Supporting Information

Stimulus-Responsive Anti-Oxidizing Drug Crystals and Its Ecological Implication

Byoung Soo Kim, Jiayu Leong, Seungjung Yu, Younghak Cho, Chang Gyun Park, Da-Hye Kim, Eunkyung Ko, Sung Gap Im, Jonghwi Lee, Young Jun Kim,* and Hyunjoon Kong**

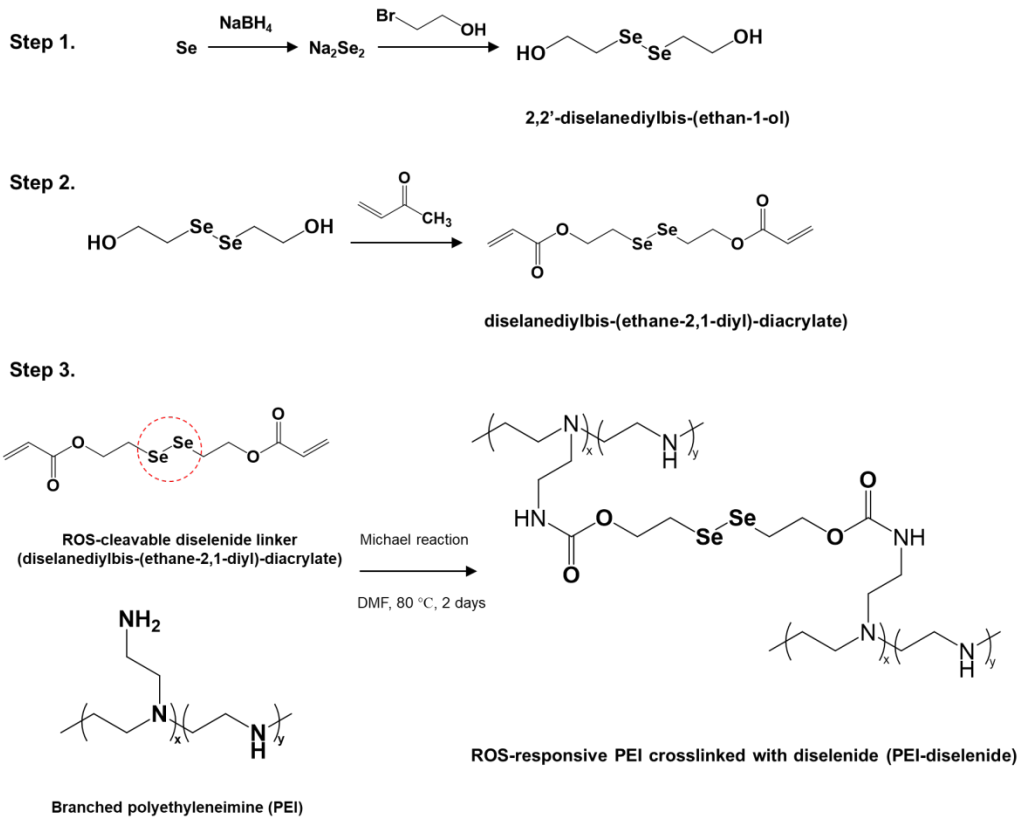

Figure S1. Synthetic scheme of the 2,2-diselanediylbis-(ethan-1-ol) (step 1), diselanediylbis-(ethane-2,1-diyl)-diacrylate (step 2), and PEI crosslinked with diselanediylbis-(ethane-2,1-diyl)-diacrylate, denoted as PEI-diselenide (step 3).

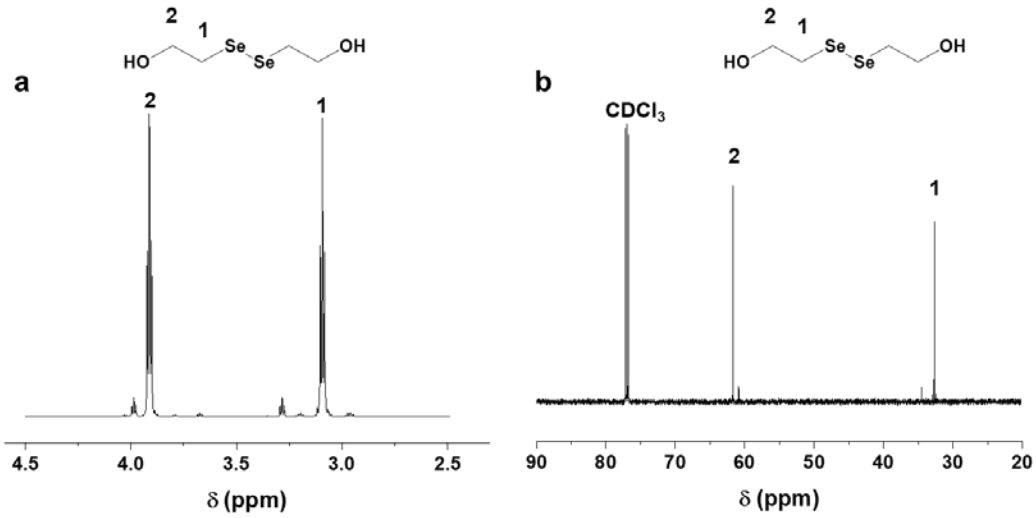

Figure S2. (a) ^{1}H NMR and (b) ^{13}C NMR spectrum of 2,2'-diselanediylbis-(ethan-1-ol).

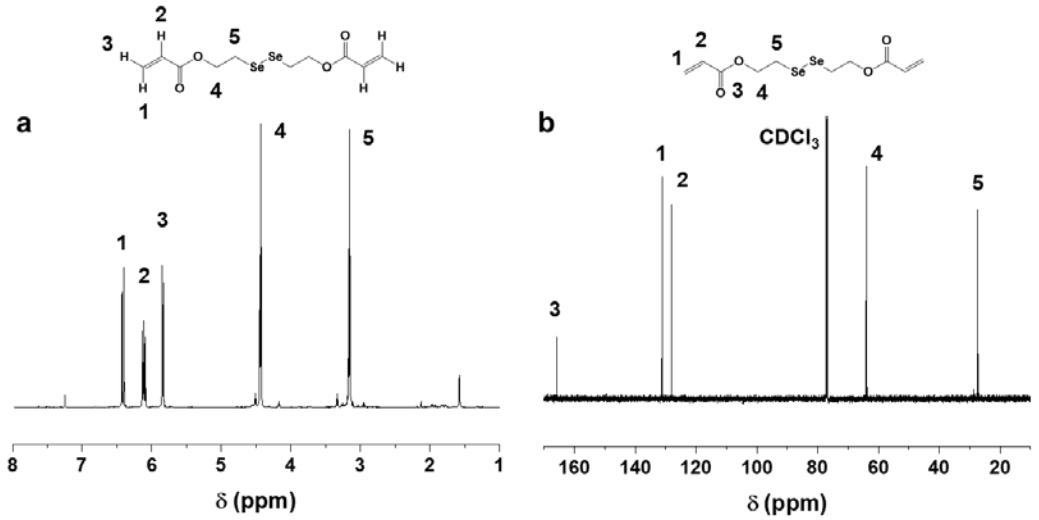

Figure S3. (a) ^{1}H NMR and (b) ^{13}C NMR spectrum of diselanediylbis-(ethane-2,1-diyl)-diacrylate.

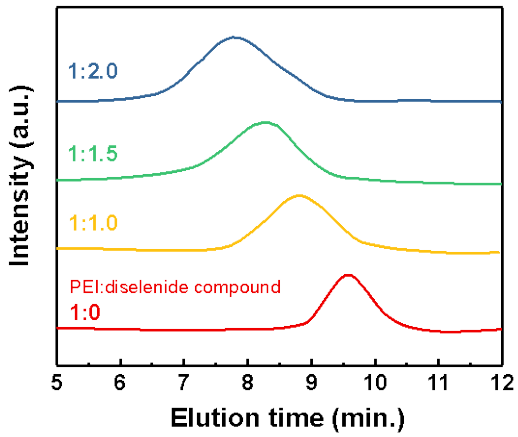

Figure S4. GPC diagrams of PEI-diselenide obtained with different molar ratios between PEI and diselanediylbis-(ethane-2,1-diyl)-diacrylate.

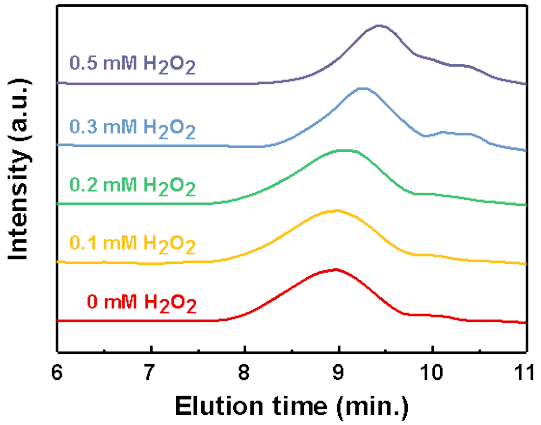

Figure S5. GPC diagrams of PEI-diselenide before and after H_2O_2 treatment (0.1, 0.2, 0.3, and 0.5 mM) for 24 h.

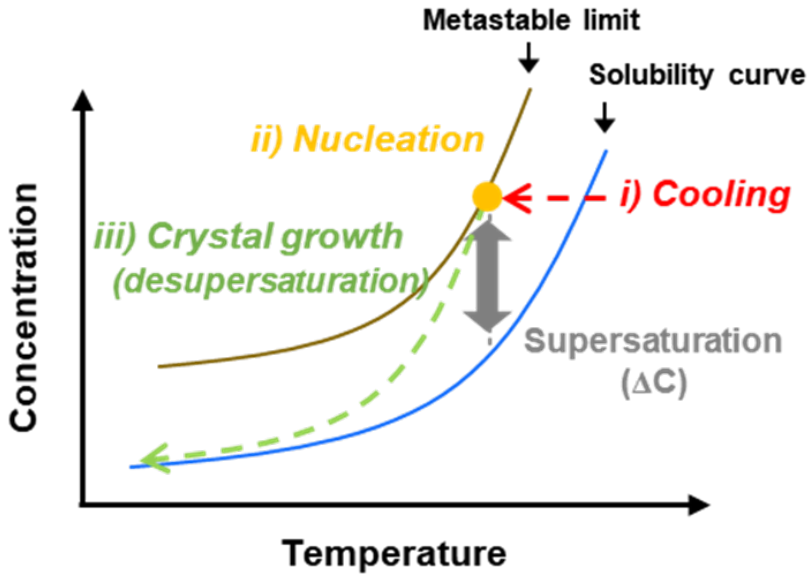

Figure S6. Schematic illustration of the polymer-directed cooling crystallization process.

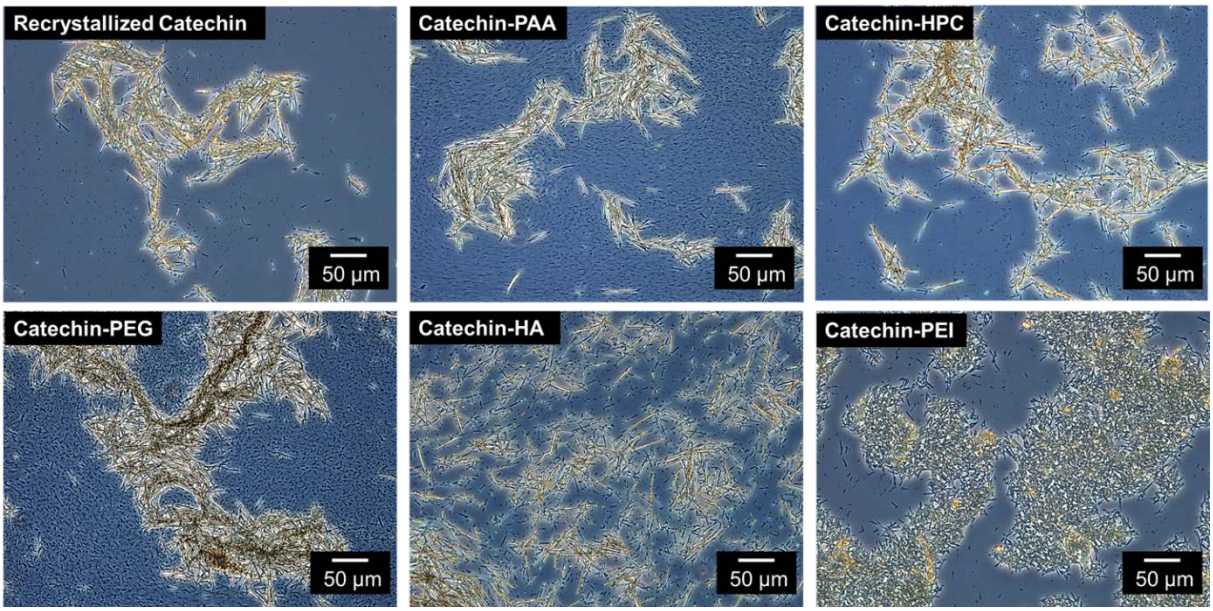

Figure S7. Optical micrographs of recrystallized catechin crystals with different polymer additives. The images were captured 24 hours after the cooling from 40 °C to 25 °C.

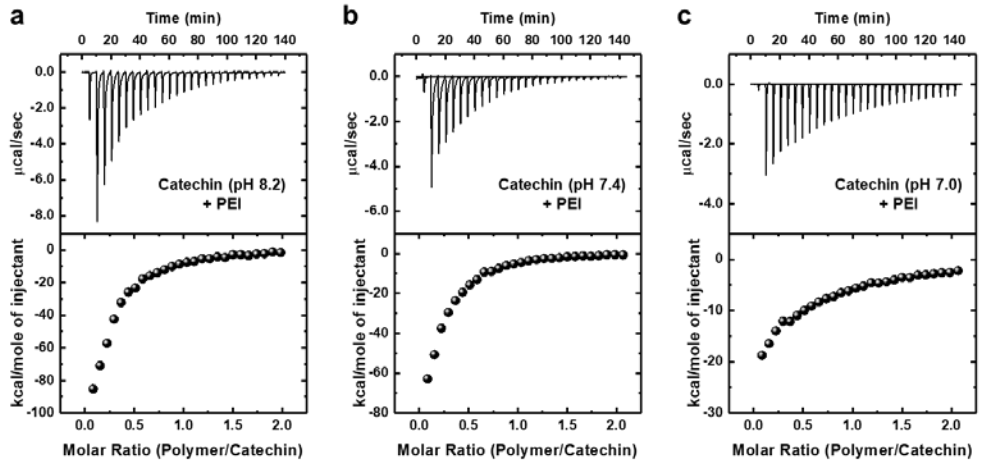

Figure S8. Representative calorimetric titrations of poly(ethylenimine) (PEI) with the catechin solution with pH of (a) 8.2, (b) 7.4, and (c) 7.0. Upper graphs represent heat flow against time for the titration of catechin, and lower graphs represent the integrated heats of each peak, normalized per mole of injectant (polymer).

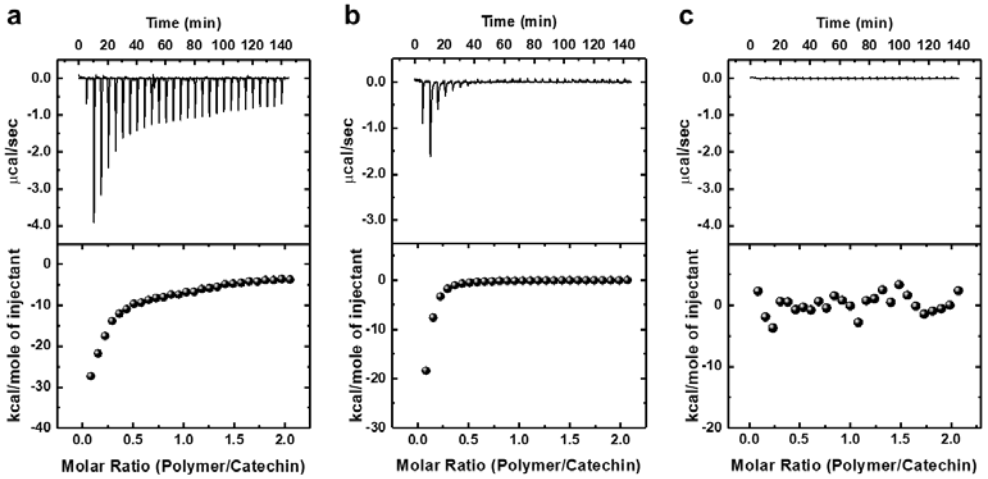

Figure S9. Representative calorimetric titrations of the catechin solution with PEI-diselenide obtained with different molar ratios between PEI and diselanediylbis-(ethane-2,1-diyl)-diacrylate: (a) 1:1, (b) 1:1.5, and (c) 1:2. Upper graphs represent heat flow against time for the titration of catechin, and lower graphs represent the integrated heats of each peak, normalized per mole of PEI-diselenide.

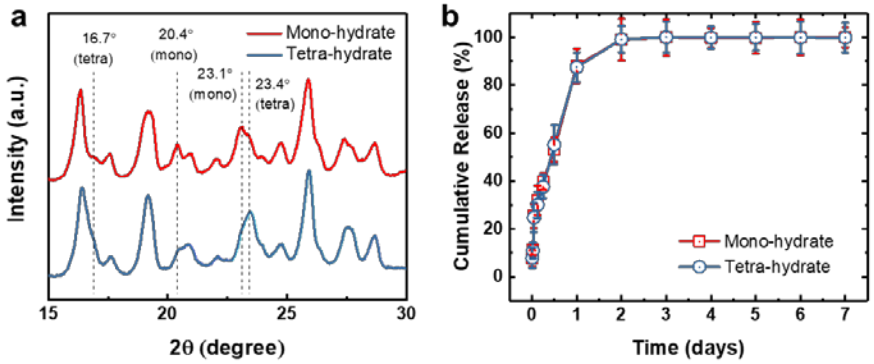

Figure S10. (a) X-ray diffraction patterns of the recrystallized catechin. (b) Cumulative release profiles of recrystallized catechin while being incubated in PBS at 37 ˚C.

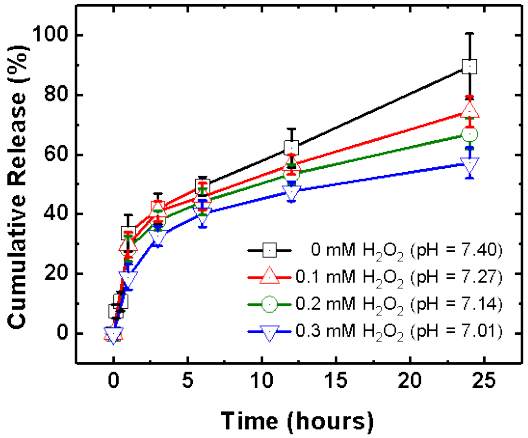

Figure S11. Cumulative release profiles of recrystallized catechin incubated with the PBS with different concentrations of H_2O_2. All samples were incubated at 37˚C. Data points and error bars represent mean values and standard deviation of 3 samples per condition, respectively.

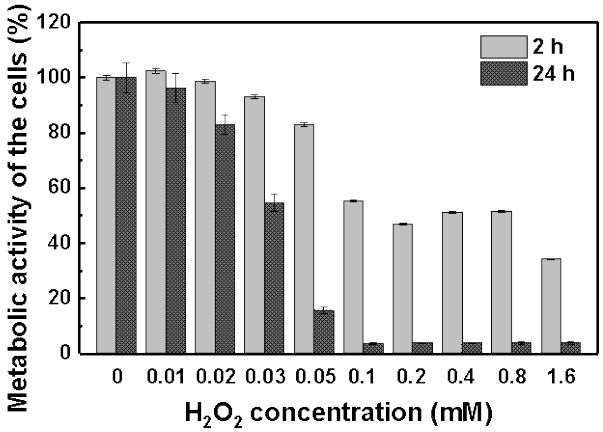

Figure S12. Analysis of metabolic activity of the C166 endothelial cells incubated with varying concentrations of H_2O_2.

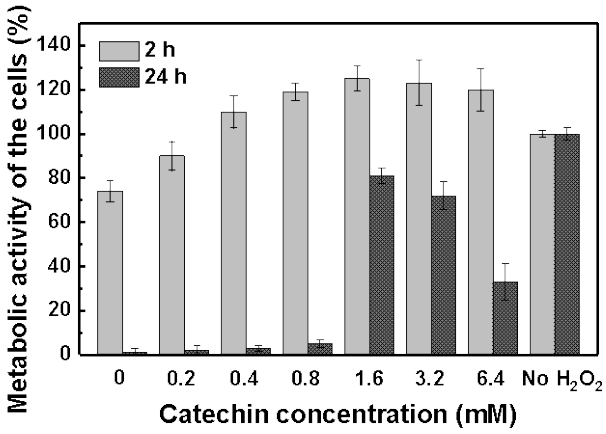

Figure S13. Analysis of the metabolic activity of the C166 endothelial cells incubated with varying concentrations of catechin. The concentration of H_2O_2 in the media was kept constant at 0.2 mM.

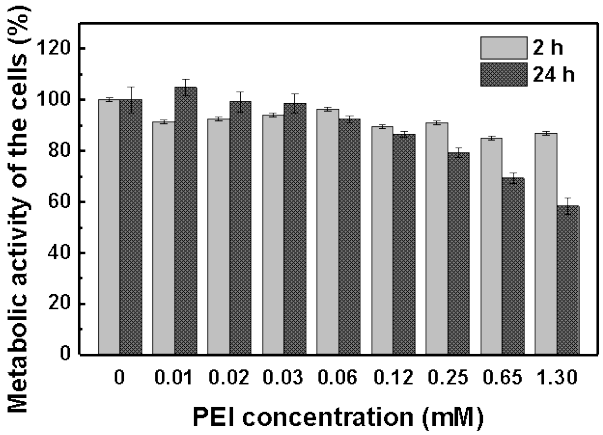

Figure S14. Analysis of cytotoxicity of PEI. Metabolic activity of C166 endothelial cells incubated with varying concentrations of PEI was examined using the MTT assay kit.

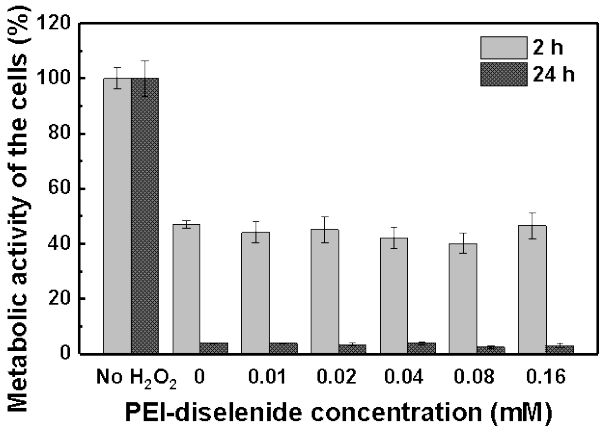

Figure S15. Analysis of the metabolic activity of the C166 endothelial cells incubated in the 0.2 mM H_2O_2 solution mixed with varying concentrations of PEI-diselenide.

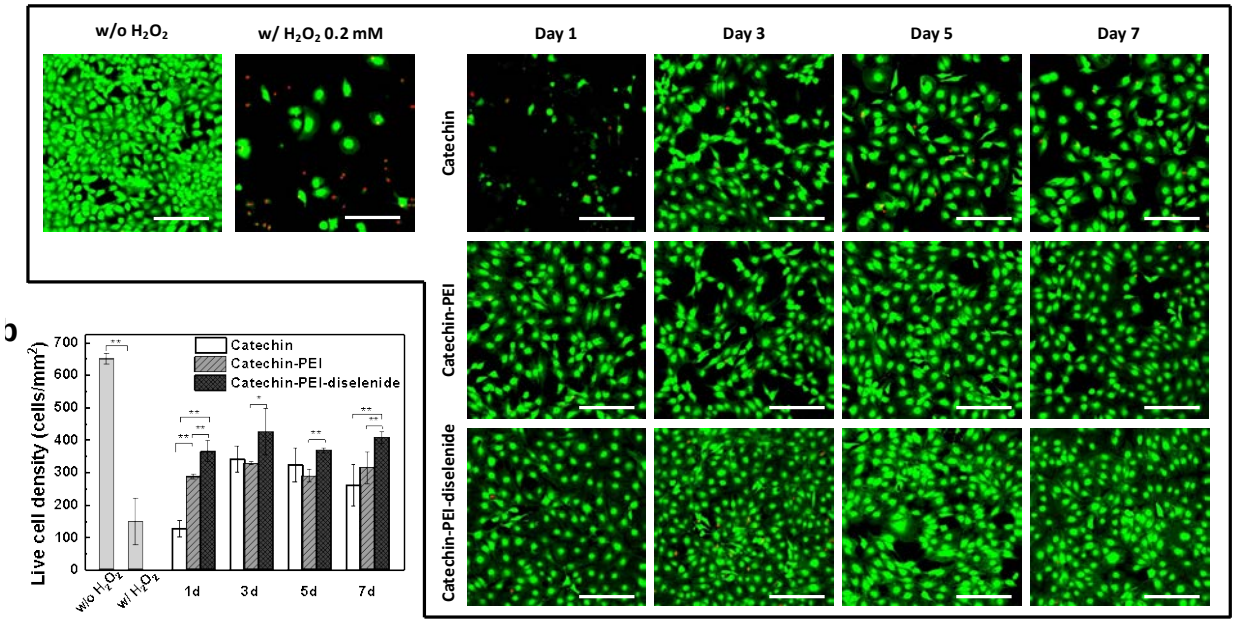

Figure S16. (a) Representative confocal images of the live and dead endothelial cells (C166) incubated in the media containing 0.2 mM H_2O_2 and catechin crystals. Green color represents live cells, and the red color represents dead cells. The scale bars represent 200 μm. (b) Quantification of the number of live cells per mm^2 using the images in (a). Data points and error bars represent mean values and standard deviation of 5 samples per condition, respectively. * and ** represent the statistical significance of the difference of values between conditions indicated with line (*$p < 0.05$, **$p < 0.01$).

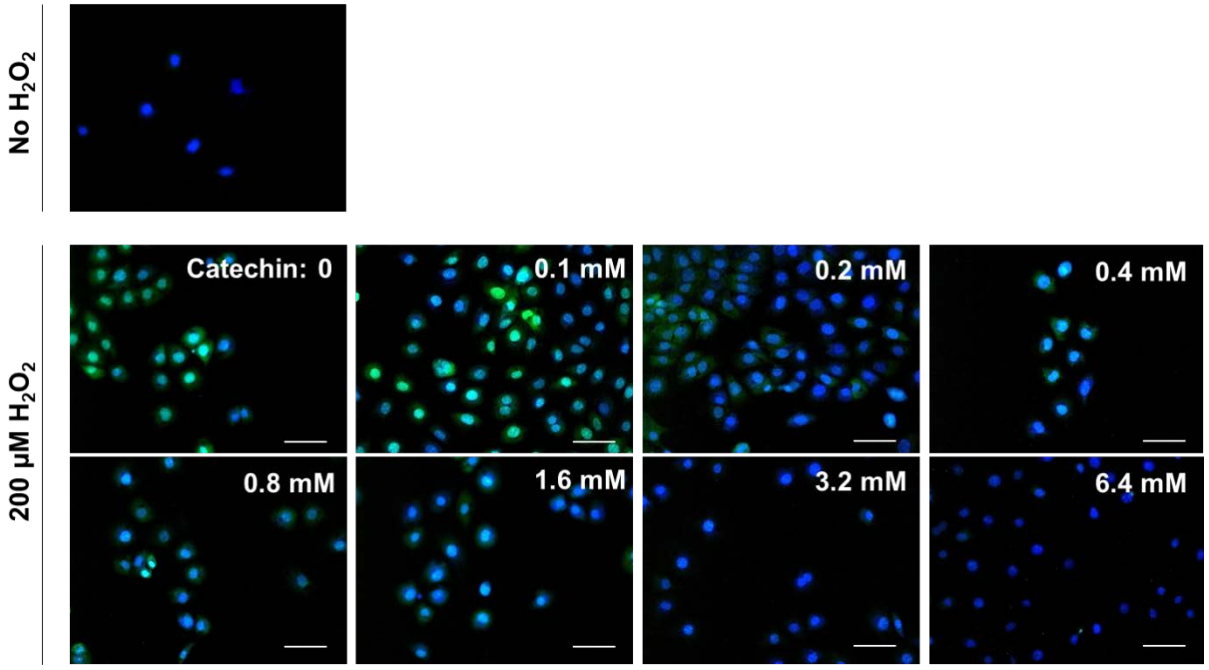

Figure S17. Representative confocal images of the oxidative stress of endothelial cells (C166) incubated in the media containing 0.2 mM H_2O_2 and different concentrations of catechin. The scale bars represent 100 µm.

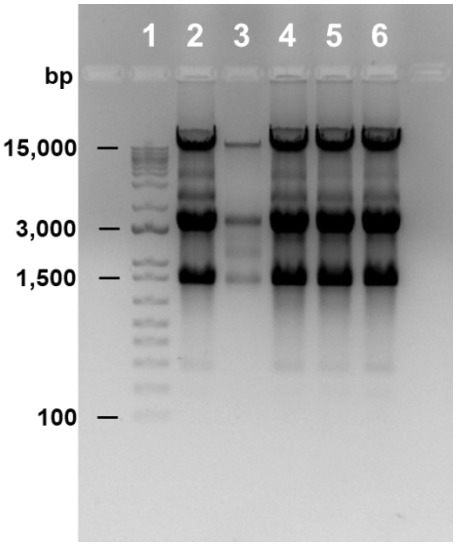

Figure S18. Agarose gel electrophoresis of extracted DNA from C166 endothelial cells. Lane 1, DNA marker, Lane 2, untreated C166 cells, Lane 3, the cells incubated with 0.2 mM H_2O_2 for 24h, Lane 4 – 6, the cells incubated with 0.2 mM H_2O_2 with 0.8, 1.6, and 3.2 mM catechin for 24h, respectively.

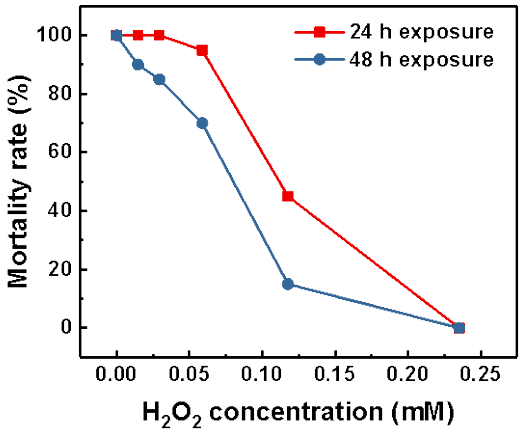

Figure S19. Mortality rate of *Daphnia magna* exposed to H_2O_2 for 24 or 48 hours with increasing concentration of H_2O_2.

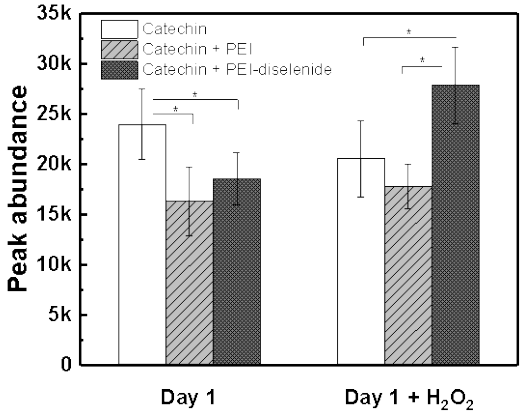

Figure S20. Mass profiles of catechin dissolved in the media containing 0.05 mM H_2O_2 and catechin crystals after 24 h. The results are represented as means ± SD, n= 10. * represents the statistical significance of the difference of values between conditions indicated with line (*p < 0.01).

Table S1. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) of PEI-diselenide obtained with different molar ratios between PEI and diselanediylbis-(ethane-2,1-diyl)-diacrylate.

Molar ratio (PEI : diselenide compound)	**M_w (g/mol)**	**M_n (g/mol)**	**M_w/M_n**
1.0 : 2.0	11,500	5,000	2.3
1.0 : 1.5	7,300	3,476	2.1
1.0 : 1.0	5,028	2,793	1.8
1.0 : 0.0 (PEI only)	800	620	1.3

Table S2. The molecular weight of PEI-diselenide incubated in aqueous media with varying concentrations of H_2O_2. The GPC was run with a buffer of acetic acid (100 mM)/sodium acetate (100 mM). Average molecular weights were measured after incubating the PEI-diselenide in the H_2O_2 solution for 24 hours.

Sample	**M_w (g/mol)**	**M_n (g/mol)**	**M_w/M_n**
PEI- diselenide	5,028	2,793	1.8
PEI-diselenide + 0.1 mM H_2O_2	3,200	1,524	2.1
PEI-diselenide + 0.2 mM H_2O_2	2,620	1,108	2.3
PEI-diselenide + 0.3 mM H_2O_2	1,740	700	2.5
PEI-diselenide + 0.5 mM H_2O_2	890	405	2.2

Table S3. Thermodynamic parameters quantified from the isothermal titration calorimetry analysis of the interaction between catechin and polymers. Titrations were run at 40 ˚C and units of the change in enthalpy (*ΔH*), and the change in Gibbs free energy (*ΔG*) are kcal/mol; the change in entropy (*ΔS*) is kcal/mol·°C ; *K* is the binding constant (10^4 M^{-1}).

Catechin solution	**Δ*H***	**Δ*G***	**Δ*S***	***K***
pH ~8.2	-55.6	-7.2	-1.2	9.7
pH ~7.4	-37.9	-7.0	-0.8	7.1
pH ~7.0	-10.6	-5.9	-0.1	2.3