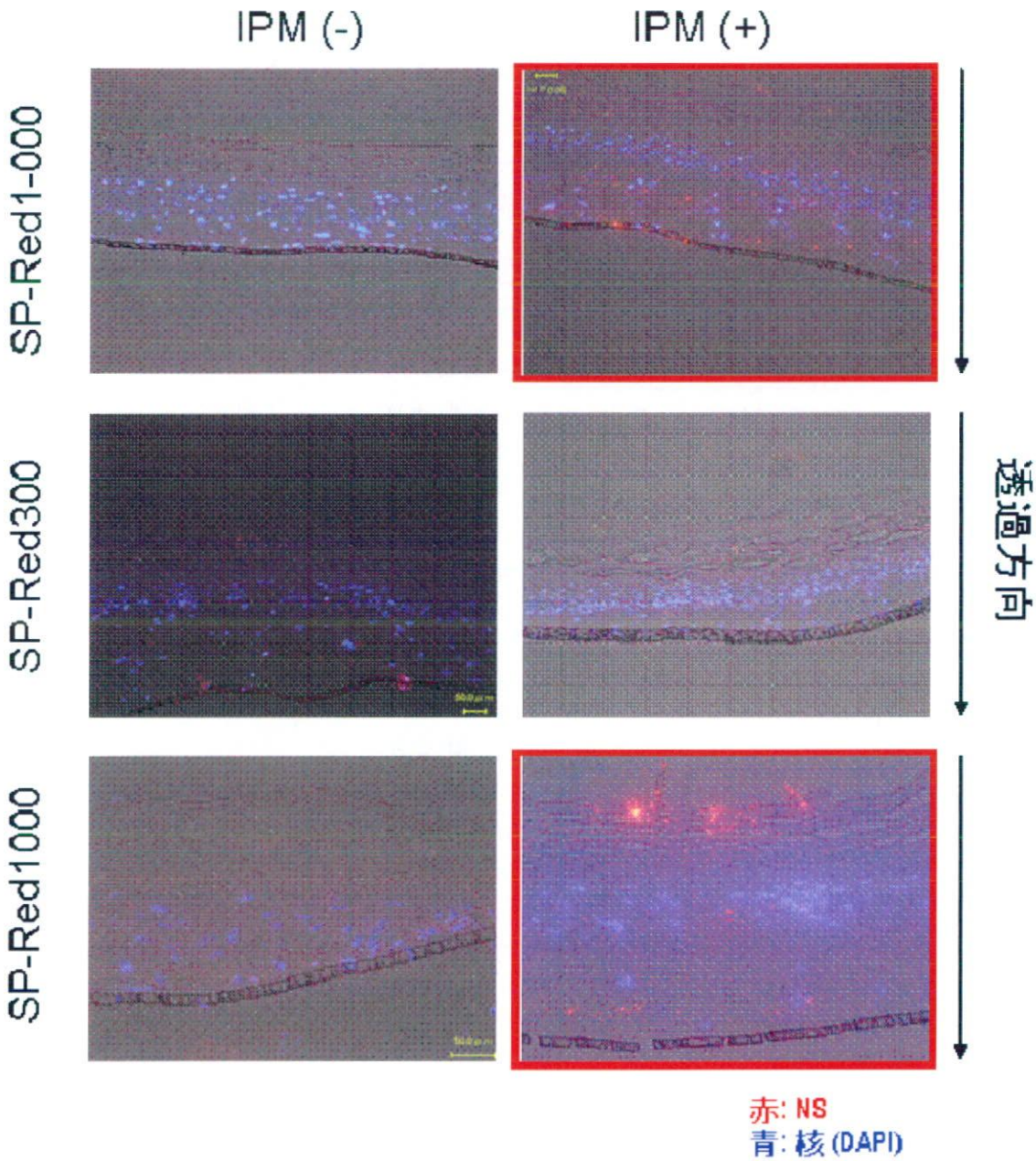
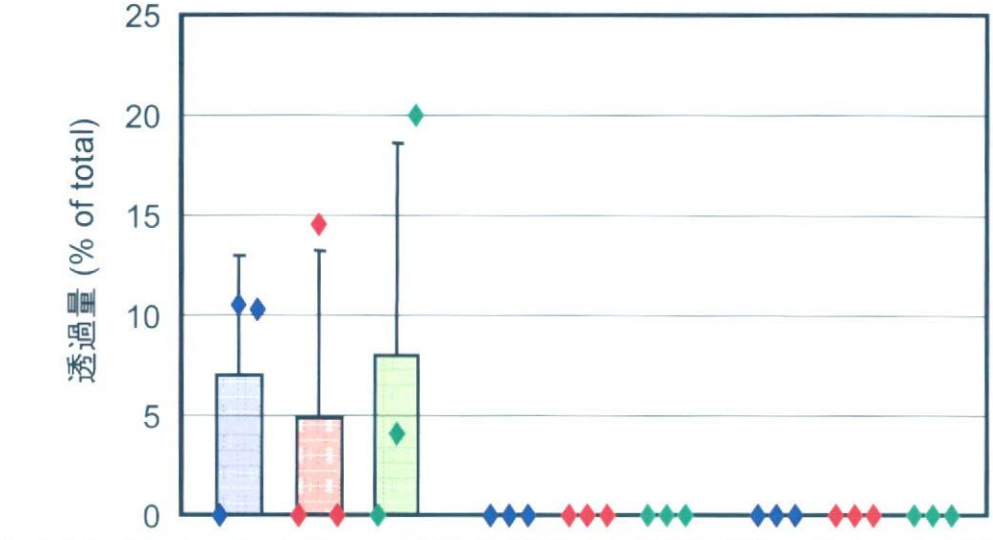


図2 ヒト三次元培養皮膚を用いたSPの皮膚透過実験



Nano-Slica	SP-FITC70			SP-FITC300			SP-FITC1000		
IPM	+	+	-	+	+	-	+	+	-
incubation(hr)	3	9	9	3	9	9	3	9	9

図3 SPのLSE透過量の定量

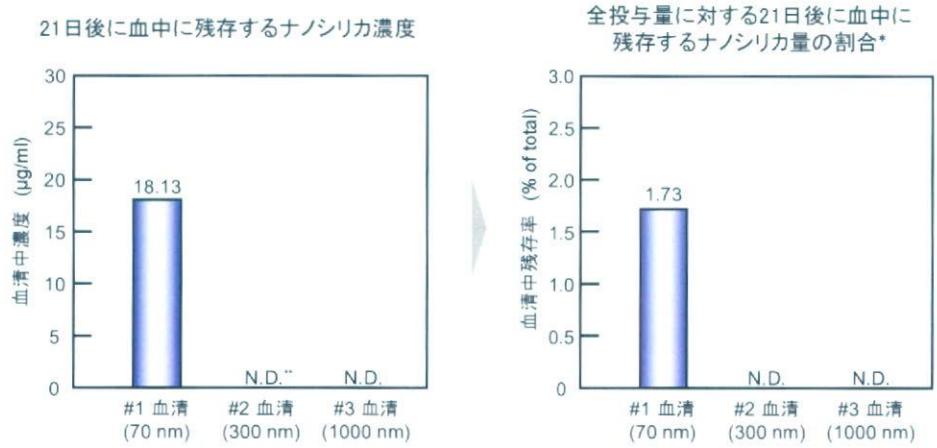


図4 ブタを用いた皮膚を塗布したSPの血中移行量の定量

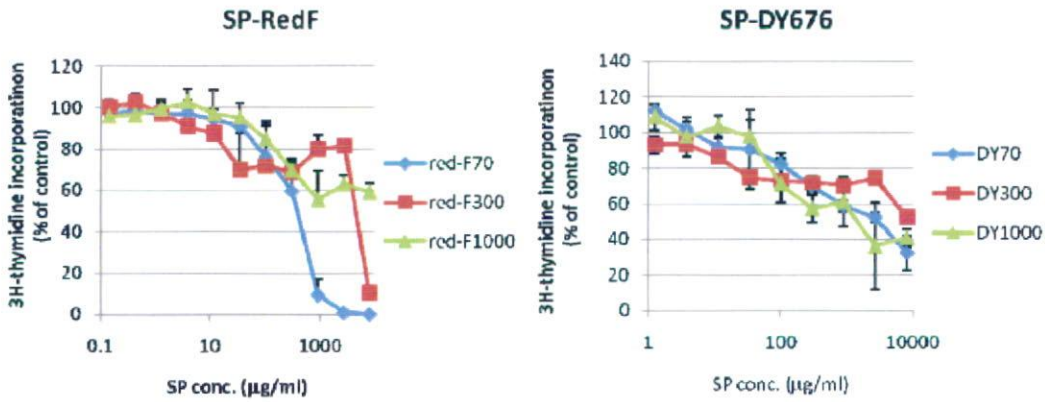


図5　シリカナノ粒子の角化細胞に対する細胞傷害性評価
各種粒径のred-F標識シリカナノ粒子あるいはDY676標識シリカナノ粒子存在下で24時間培養したHaCaT細胞の^{3}H-thymidine取り込みを測定した。

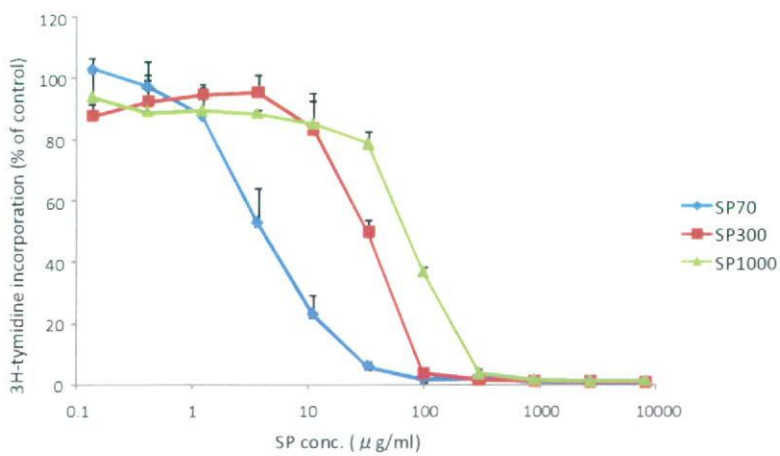


図6　シリカナノ粒子のランゲルハンス細胞に対する細胞傷害性評価
各種粒径のred-F標識シリカナノ粒子存在下で24時間培養したXS52細胞の^{3}H-thymidine取り込みを測定した。

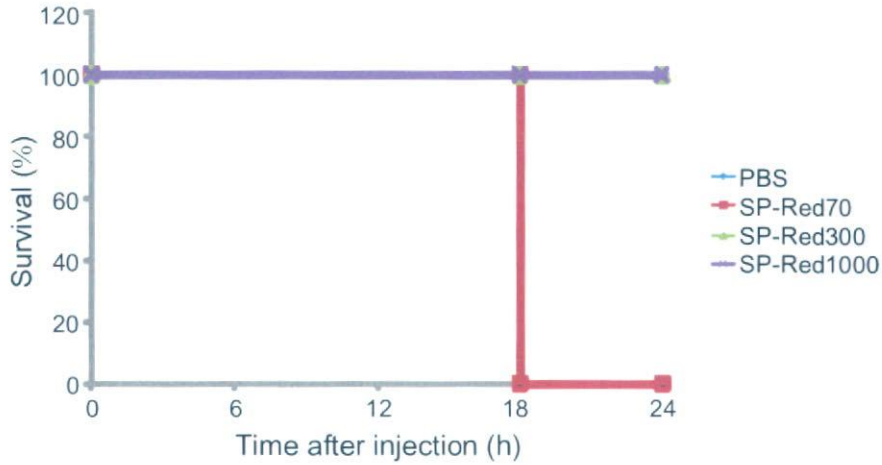


図7 in vivoにおけるシリカナノ粒子の急性毒性

各粒子径のred-F標識シリカナノ粒子を2.0mg/mouseで尾静脈投与後、各観察時間における生存率を示した。観察時間は投与後0 ,6 ,18 ,24 hr。

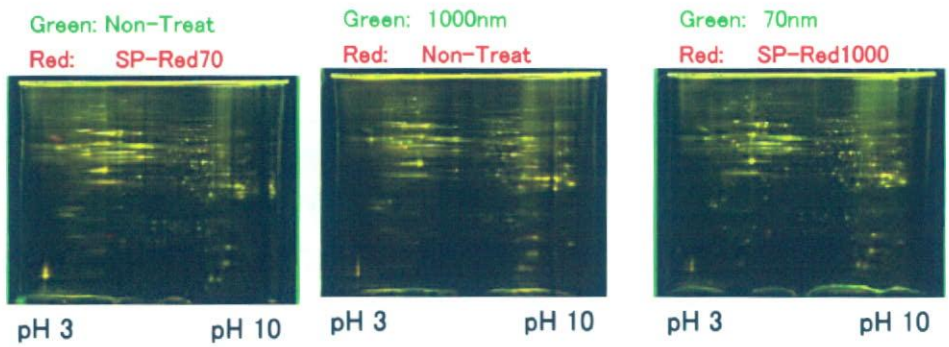


図8 2D-DIGEによるシリカナノ粒子のトキシコプロテオーム解析
HaCaT細胞を70nm又は1000nmの未標識シリカナノ粒子100μg/ml存在下で24hr培養後、細胞溶解液(whole cell lysate)を作製した。シリカナノ粒子未処理および、シリカナノ粒子処理細胞溶解液50μgをそれぞれcy3およびcy5で標識し、それらを混合して2次元電気泳動を行った。

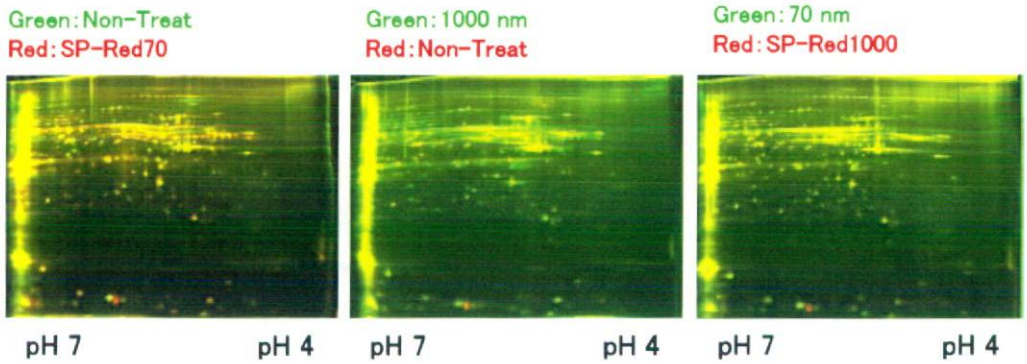


図9 オルガネラ(核)レベルでのシリカナノ粒子のトキシコプロテオーム
HaCaT細胞を70nm又は1000nmのred-F標識シリカナノ粒子100μg/ml存在下で24hr培養後、その細胞溶解液から核を分画しサンプルとした。シリカナノ粒子未処理およびシリカナノ粒子処理細胞の核画分サンプル50μgをそれぞれcy3およびcy5で標識し、それらを混合して2次元電気泳動を行った。

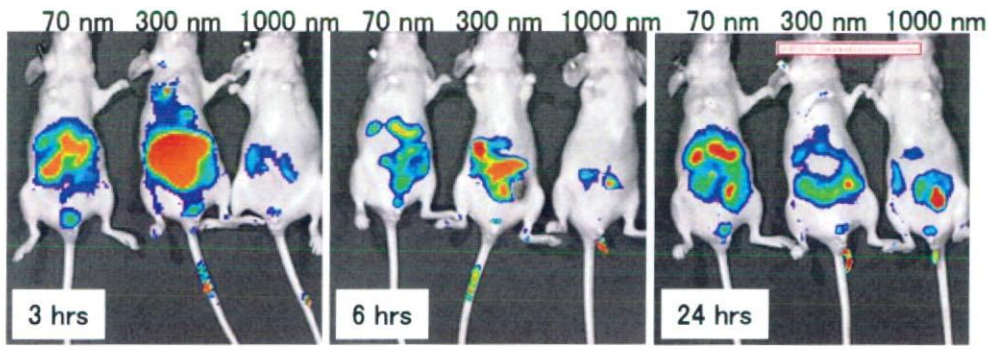


図10 In vivo imaging によるナノマテリアルの挙動解析
各粒子径のDY676ラベルシリカナノ粒子を約7 x10^{10}particle/mouseとなるように尾静脈投与し、投与後3, 6, 24 hrの粒子の挙動をin vivo imager (Xenogen IVIS 200 imaging system)にて撮影した。

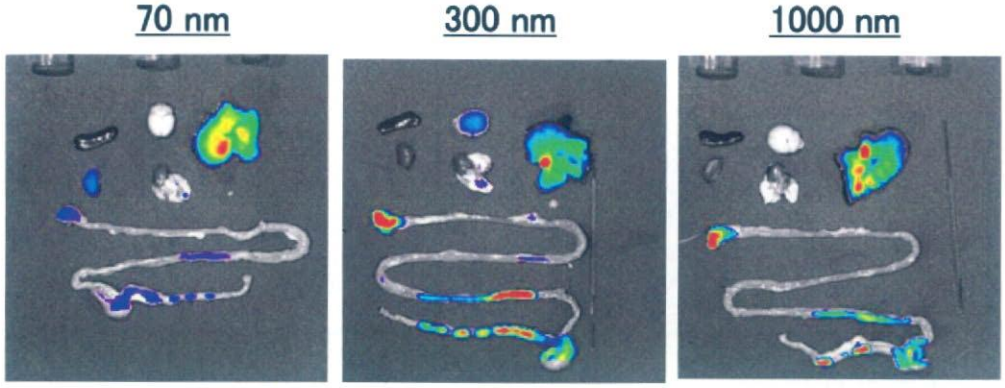


図11　ex vivo imaging 解析 (24hr)
各粒子径のDY676ラベルシリカナノ粒子を約7 x10^{10}particle/mouseとなるように尾静脈投与し、投与後24hrの粒子の各組織への集積をex vivoにて撮影した。

	カタログ値	Z-average D. (nm)	Peak Top D. (nm)	Zeta Potential (mV)
sicastar® non-labeled	70nm	77.7	85.3	-55.2
	300nm	322	336	-62.1
	1000nm	1140	896	-67.0
sicastar®-redF	70nm	82.8	88.4	-55.8
	300nm	354	371	-60.3
	1000nm	1400	800	-72.2
sicastar®-F (DY-676)	70nm	66.1	74	-51.6
	300nm	350	367	-61.7
	1000nm	1290	1320	-81.1
	70nm	101	113	-29.1
	300nm	317	349	-41.5
	1000nm	1270	875	-37.8

図12　ZetaSizerによるシリカナノ粒子の粒径と表面電荷測定
各粒子径のシリカナノ粒子を蒸留水にて100倍に希釈し、粒子径を動的光散乱法にて、表面電荷(ゼータ電位)をレーザードップラー電気泳動法にて測定した。

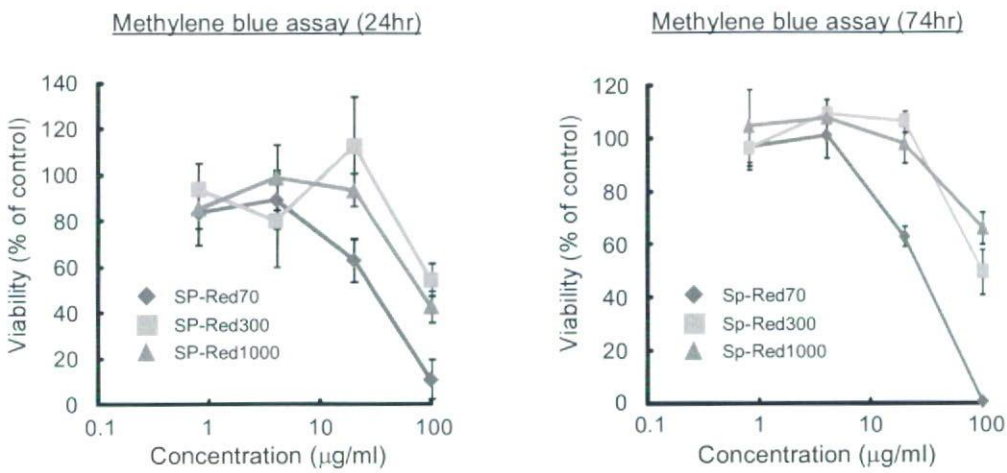


図13 Cytotoxicity of nanoparticles on macrophage cells (RAW264.7). Cells were treated with different concentrations of nanoparticles for 24 or 74 hr. At the end of the incubation period, cellular viability were determined by the Methylene blue assay, respectively. The subtracted OD value of control cells (nanoparticle-free medium) was taken as 100% and then calculated as the percentage of reduction of OD in nanoparticle-exposed cells. (n = 6)

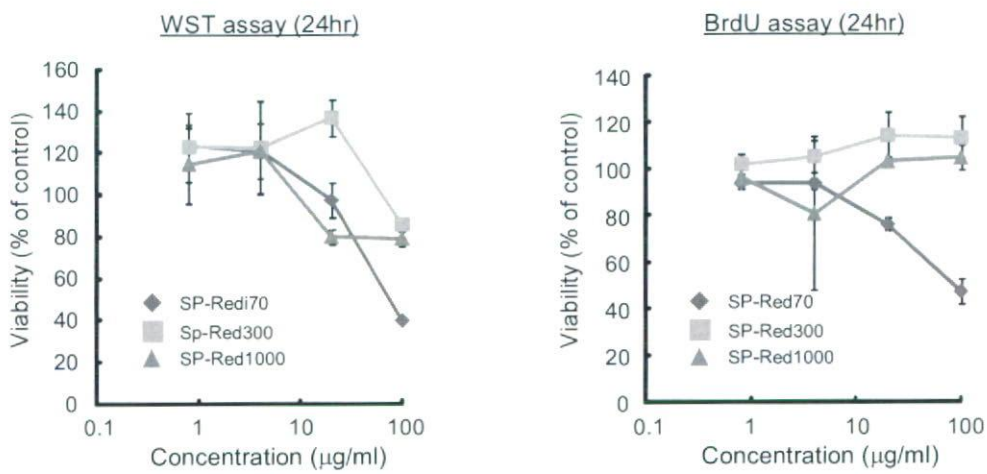


図14 Cytotoxicity of nanoparticles on RAW264.7 cells. Cells were treated with different concentrations of nanoparticles for 24 hr. At the end of the incubation period, cellular viability were determined by WST-1 assay or BrdU assay, respectively. The subtracted OD value of control cells (nanoparticle-free medium) was taken as 100% and then calculated as the percentage of reduction of OD in nanoparticle-exposed cells. (n = 3-4)

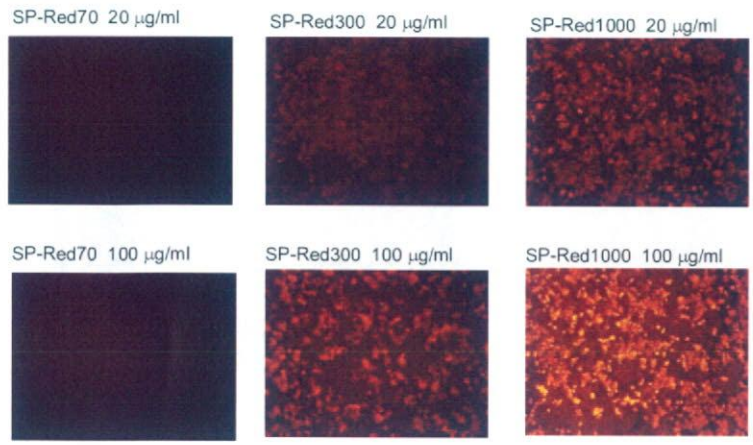


図15 Fluorescence microscope observation of RAW264.7 cells treated with various nanoparticles. Cells were treated with different concentrations of nanoparticles for 24 hr. At the end of the incubation period, cells were observed under fluorescence microscope.

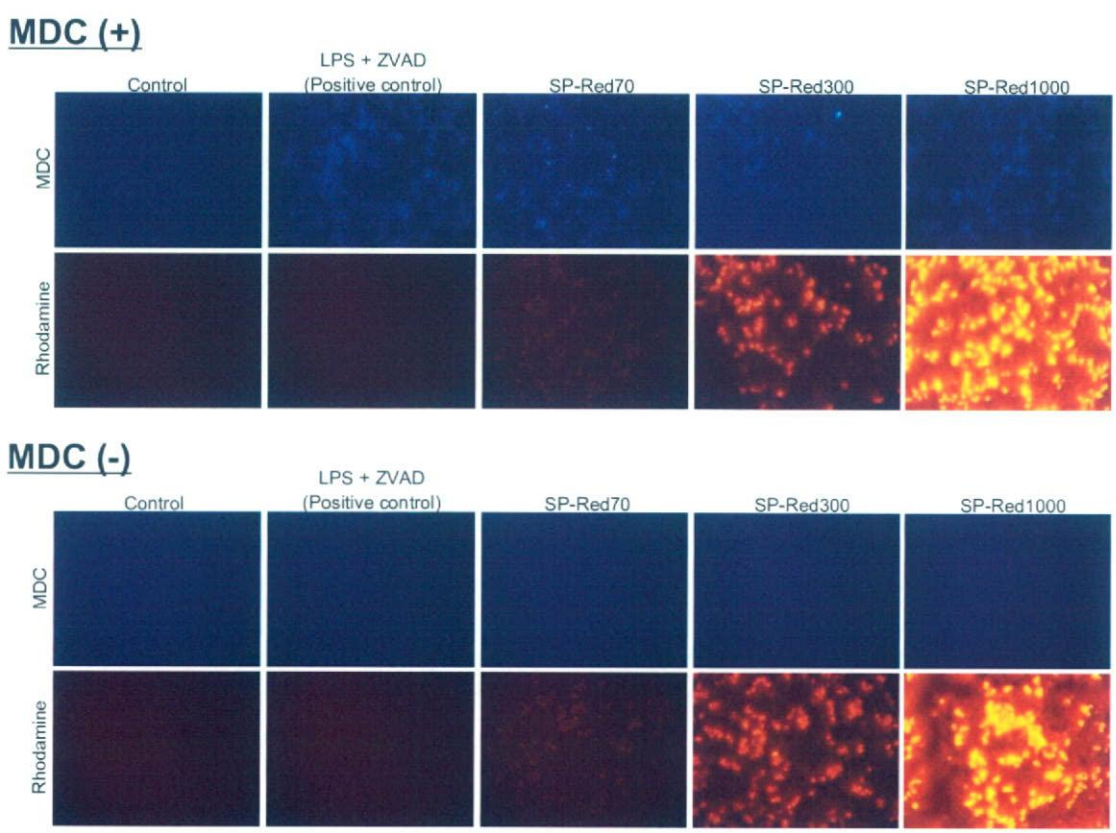


図16 MDC staining of RAW264.7 cells treated with various nanoparticles. Cells were treated with 100 μg/ml of nanoparticles or LPS (100 ng/ml) + ZVAD (50 μM) for 8 hr. At the end of the incubation period, representative control cells and nanoparticles treated cells labeled with MDC are shown.

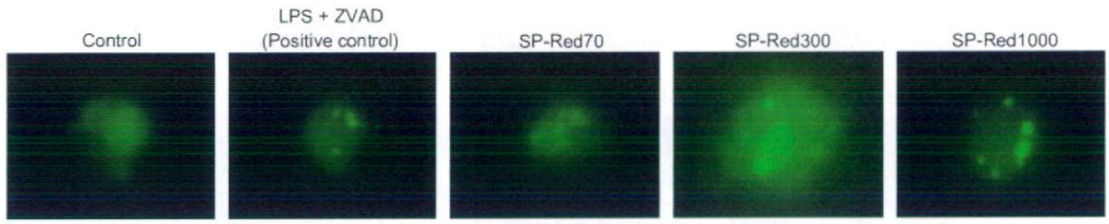


図17 Nanoparticles induces autophagy on RAW264.7 cells. Cells that had been transfected with the expression plasmid of GFP-LC3 were treated with LPS + ZVAD or 100 μg/ml of nanoparticles for 12 hr. The cells were analyzed under fluorescence microscope.

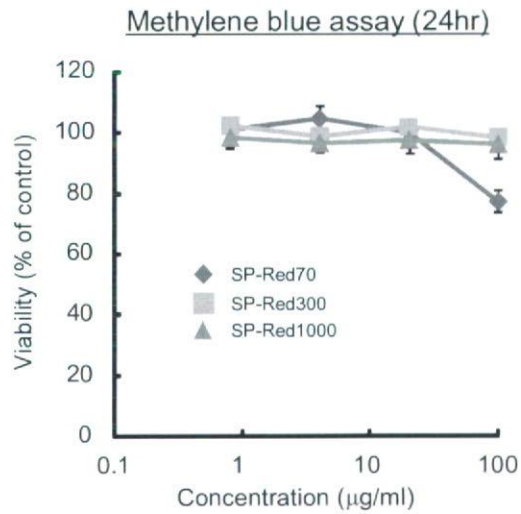


図18 Cytotoxicity of nanoparticles on dendritic cells (DC2.4). Cells were treated with different concentrations of nanoparticles for 24 hr. At the end of the incubation period, cellular viability were determined by the Methylene blue assay. The subtracted OD value of control cells (nanoparticle-free medium) was taken as 100% and then calculated as the percentage of reduction of OD in nanoparticle-exposed cells. (n = 6)

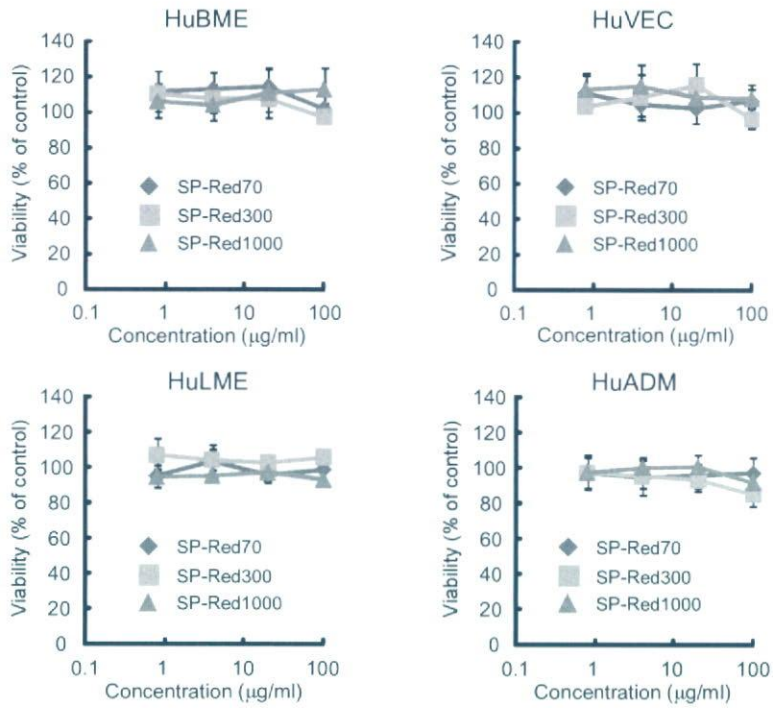


図19 Cytotoxicity of nanoparticles on vascular endothelial cells (HuBME, HuVEC, HuLME, or HuADM). Cells were treated with different concentrations of nanoparticles for 24 hr. At the end of the incubation period, cellular viability were determined by the Methylene blue assay, respectively. The subtracted OD value of control cells (nanoparticle-free medium) was taken as 100% and then calculated as the percentage of reduction of OD in nanoparticle-exposed cells. (n = 6)

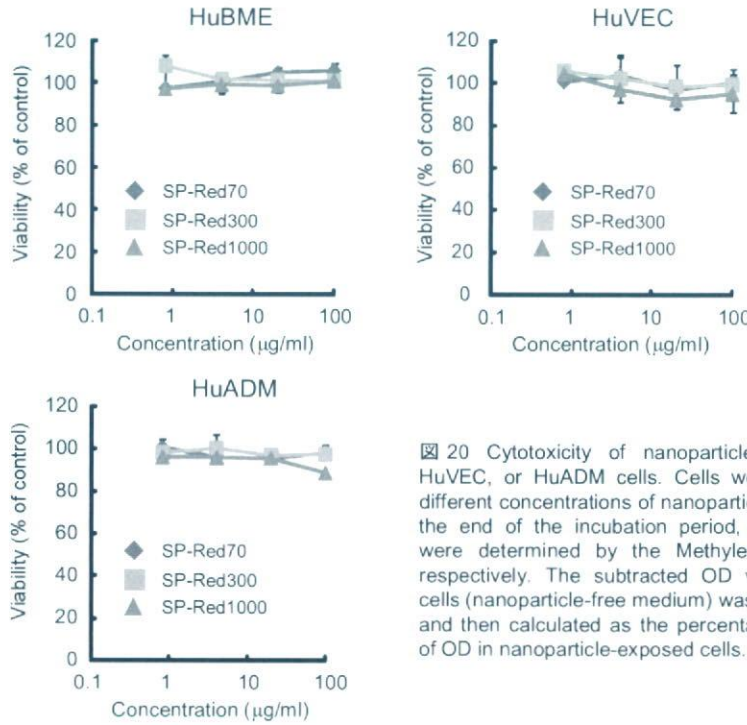


図 20 Cytotoxicity of nanoparticles on HuBME, HuVEC, or HuADM cells. Cells were treated with different concentrations of nanoparticles for 72 hr. At the end of the incubation period, cellular viability were determined by the Methylene blue assay, respectively. The subtracted OD value of control cells (nanoparticle-free medium) was taken as 100% and then calculated as the percentage of reduction of OD in nanoparticle-exposed cells. (n = 6)

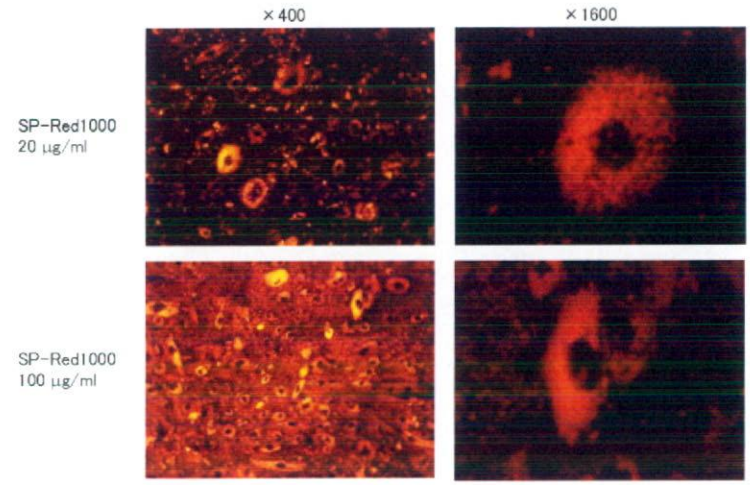


図21 Uptake of nanoparticles by HuBME cells. Cells were treated with different concentrations of nanoparticles for 24 hr. At the end of the incubation period, cells were observed under fluorescence microscope. Magnification, × 400, × 1600.

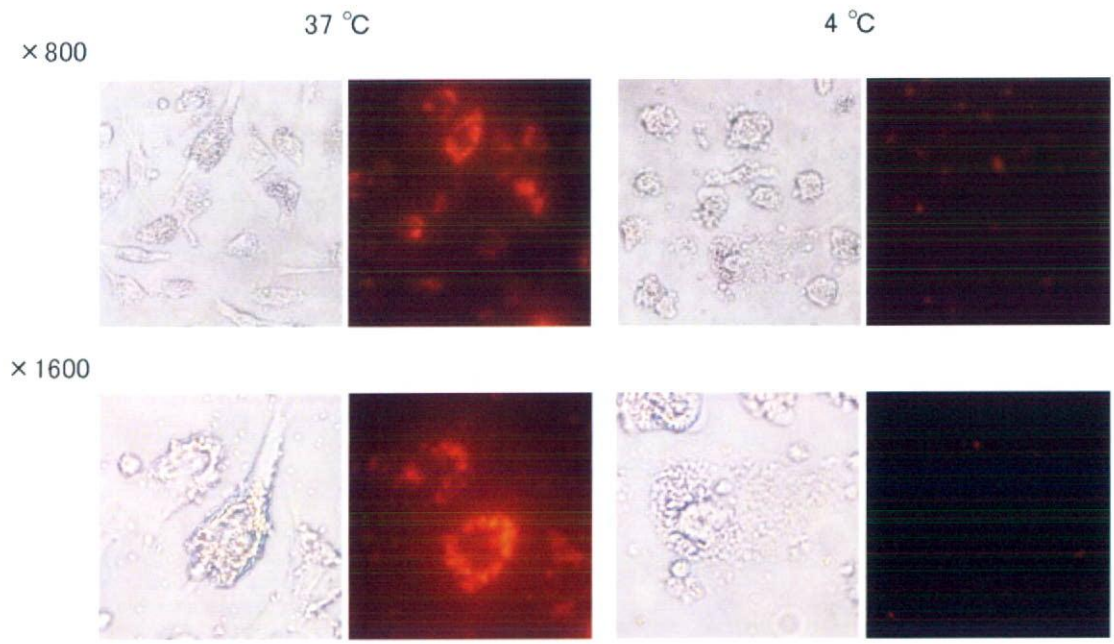


図22 Uptake of nanoparticles by HuBME cells at 37 °C or 4 °C. HuBME cells were preincubated at 37 °C or 4 °C for 1 hr and added 100 μg/ml of Si1000. After 8 hr incubation at 37 °C or 4 °C, cells were observed under fluorescence microscope. Magnification, × 800, × 1600.

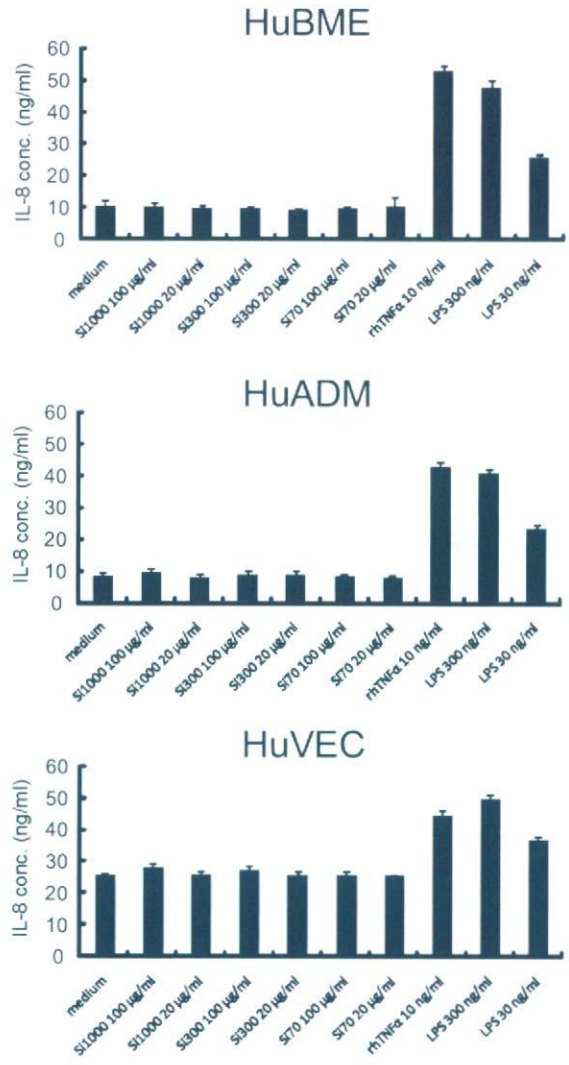


図23 IL-8 concentration in culture supernatant on nanoparticles-treated endothelial cells. HuBME, HuADM, or HuVEC cells (5×10^3 cells) were treated with different concentrations of nanoparticles, rhTNF α (10 ng/ml), or LPS (300, 30 ng/ml) for 24 hr. IL-8 concentration in culture supernatant was determined by ELISA, respectively. (n = 6)

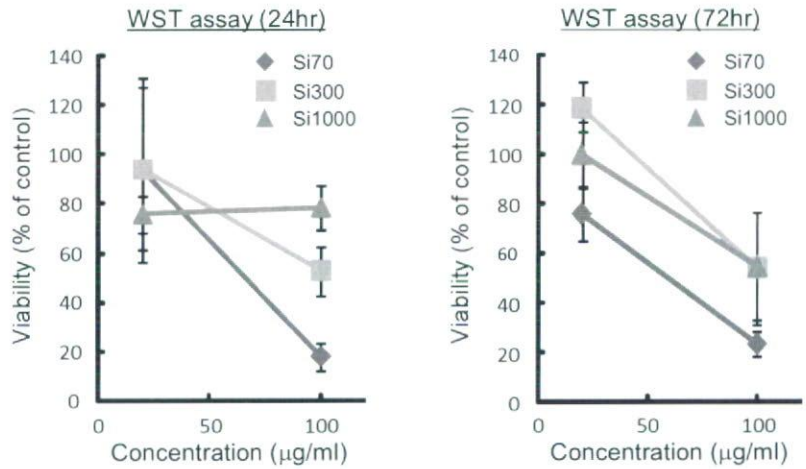


図24 Cytotoxicity of nanoparticles on neuron stem cells. Cells were treated with different concentrations of nanoparticles for 24 or 72 hr. Cellular viability were determined by the WST-1 assay, respectively. (n = 3)

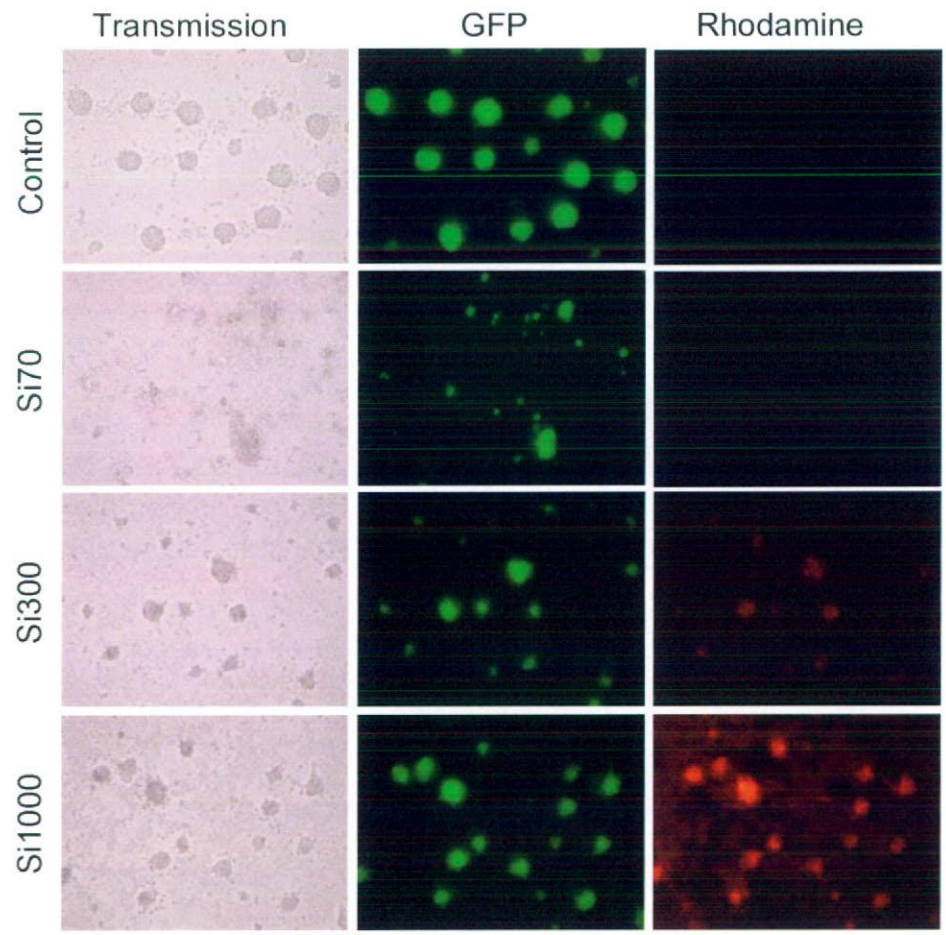


図25 Effect of nanoparticles on neural stem cells. Cells were treated with 100 μg/ml of nanoparticles for 24 hr. At the end of the incubation period, cells were observed under fluorescence microscope.

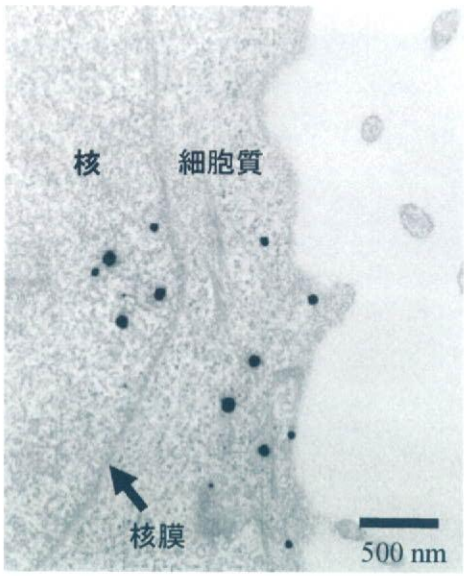


図26-1　培養細胞に 70 nm シリカを 30 μg/ml 処置
細胞質および核内にシリカ（黒いドット）が侵入している

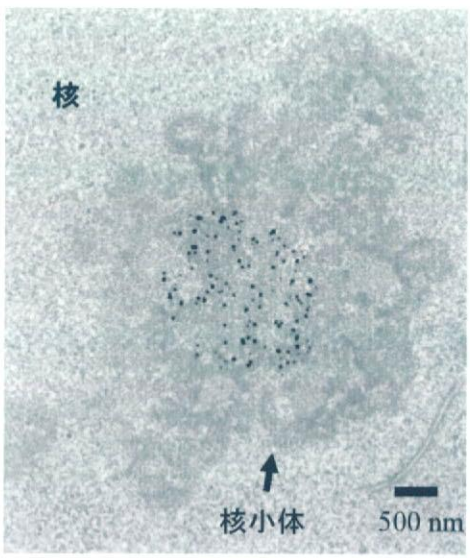


図26-2　培養細胞に 70 nm シリカを 100 μg/ml 処置
核小体内にシリカが侵入している

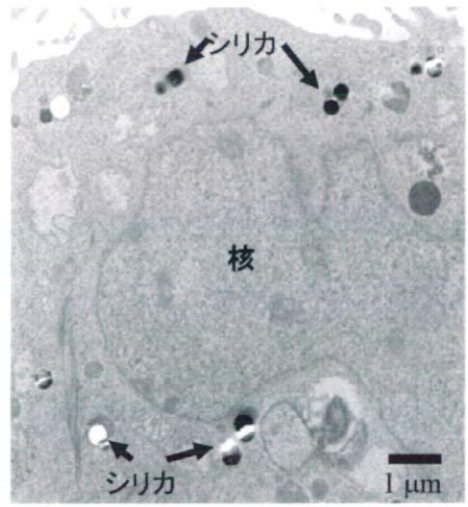


図27-1　培養細胞に 300 nm シリカを 30 μg/ml 処置
細胞質内にシリカが侵入している

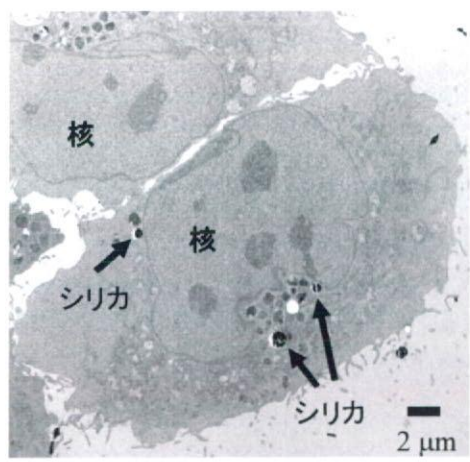


図27-2　培養細胞に 1000 nm シリカを 30 μg/ml 処置
細胞質内にシリカが侵入している

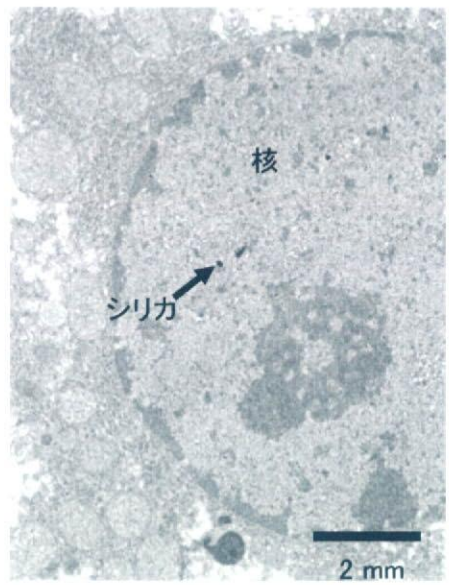


図28　マウス肝細胞、70 nm シリカを10 mg/kg 投与
核内にシリカが侵入している

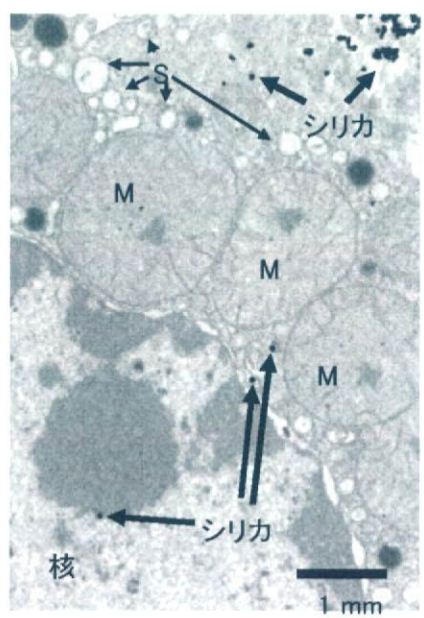


図29　マウス肝細胞、70 nm シリカ 100 mg/kg を投与、無染色
細胞質と核小体にシリカが侵入している。ミトコンドリア(M)内に沈着物、
細胞質に小管状物質(S)が見られる

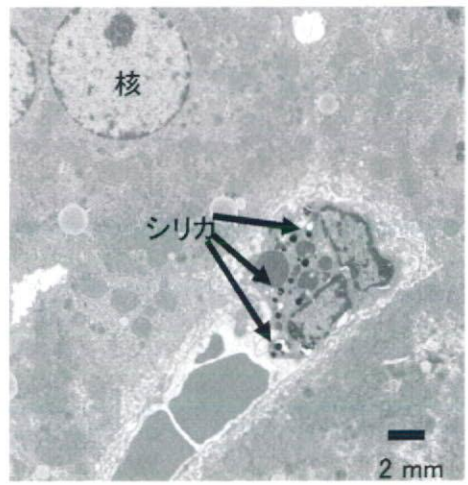


図30-1　マウス肝細胞　300 nm シリカ 10 mg/kg 投与
シリカはクッパー細胞に貪食されている

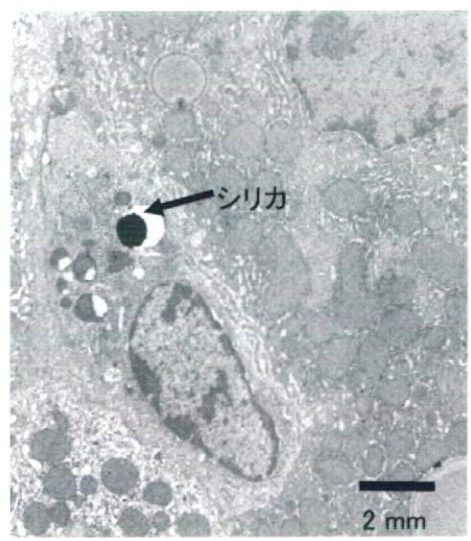


図30-2　マウス肝細胞　300 nm シリカ 10 mg/kg 投与
シリカはクッパー細胞に貪食されている

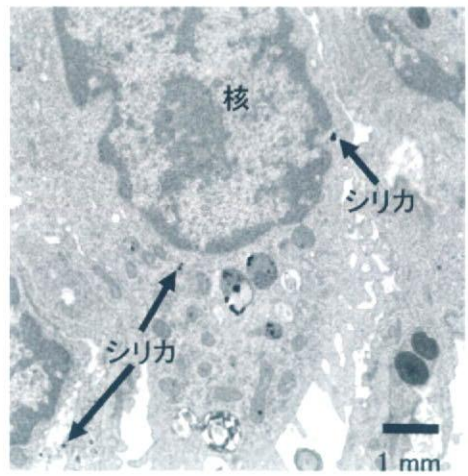


図31-1　マウス肺、70 nm シリカ 100 mg/kg投与、無染色
肺胞上皮II型細胞に侵入している

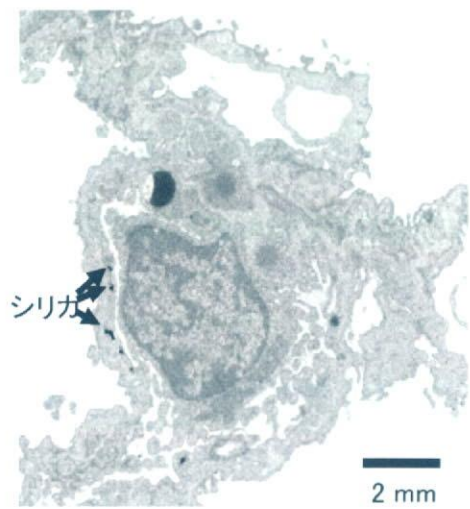


図31-2　マウス肺、70 nm シリカ 100 mg/kg投与、無染色
毛細血管内皮細胞に侵入している

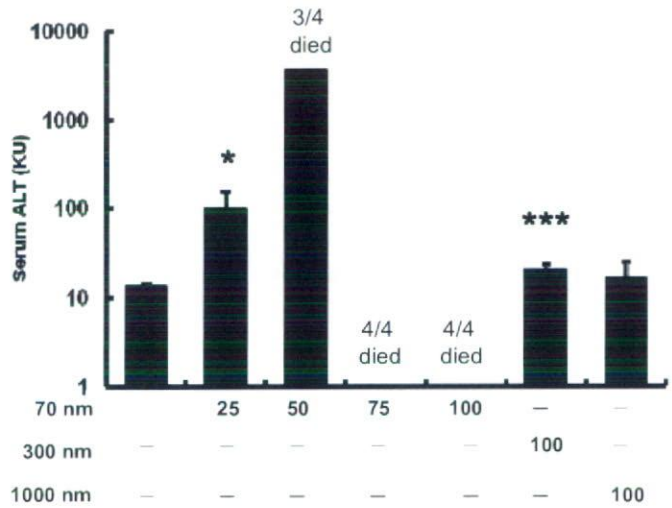


図32　Acute liver injury in mice treated with labeled nano-silica.
Mice were injected labeled nano-silica intraveneously. After 24 hours, Serum ALT level of mice was measured. Each point represents the mean ±S.D.(n=4) * : $p <$ 0.05 vs. Control　　*** : $p <$ 0.005 vs. Control

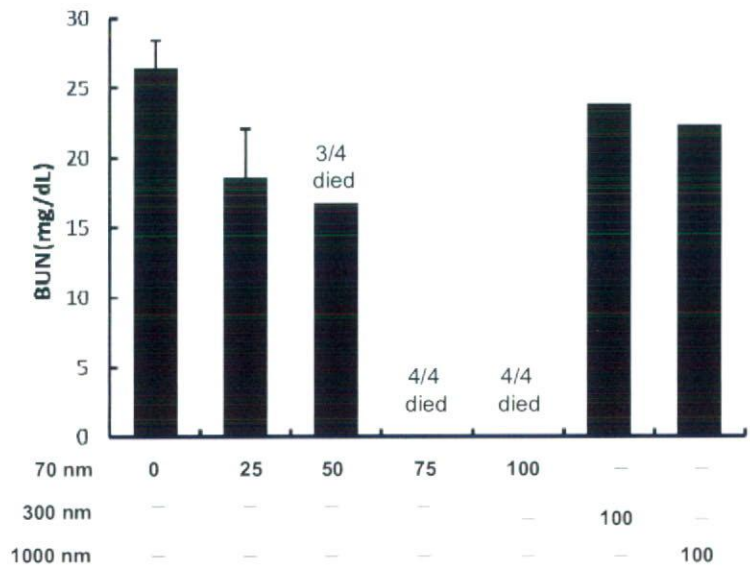


図33　Acute liver injury in mice treated with labeled nano-silica.
Mice were injected labeled nano-silica intraveneously. After 24 hours, Serum BUN level of mice was measured. Each point represents the mean ±S.D.(n=4)

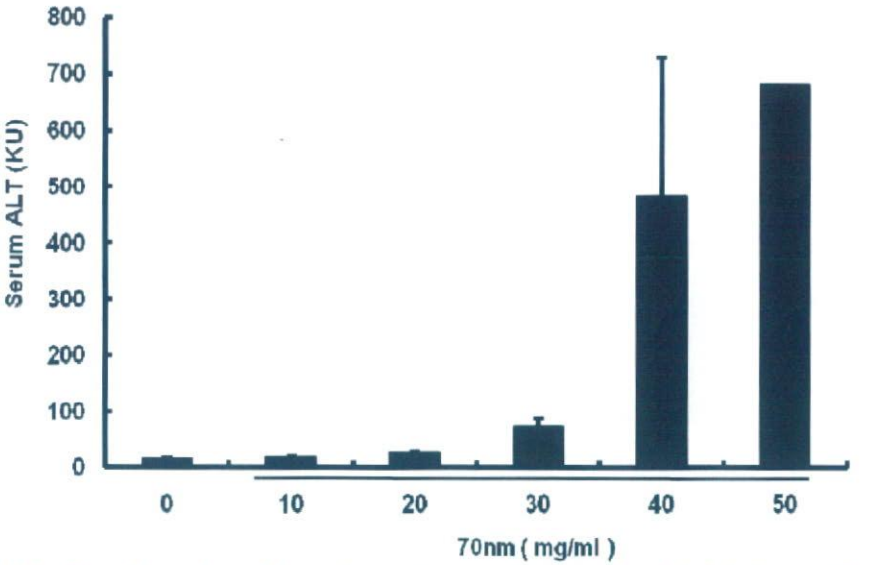


図34 Dose dependency of acute liver injury in mice treated with labeled nano-silica. Mice were injected labeled 70 nm silica intraveneously (0 to 50 mg/kg body weight). After 12 hours, Serum level in mice was measured. Each point represents the mean ±S.D.(n=4)

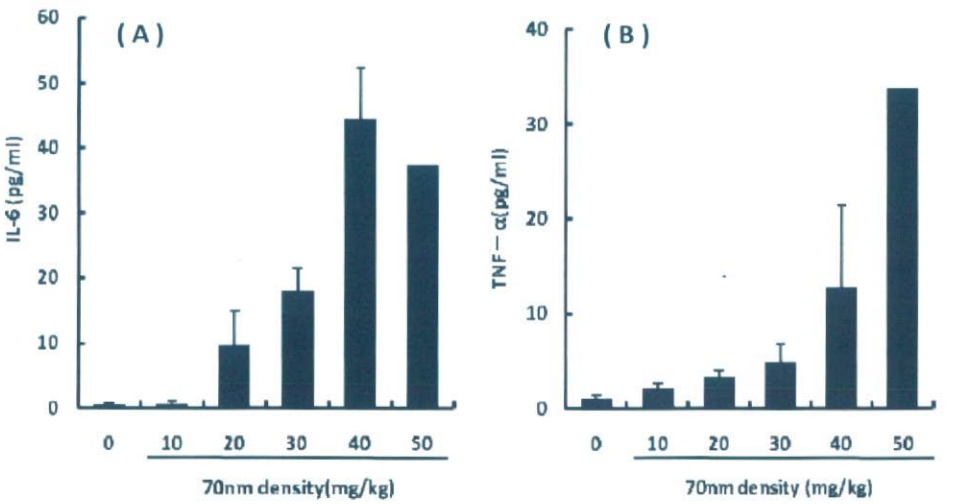


図35 Dose dependency of acute liver injury in mice treated with labeled nano-silica. Mice were injected labeled 70 nm silica intraveneously (0 to 50 mg/kg body weight). After 12 hours, concentration of IL-6 (A), TNF-α (B) in mice was measured. Each point represents the mean ±S.D.(n=4)