SUPPORTING INFORMATION

pH-Controlled Gas-Generating Mineralized Nanoparticles: A Theranostic Agent for Ultrasound Imaging and Therapy of Cancers

Kyung Hyun Min,[1,2] Hyun Su Min,[1] Hong Jae Lee,[3] Dong Jin Park,[4] Ji Young Yhee,[1] Kwangmeyung Kim,[1] Ick Chan Kwon, [1,*] Seo Young Jeong,[4] Oscar F. Silvestre,[2] Xiaoyuan Chen,[2] Yu-Shik Hwang,[3] Eun-Cheol Kim,[5] and Sang Cheon Lee[3,*]

[1] Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea

[2] Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, Maryland 20892, USA.

[3] Department of Maxillofacial Biomedical Engineering & Institute of Oral Biology, School of Dentistry, Kyung Hee University, Seoul 130-701, Republic of Korea

[4] Department of Life and Nanopharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea

[5] Department of Maxillofacial Tissue Regeneration and Research Center for Tooth and Periodontal Regeneration (MRC), School of Dentistry, Kyung Hee University, Seoul 130-701, Republic of Korea

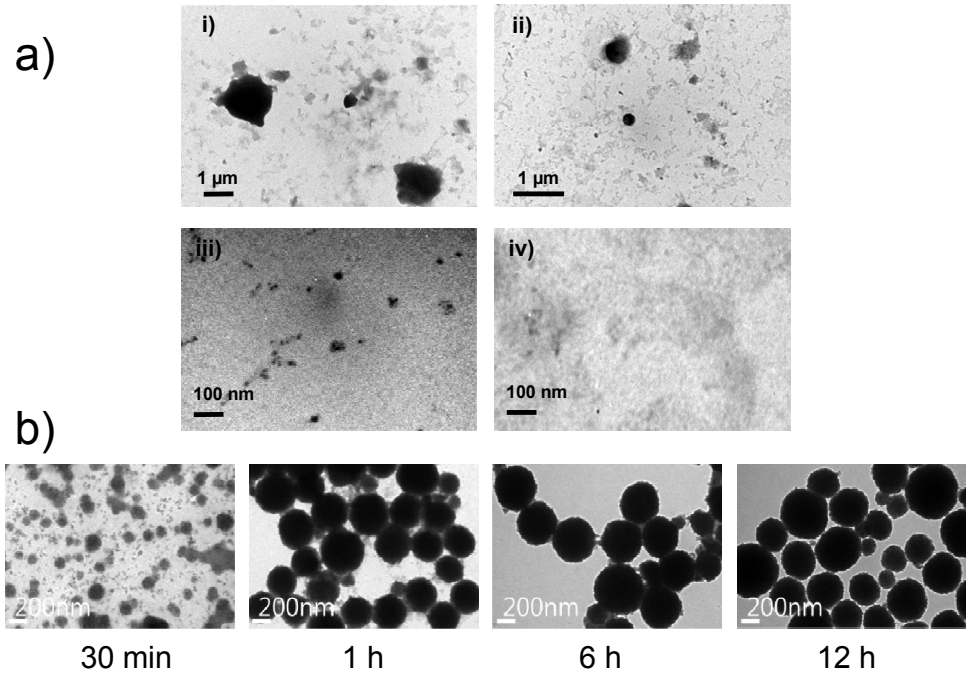

Figure S1. a) TEM images of DOX-$CaCO_3$-MNPs at various moral feed ratios ([Asp] : [Ca^{2+}] : [CO_3^{2-}]). i) 1:5:5, ii) 1:2:2, iii) 1:0.5:0.5, and iv) 1:0.2:0.2 (mineralization time: 12 h). b) TEM images of DOX-$CaCO_3$-MNPs prepared at feed ratio of [Asp] : [Ca^{2+}] : [CO_3^{2-}] = 1:1:1 at various mineralization periods.

Table S1. Mean hydrodynamic diameter, polydispersity, and morphology of DOX-$CaCO_3$-MNPs at various mineralization time.

Mineralization time (h)	Mean diameter [a] (nm)	Polydispersity factor (μ_2/Γ^2) [a]	Morphology [b]
0.5	145.2 ± 55.6	0.46 ± 0.13	irregular
1	180.2 ± 17.2	0.28 ± 0.08	sphere
6	217.4 ± 10.8	0.25 ± 0.03	sphere
12	240.8 ± 8.1	0.21 ± 0.01	sphere

[a] Measured by dynamic light scattering.
[b] Estimated by TEM.

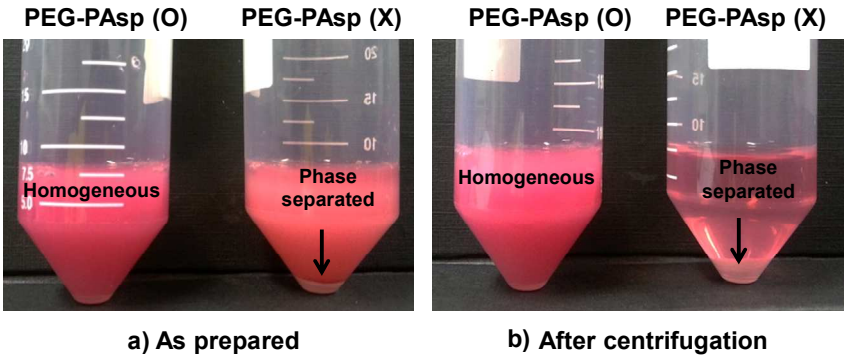

Figure S2. Effects of PEG-PAsp on the preparation of stably dispersed DOX-loaded mineralized nanoparticles.

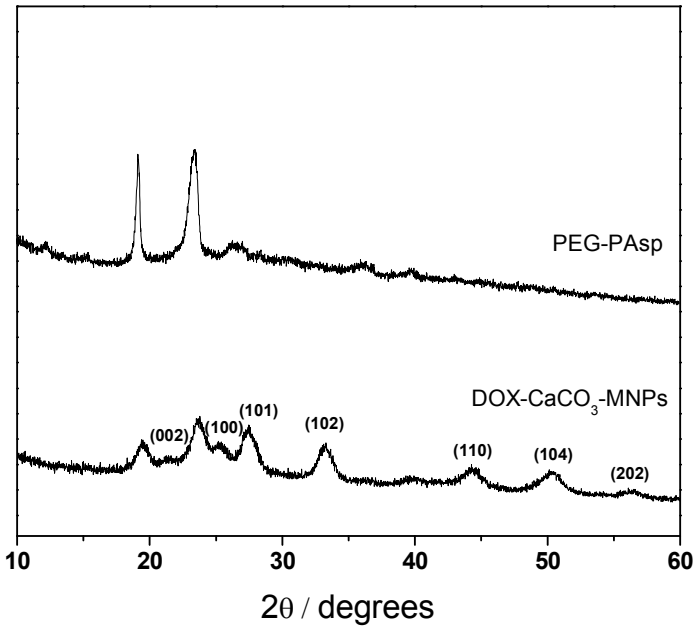

Figure S3. The powder XRD pattern of PEG-PAsp and DOX-$CaCO_3$-MNPs.

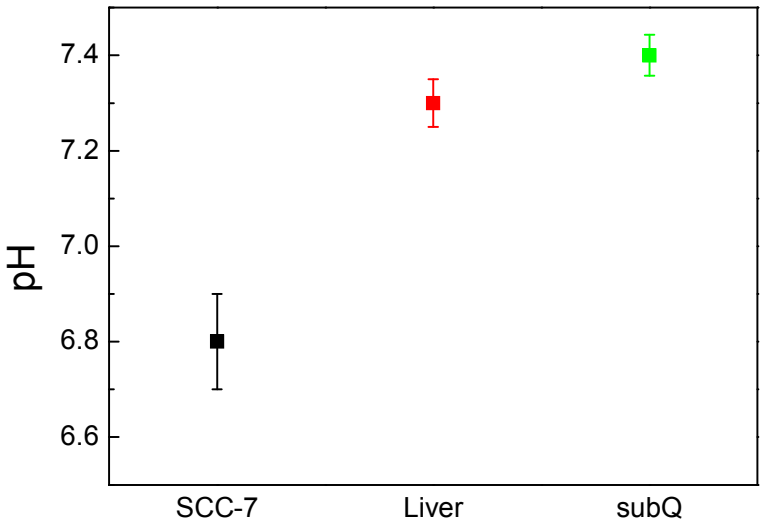

Figure S4. pH values of SCC-7 tumors, the liver, and the subcutaneous tissue (subQ).

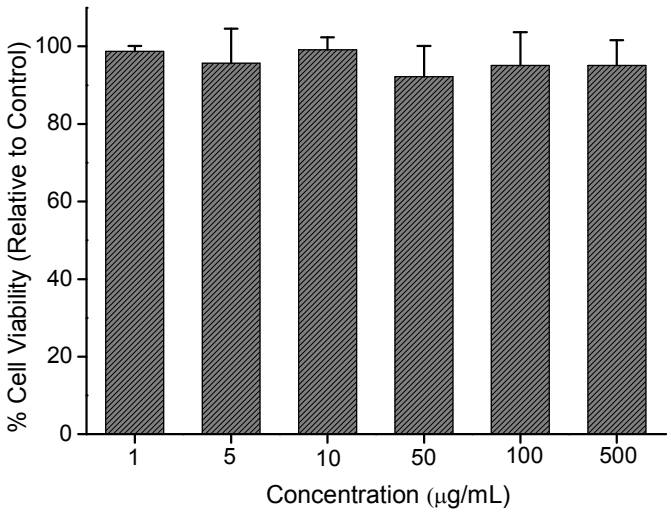

Figure S5. Viability of SCC-7 cells at various concentration of DOX-free $CaCO_3$-MNPs (n=3).

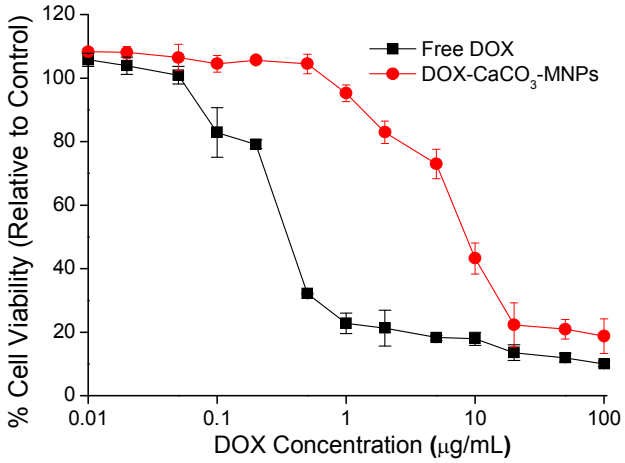

Figure S6. *In vitro* cytotoxicity of free DOX and DOX-$CaCO_3$-MNPs against SCC-7 cells (n=3).

Table S2. IC_{50} values of free DOX and DOX-$CaCO_3$-MNPs.

Sample	IC_{50} (μg/mL)
Free DOX	0.27
DOX-$CaCO_3$-MNPs	5.76

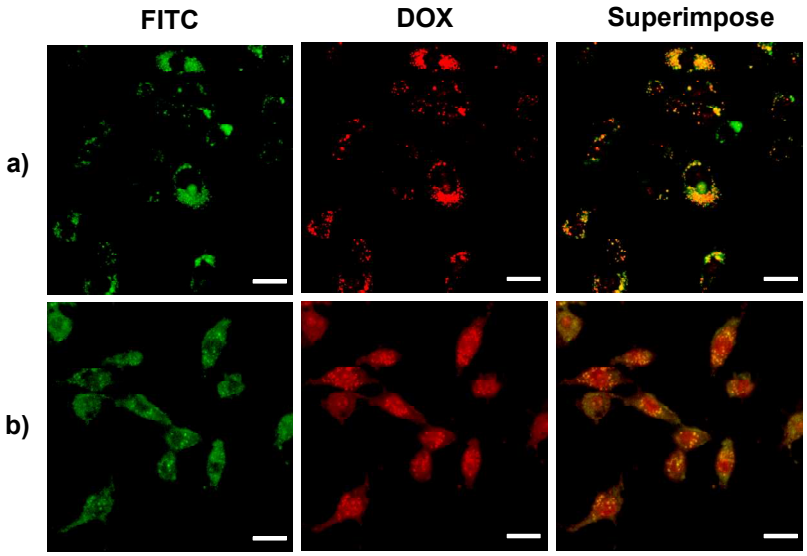

Figure S7. CLSM images of live SCC-7 cells treated with FITC-incorporated DOX-$CaCO_3$-MNPs (DOX=5 μg/mL). a) after 1 h incubation and b) after 6 h incubation. (Green fluorescence is associated with FITC; the red fluorescence is expressed by free DOX, released DOX, and DOX retained within $CaCO_3$-MNPs.). Scale bar: 20 μm.

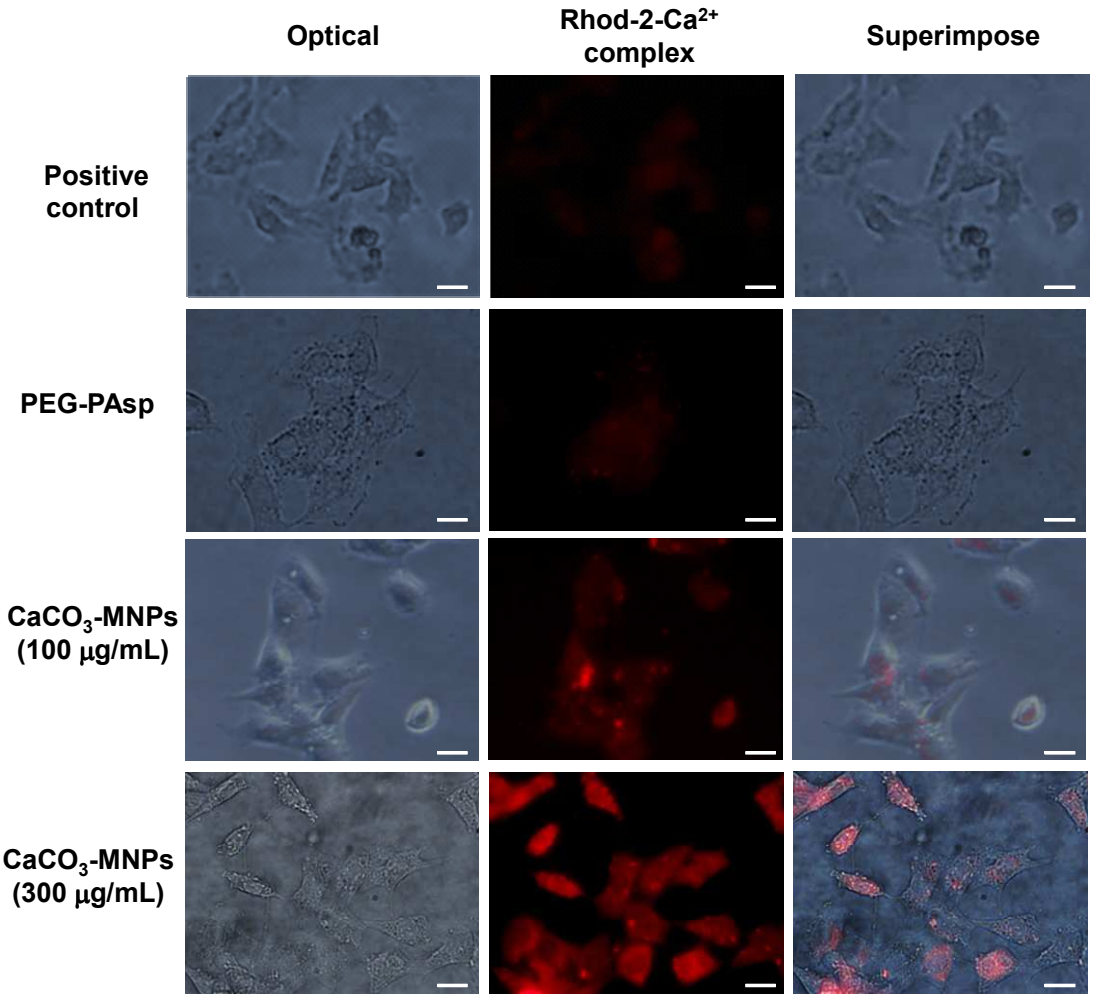

Figure S8. Fluorescence image of Rhod-2 labeled SCC-7 cells after the addition of $CaCO_3$-MNPs. Scale bar: 20 μm.

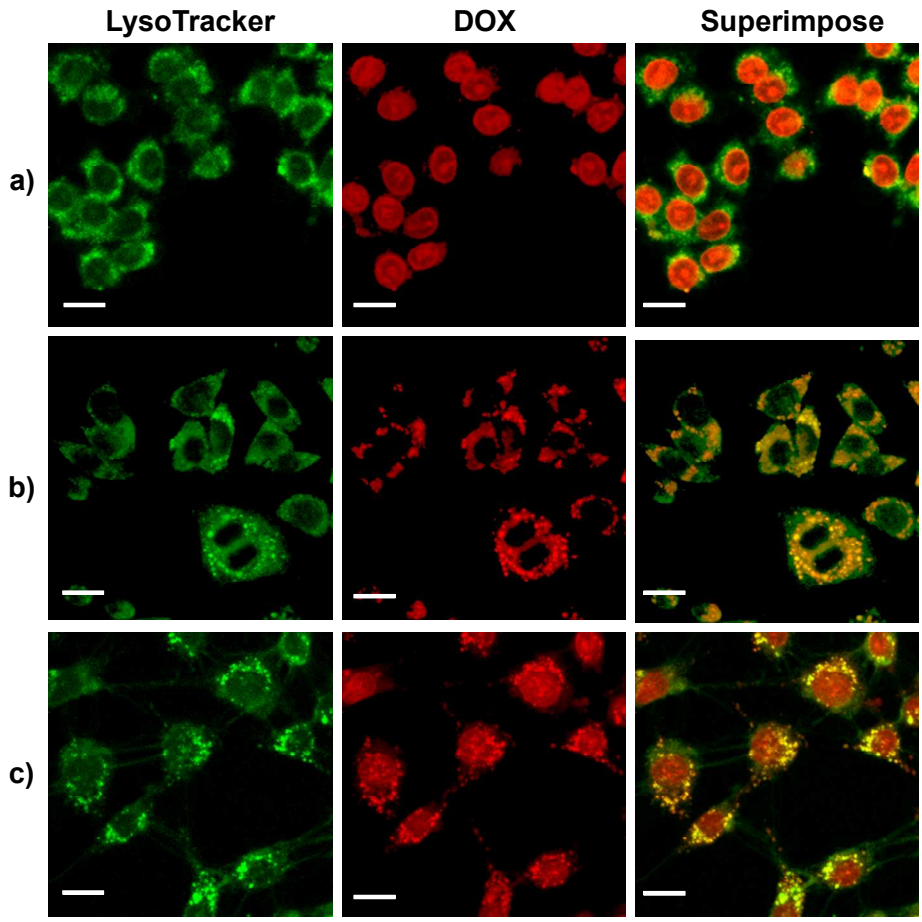

Figure S9. CLSM images of live SCC-7 cells treated with LysoTracker (50 nM), free DOX (5 μg/mL), and DOX-$CaCO_3$-MNPs (DOX=5 μg/mL). a) Free DOX for 1 h incubation, b) DOX-$CaCO_3$-MNPs for 1 h incubation, c) DOX-$CaCO_3$-MNPs for 6 h incubation (Green fluorescence is associated with LysoTracker; the red fluorescence is expressed by free DOX, released DOX, and DOX retained within $CaCO_3$-MNPs.). Scale bar: 20 μm.

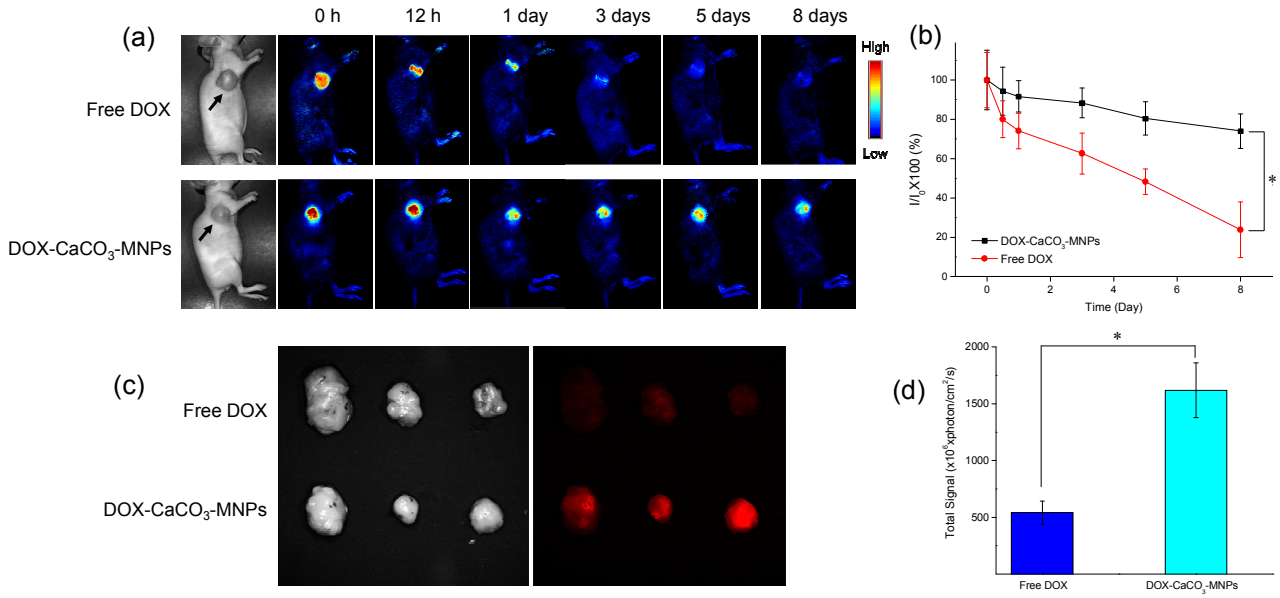

Figure S10. a) *In vivo* time-dependent non-invasive fluorescence imaging of SCC-7 tumor-bearing mice after intratumoral injection of free DOX and DOX-$CaCO_3$-MNPs. Solid arrows indicate the tumors. b) Time-dependent changes of the ratio of fluorescence intensities (I/I_0) as a function of time, where I and I_0 indicate the fluorescence intensity of the tumor at pre-determined time intervals and the fluorescence intensity of the tumor immediately after injection, respectively. c) *Ex vivo* fluorescence imaging of isolated tumors from three mice at 8 days post-injection of free DOX and DOX-$CaCO_3$-MNPs. d) Quantification of total fluorescence photon counts per each tumor (n = 3 mice per group) treated with free DOX or DOX-$CaCO_3$-MNPs. *$P < 0.01$.