***Title of Research:***

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# Construction of the novel *in vitro* evaluation systems based on the genotoxic mechanisms of nanomaterials

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***Collaborators:***

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***Summary of Research:*** To establish new in vitro evaluation systems for lung/skin toxicity of nanomaterials, we tried to establish assay models as listed below.

① A novel in vitro genotoxicity assay model to asses lung toxicity using a co-culture system.
② A novel in vitro assay model to assess skin toxicity using 3D human skin reconstitution models.
③ A novel in vitro assay model using 3D culture techniques such as tissue-slice and spheroid.

For the lung toxicity test, we used a co-culture system of fibroblast cells established from mice lungs (GDL1 cells) and murine macrophage cells (RAW264.7). Mutation frequencies induced in GDL1 by both MGT and MWCNT were significantly greater in the coexistence of RAW264.7 than in its absence. Mutation spectra observed in GDL1 co-cultureed with RAW264.7 were distinguished from those seen in single-cultured GDL1, and similar to those observed in mice lungs exposed to these nanomaterials in vivo. Moreover, the levels of oxidative- and inflammatory-related DNA adducts, 8-oxo-dG and $\varepsilon$ dC, in GDL1 exposed to MGT or MWCNT exposure were greater in the co-culture condition than in the single-culture condition. Therefore, it is suggested that the co-culture assay model can be considered as a suitable evaluation system for lung toxicity of nanomaterials.

As a 3D skin model, we selected the LabCyte EPI model. The cytotoxicity of gold nanoparticles was assessed by measuring the activity of lactate dehydrogenase leaking into the culture medium. Significant cell death was induced in a clear dose-dependent manner. On the other hand, the same gold nanoparticles showed more potent cytotoxicity against monolayered cultured HepG2 cells than the 3D skin model. It is thus suggested that the LabCyte EPI model may be useful as a novel in vitro system to assess skin toxicity of nanomaterials, and that in this model reconstituted skin may possess a barrier function similarly to human and animal in vivo skin.

***Timeline:*** March 1st, 2015 – February 29th, 2016

***Topics:*** “Construction of the novel in vitro evaluation systems based on the genotoxic mechanisms of nanomaterials “ Presented at the poster session of the Annual Conference of New JCIA-LRI

***Publications:***

Journals:

1. Ishino Kousuke, Kato Tatsuya, Kato Mamoru, Shibata Tatsuhiro, Watanabe Masatoshi, Wakabayashi Keiji, Nakagama Hitoshi, Totsuka Yukari. “Comprehensive DNA adduct analysis reveals pulmonary inflammatory response contributes to genotoxic action of magnetite nanoparticles.” International Journal of Molecular Sciencs. 2015;16:3474-92.
2. Koichiro Hayashi, Wataru Sakamoto, Toshinobu Yogo “Smart Ferrofluid with Quick Gel Transformation in Tumors for MRI-Guided Local Magnetic Thermochemotherapy” Advanced Functional Materials 2016; 26: 1708–1718.

Meetings:

1. Saho Hashimoto, Sou Yamaguchi, Kanako Kojima, Nao Furuta, Tadashi Nittami, Kazuaki Kawai, Hiroshi Kasai, and Masatoshi Watanabe. “Cellular effects of magnetic nanoparticles as determined by cell type and surface coating.” The 74th Annual Meeting of the Japanese Cancer Association, Nagoya, Oct.8-10, 2015.
2. Nao Furuta, Saho Hashimoto, Jieun Seo, Kanako Kojima, Sou Yamaguchi, Tadashi Nittami, and Masatoshi Watanabe. “Effect of magnetic nanoparticles on cancer stem-like cells from human lung and prostate cancer cell lines.” The 74th Annual Meeting of the Japanese Cancer Association, Nagoya, Oct.8-10, 2015.
3. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo “Multifunctional Nanoparticles for MRI-Guided Magnetic Thermochemotherapy” BIT’s 2nd Annual World Congress of Smart Materials-2016, Singapore, Mar. 2016 (invited).
4. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo “Core-Shell Nanoparticles for the Combination of Magnetic Hyperthermia and Chemotherapy” ISETS '15 International Symposium on EcoTopia Science 2015 - Innovation for Smart Sustainable Society -, Nagoya, Nov. 2015.
5. Koichiro Hayashi “Clustered Magnetic Nanoparticles-Polymer Core-Shell Nanoparticles for Magnetic Thermochemotherapy” The 2015 Energy, Materials, and Nanotechnology (EMN) Meeting, Phuket, Thailand, May, 2015 (invited).
6. Koichiro Hayashi, Yusuke Sato, Takuma Maruhashi, Wataru Sakamoto, Toshinobu Yogo “Synthesis of multifunctional hybrid nanoparticles for imaging and therapy” Tokyo, The Ceramic Society of Japan Annual Meeting 2016, Mar. 2016 (invited).
7. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo “Alternating Magnetic Field-Responsive Smart Core-Shell Nanoparticles for Magnetic Thermochemotherapy” Toyama, The Ceramic Society of Japan The 28th Fall Meeting, Sep. 2015.