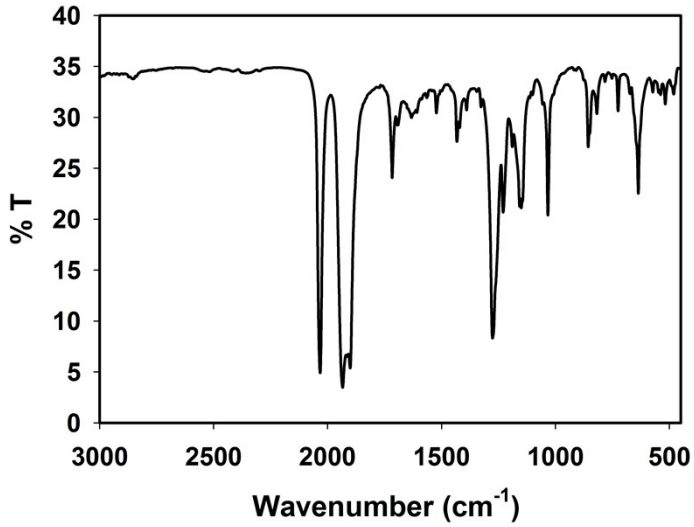

Fig. S2. IR spectra of [Re(CO)$_3$(phen)(pyAl)](CF$_3$SO$_3$) (**1**) (in KBr disk)

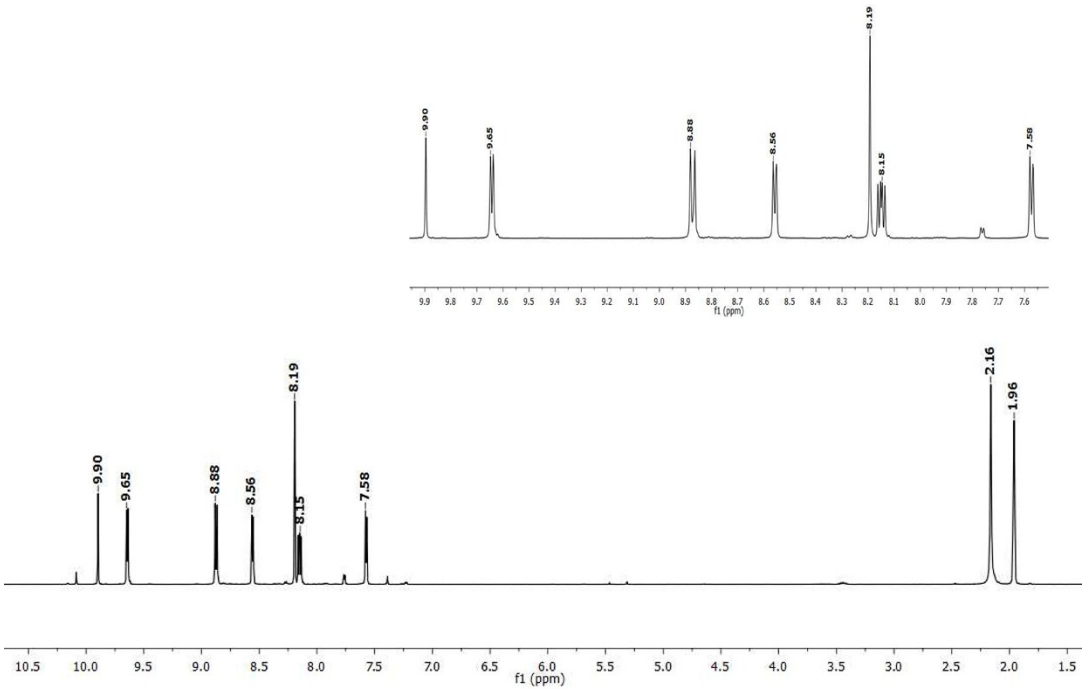

Fig. S3. ^{1}H NMR of $[Re(CO)_3(phen)(pyAl)](CF_3SO_3)$ (**1**) at 298 K in CD_3CN

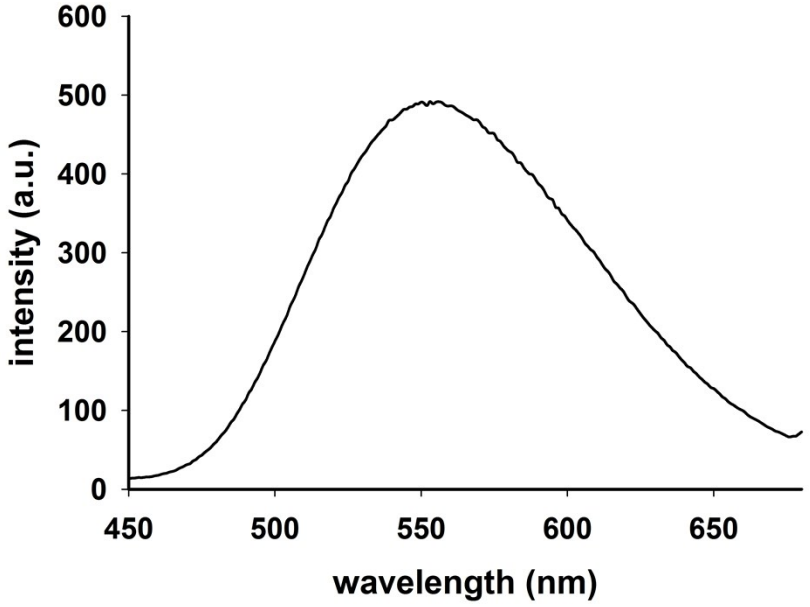

Fig. S4. Emission spectrum of ReCMC at 298 K in PBS solution (λ_{ex} = 350 nm and λ_{em}= 550 nm)

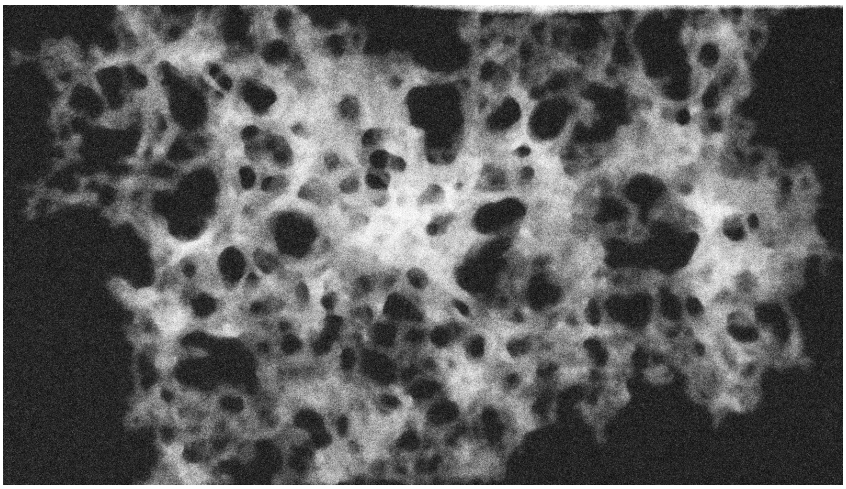

Fig. S5. SEM image of ReCMC composite material

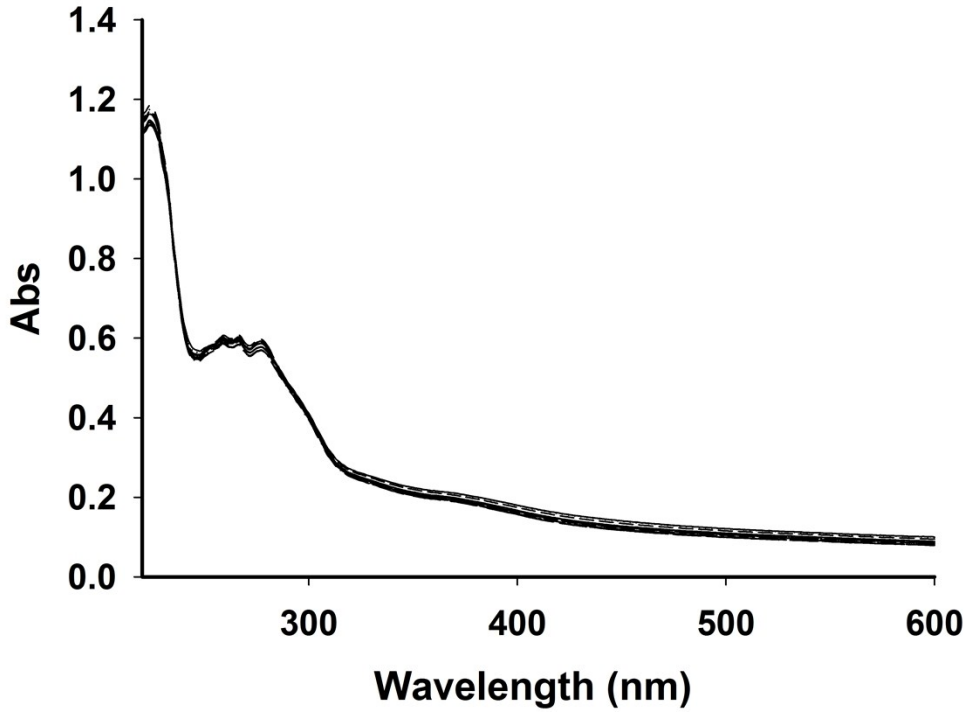

Fig. S6. UV-Vis spectral traces for ReCMC composite material in PBS over 24 h

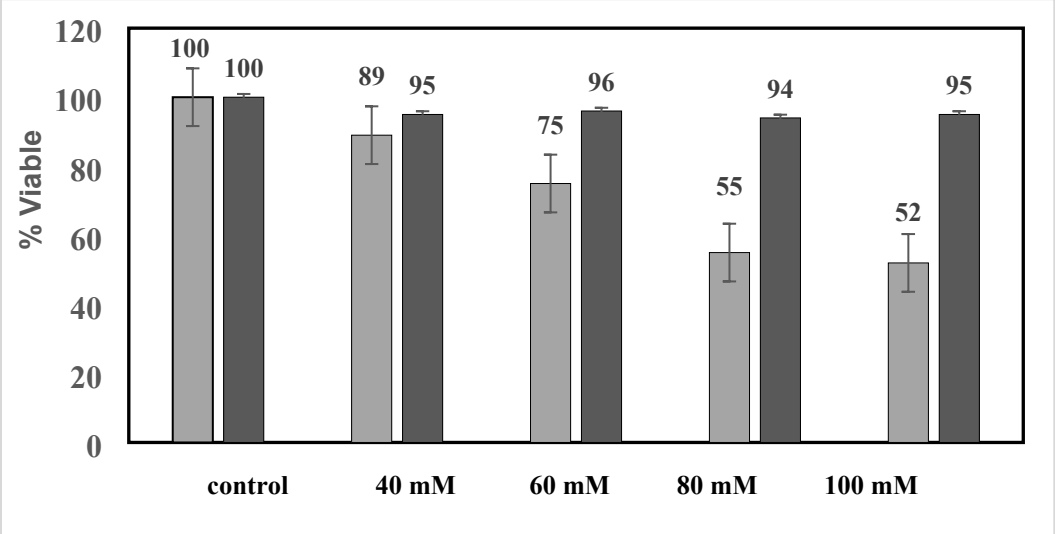

Fig. S7. Results of the MTT assay with HT-29 cells utilizing complex **1** exhibiting dose-dependent eradication of the cells upon CO delivery under light (grey bars). The dark bars represent the cell viability in presence of **1** under dark condition.

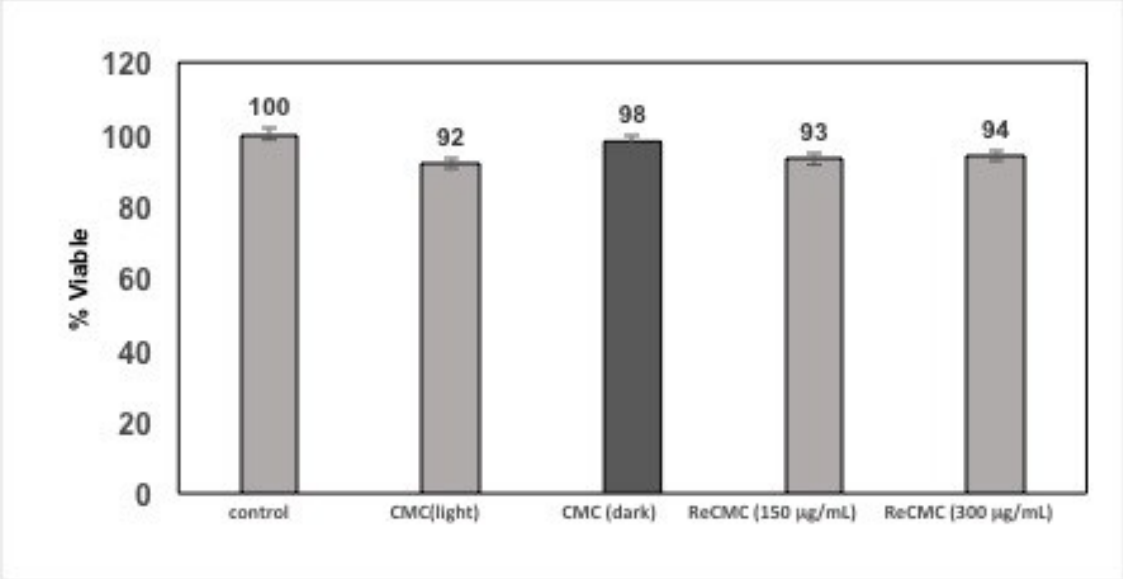

Fig. S8. Results of the MTT assay with HEK-293 cells utilizing ReCMC composite material. The results clearly show that both CMC and ReCMC hardly affect the viability of the normal cells both in the dark and under illumination.

References:

1. D. D. Perrin, W. L. F. Armarego, *Purification of Laboratoty Chemicals*; Pergamon Press: Oxford, U.K., 1988.

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