

製造・使用から廃棄までの全ライフサイクルにわたる 管理策の策定とその評価法や手法の標準化のための CNTの生物模倣的な分解技術の開発

Biotic and Biomimetic Degradation of CNTs

- Our Scope for the Voluntary Lifecycle Management of CNTs in Production, Use and Waste

目的 (Objectives) :

1. ライフサイクル全般にわたる自主管理策の策定のための必須の知見を得る
Essential Knowledge for Entire Lifecycle Management of CNT
2. REACHやTSCAなどの化学物質管理策や環境規制策を見据えたナノ材料登録に活用する
Crucial for Advocative Registration of the CNTs for the Chemical Substances Management System as REACH and TSCA
3. 手法や計測・評価法を標準化する
Standardization of the methodology and evaluation of CNT degradation

概要 (Summary of Our Task) :

1. SGCNTは肺マクロファージや肝臓クッパー細胞で生分解される
SGCNT is Biodegraded by Immune Cells such as Macrophage in Lung, by Kupffer Cell in Liver.
2. SGCNTは市販の塩素系漂白剤で分解できる
SGCNT is Degraded by Commercial Bleaching
3. SGCNTを生分解できる土壌微生物 (バクテリア) の探索
In Search of the Soil Microbe (Bacteria) which capable of degrade CNTs.

ゼオン独自の自主管理策の策定のための レギュラトリーサイエンス共同研究

Joint Researches in regulatory science area for Establishing ZEON's Voluntary Management System

ZEONのナノテクノロジー Nanotechnol. In ZEON

総合開発センター CNT研究所

CNT Research Laboratory
@ R&D Center

ゼオンナノテクノロジー ZEON Nanotechnol.

カーボンナノチューブ
複合材料研究拠点 (TACC)
Team of Application for Carbon
Nanotubes Composite
(AIST, ZEON Corp, Sunarrow
Ltd.)

- 吸入暴露試験：炎症がエンドポイント
Inhalation Toxicity (OECD/WPMN Sponsorship Program)
J. Nakanishi, PhD. @ Research Institute of Science for Safety and Sustainability, AIST (NOAEL = 0.03 mg/m³)
- GLP適合試験所による長期吸入暴露試験
Long-term Inhalation Toxicity at GLP Testing Laboratory
- 炎症・中皮腫試験 (腹腔内投与)
Carcinogenic Toxicity by Intra-Peritoneal Adm. of CNTs
Prof. S. Toyokuni, MD. @ Medical School, Nagoya Univ.
- 発がん性試験 (気管内投与)
Carcinogenic Toxicity by Intra-Pulmonary Injection of CNTs
Prof. H. Tsuda, MD. (Toxicologist) @ Nagoya City Univ.
- 変異原性試験 (哺乳類細胞)
Mutagenicity test using Mammalian Cells by GLP Testing Laboratory
- 活性汚泥法試験：Biodegradation by Activated Sludge
Chemicals Evaluations and Research Institute, Japan (CERI)
- 生分解および化学分解
Biodegradation and Chemical Degradation of CNTs
M. Zhang, PhD. @ CNT Application Research Center, AIST
- 環境生分解性
Environmental degradation of CNTs
Prof. K. Hori, PhD. @ Nagoya Univ. + Friend Microbes Inc.,

近赤外吸収法によるSGCNTの肺からの排出の観察

Clearance of SGCNT from Lung Monitored by Near-IR Absorption

Clearance of single-wall carbon nanotubes from the mouse lung: a quantitative evaluation By Minfang Zhang, et. al., *Nanoscale Adv.*, 2020, 2, 1551

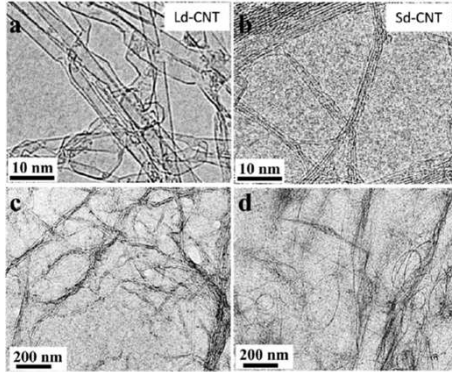
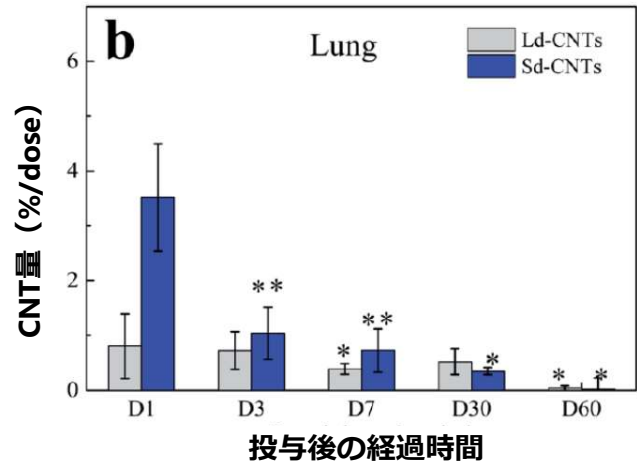


Fig. 1 Characteristics of large-diameter carbon nanotubes (Ld-CNTs) and small-diameter carbon nanotubes (Sd-CNTs). Transmission electron microscopy (TEM) images of Ld-CNTs (a), Sd-CNTs (b), and dispersions of Ld-CNTs (c) and Sd-CNTs (d) in BSA.

Ld-CNTs = 大径のカーボンナノチューブ
Sd-CNTs = 小径のカーボンナノチューブ

肺におけるCNT量の変化

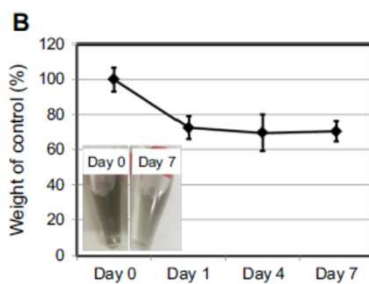
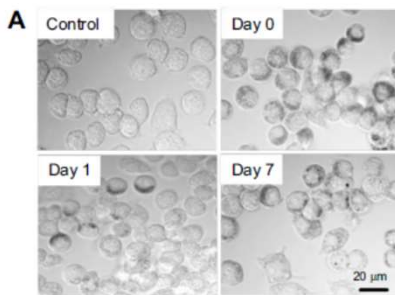


免疫細胞によるSGCNTの生分解実証論文

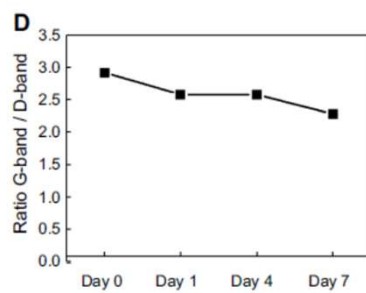
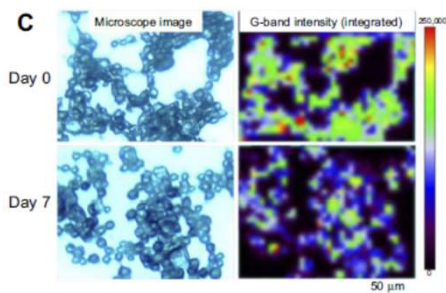
Biodegradation of Super Growth CNT by Immune Cells

Minfang Zhang, et al., *International Journal of Nanomedicine* 2019:14 2797–2807

マイクロファージ細胞 (RAW264.7) によるSGCNTの生分解



- A) RAW264.7細胞 (Control) とそのSGCNTを貪食した後各時点の光学顕微鏡写真; 黒い粒はCNTである。
- B) 近赤外光吸収法により測定した細胞内CNTの量の経時変化とその細胞溶解液の写真
- C) 固定したSGCNTを貪食した細胞の光学顕微鏡写真 (左) とその測定したラマンスペクトルGバンドの強度のマッピング写真 (右)
- D) 細胞溶解液を測定したラマン強度G/D比率の変化



Degradation of SG-CNTs in RAW 264.7 macrophages.

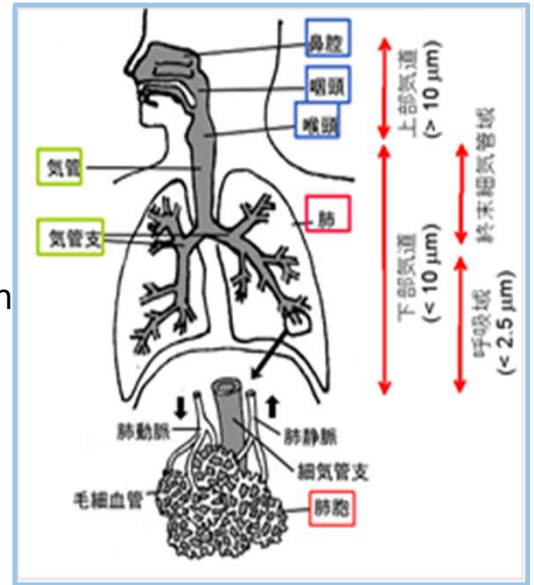
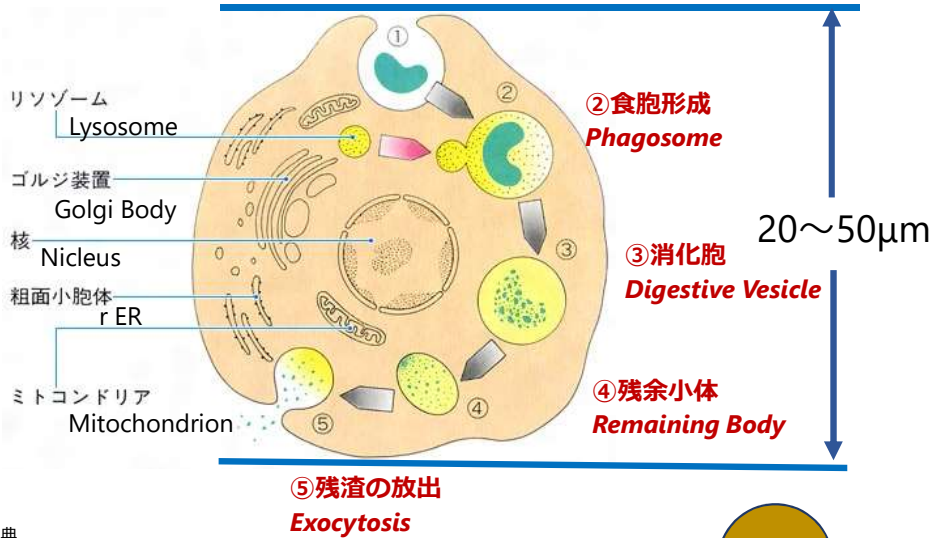
- (A) DIC images of control RAW 264.7 cells incubated with SGCNTs for 24 h (Day 0), and images taken on Day 1 and Day 7, obtained by confocal microscopy. CNTs appear as black spots.
- (B) The intracellular SGCNT levels in RAW 264.7 cells at the indicated time points were estimated from the optical absorbance of the cell lysate at 750 nm. Data represent the percentages of SGCNTs relative to the starting concentration (Day 0) and are expressed as the mean \pm SD of three independent replicates. The insets show the cell lysates at Day 0 and Day 7.
- (C) Microscopy Raman mapping images of G-band intensities of SGCNTs in RAW 264.7 cells fixed with glutaraldehyde at Day 0 and Day 7 (right), along with the corresponding microscopy images (left).
- (D) The ratio of G-band and D-band intensities for cell lysates obtained at each time point.

https://www.aist.go.jp/aist_j/press_release/pr2017/pr20170912/pr20170912.html

貪食細胞による異物の分解プロセス

Phagocytosis and Degradation of Foreign Matter by Phagocytic Cells

①食細胞活動による異物取り込み
Phagocytosis: Take in a Foreign Matter



出典
<https://kotobank.jp/image/dictionary/nipponica/media/81306024004928.jpg>



マクロファージを35µとしたときのPM2.5とPM10
PM2.5 and PM10 comparing with macrophage (35µ)

国環研HPより

HP of the National Institute of Environmental Studies (NIES)

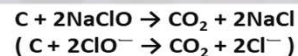
CIO⁻が生物模倣的にCNTを分解

CNT Degradation by CIO⁻ is "Biomimetic"

CNTは濃硫酸や濃硝酸の中でも分解されにくい
CNT could survive even in conc. H₂SO₄ and HNO₃



数滴の塩素系漂白剤で容易に二酸化炭素に酸化される
Possible to degrade with few drops of Chlorine Bleach → CO₂

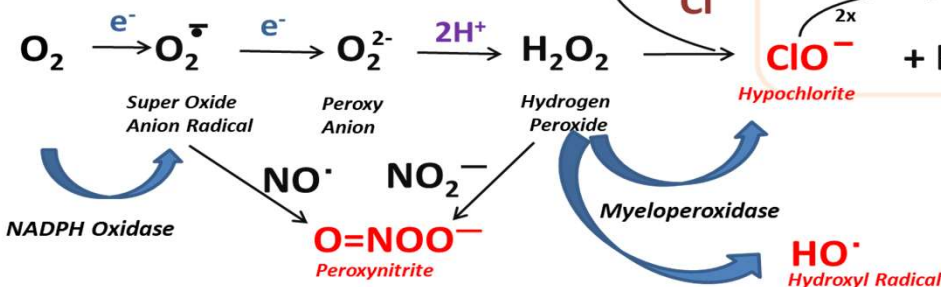


免疫細胞によるCNT分解はNaClの添加によって加速される

CNT degradation by immune cell is effectively accelerated by addition of small amount of NaCl Kotchey et al., Adv. Drug Delivery Rev. 65, 1291(2013)

人体の塩分濃度は0.9%、海水の1/3~1/4

NaCl content of human body is 0.9%, 1/3~1/4 of seawater



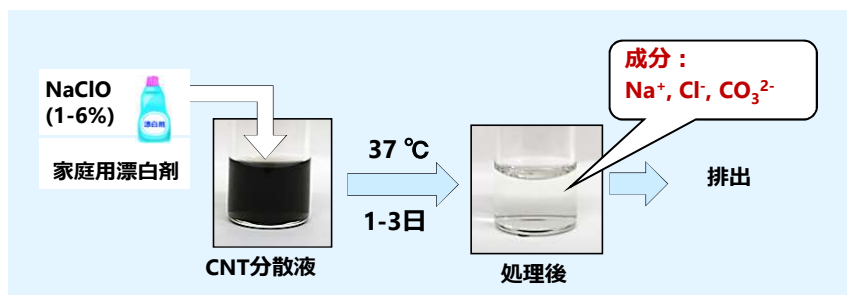
免疫反応系における活性酸素種
Reactive Oxygen Species (ROS)
in immunokinetic process

市販の塩素系漂白剤によるCNTの化学分解

Chemical Degradation of CNTs using Commercially Available Bleaching

2019/02/04 AIST Press Release

https://www.aist.go.jp/aist_j/press_release/pr2019/pr20190204/pr20190204.html



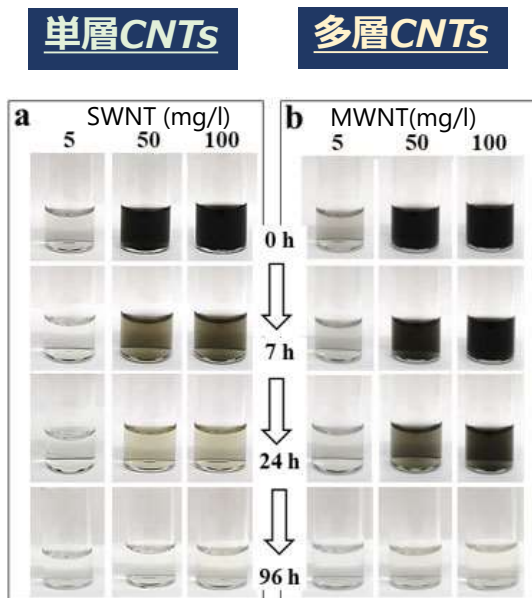
管理策への応用

Possible application in occupational field



作業服：
焼却処理⇒ブリーチング

Working Clothes
Incineration ⇒ Bleaching



M. Zhang, et al., *ACS Appl. Nano Mater.* 2019, 2,7, 4293-4301

SGCNTに関する リスクマネジメントのための情報

Quick Summary for the Risk Management of SGCNT

- ◆ SGCNTは発がん物質に分類されていません (IARC)
SGCNT is not carcinogenic.
- ◆ SGCNTは哺乳類細胞に変異原性を示しません (GLP適合試験所に委託)
SGCNT is not mutagenic to a mammalian cell.
- ◆ SGCNTに生殖毒性は認められていません (OECD)
SGCNT is not reproductive toxicity.
- ◆ SGCNTは難分解性ではありません
SGCNT is not persistent.
 - ▶ SGCNTに活性汚泥法での生分解性はありません、しかし、
SGCNT is not biodegradable when using the active sludge method of OECD test guideline, however,
SGCNTは植物や動物の酸化酵素、免疫細胞を用いた生物的な方法で分解できます
SGCNT is degraded by the biotic method of using immune cells and enzyme.
 - ▶ SGCNTを分解する土壌微生物 (バクテリア) が存在します
SGCNT could be degraded by soil microbe (bacteria) .
 - ▶ SGCNTは生体模倣的な方法である市販の塩素系漂白剤による処理で分解できます
SGCNT is degraded by the biomimetic method using commercially available bleach.
- ◆ SGCNTは生体蓄積性はありません (OECD)
SGCNT is not bioaccumulative.