News Release

"*Light* hope to cancer treatment" : New cell death "Photochemosis" concept

Detailed elucidation of the cell death mechanism of near-infrared phototherapy

Key Points

- **The mechanism of cell death in NIR-PIT, "photochemosis", was elucidated in detail using a new imaging technique called scanning electron-assisted dielectric microscope (SE-ADM) developed by Dr. Ogura, a chief researcher at AIST, Japan.**
- **Actin filaments that underlie the cell membrane play an important role, and that the photochemical reaction of the IR700 causes the aggregation of actin filaments by NIR irradiation, resulting in the rupture of the cell membrane and cell death.**
- **The cell death mechanism of "Photochemosis" defines NIR-PIT as a new, independent cancer treatment technology, and the "fifth cancer treatment", because the cell death mechanism is completely different from the existing cancer treatment technologies of surgery, chemotherapy, radiation therapy, and cancer immunotherapy.**
- **The details of "photochemosis" have been clarified, providing academic support for multidisciplinary treatment theories, such as combination therapy with other cancer treatment techniques in the future.**

Summary

Dr. **Kazuhide Sato**, MD, PhD, Specially Appointed Lecturer, Graduate School of Medicine, Nagoya University, Institute for Advanced Research, Nagoya University, JST-FOREST 1st term, and Dr. **Toshihiko Ogura**, PhD, Senior Chief Researcher, and Dr. **Tomoko Okada**, PhD, National Institute of Advanced Industrial Science and Technology (AIST), and their research group have achieved a mechanism of cell death in near-infrared photoimmunotherapy (NIR-PIT) using a newly developed scanning electron-assisted dielectric microscope (SE-ADM), and have named this cell death **"photochemosis"** and clarified the detailed mechanism as a new type of cell death. This research result was achieved with the support of JST CREST [Extracellular Particles]　JPMJCR19H2.

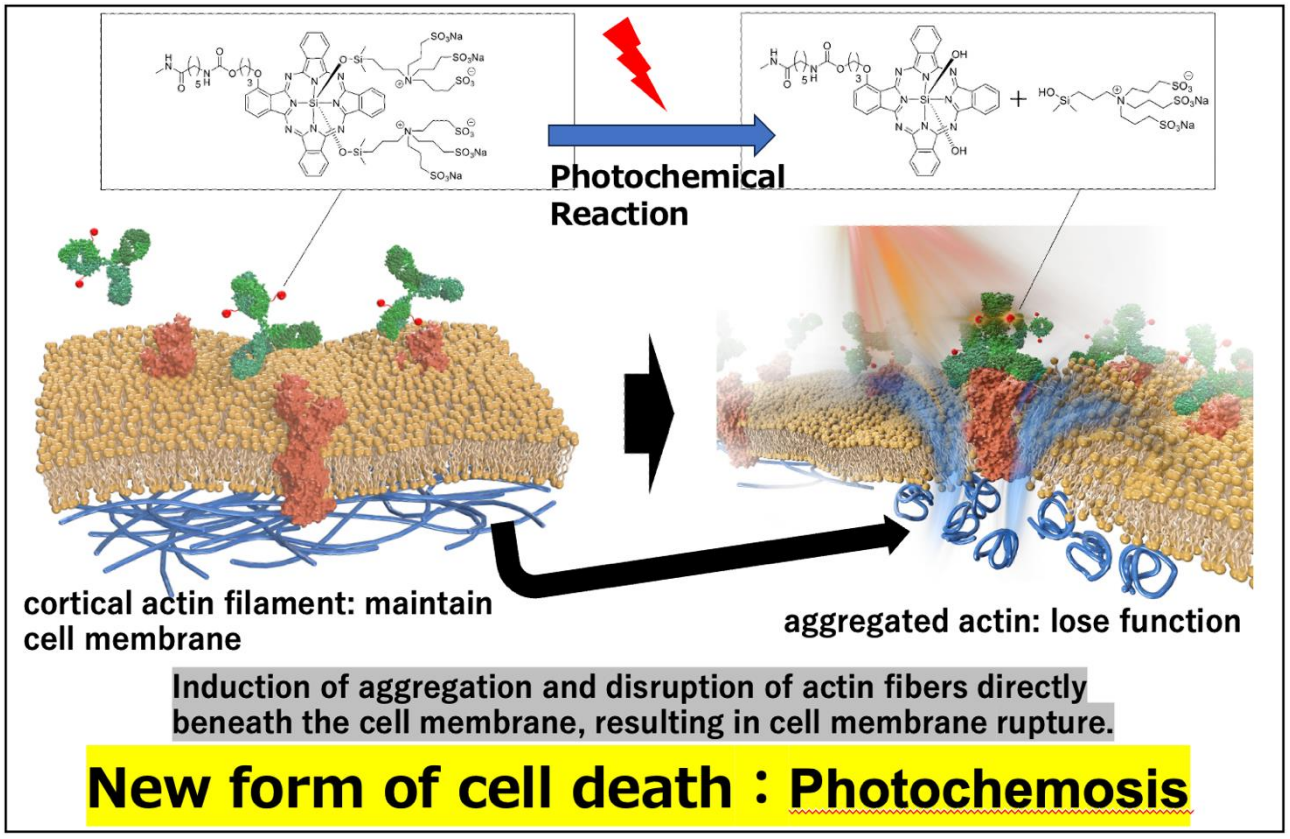
NIR-PIT was approved in Japan in 2020 as the world's first treatment for recurrent head and neck cancer, and is being developed globally in Asia, India, the Middle East, Africa, etc. It is expected to be further developed as a highly selective treatment using NIR-light, but the details of its cell death mechanism have not yet been clarified, and the differences between it and the four conventional

cancer treatment technologies of surgery, chemotherapy, radiotherapy, and cancer immunotherapy, as well as the conventional photodynamic therapy (PDT) using light, were unclear. The first author and corresponding author, Sato, had already demonstrated in ACS Central Science in 2018 that the origin of cell death in NIR-PIT is the hydrophilic side chain axial ligand ($C_{14}H_{34}NO_{10}S_3Si$; silanol) of the light absorber IR700 dissociating from the conjugate through photochemical ligand reactions dissociate from the conjugate, the remaining structure, including the antibody, rapidly becomes hydrophobic and aggregates (Sato K, et al, ACS Central Science, 2018, Nov 28;4(11):1559-1569.), but the details of this photochemical reaction and its effect on the cell membrane, which is the site of action, and cell death were still unknown.

In this study, the cells treated with NIR-PIT were observed in detail using a scanning electron-assisted dielectric microscope (SE-ADM), a new imaging technology developed by Dr. Ogura at AIST, and combined with biochemical and cell biological analysis, it was found that the photochemical reaction of the photosensitizer IR700 causes the aggregation of actin filaments just below the cell membrane, destroying its membrane support function, and water flows into the cell according to the osmotic pressure difference between inside and outside the cell, causing the cell to swell and die. We have named this new type of cell death, which differs from the cell death that has been reported so far, **“photochemosis”**. This mechanism differs from the cell death mechanism reported in PDT, which is known as conventional phototherapy, and it is expected to provide scientific support for the further spread and implementation of NIR-PIT, proving the uniqueness of NIR-PIT as a phototherapeutic treatment.

By establishing and proving the new cell death mechanism **“Photochemosis”**, NIR-PIT will contribute to cancer patients as a new, independent cancer treatment technology, **“the fifth cancer treatment”**, and will provide theoretical support for multidisciplinary treatment, which is a combination of various cancer treatment technologies in the future, because the cell death mechanism of NIR-PIT is completely different from the existing cancer treatment technologies of surgery, chemotherapy, radiation therapy, and cancer immunotherapy.

The results of this research were published in the American Chemical Society journal **ACS nano** on February 18, 2025 (February 19, 2025 JST).



Overview of this research

Research Background

Recently, a new nano-imaging technique called scanning electron-assisted dielectric microscope (SE-ADM) has been developed **(Figure 1)**. This technique allows us to observe various biological samples in aqueous media with a spatial resolution of 8 nm without radiation damage (Ogura T, et al, Microscopy and Microanalysis, 2023、29, 1037-1046). This analysis makes it possible to image living cells at the nanoscale, and it can detect new changes that have not been reported before (Ogura T and Okada T, Sci. Rep., 2016、6, 29169).

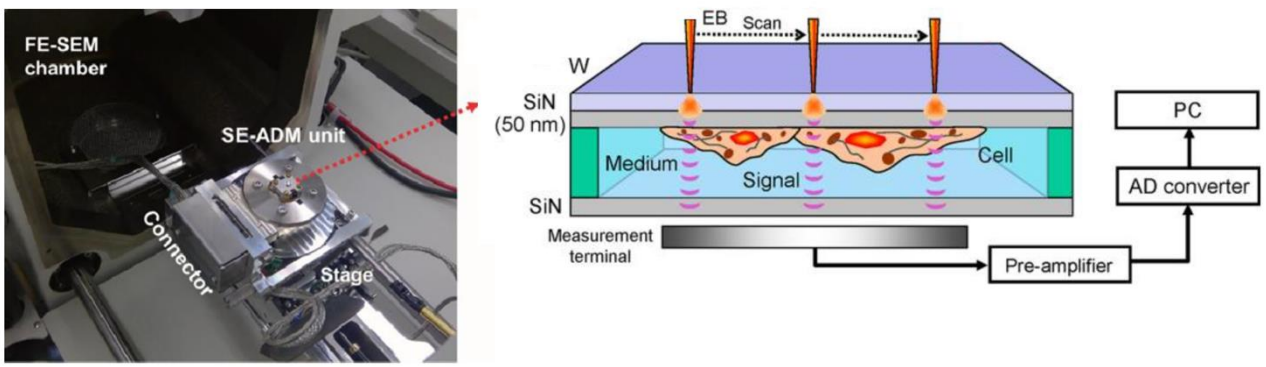


Figure1. Scheme of SE-ADM

In recent years, the development of light targeting therapy has been attracting attention as a new treatment technology. Among these, NIR-PIT, a new cancer treatment method reported by Dr. Hisataka Kobayashi, MD, PhD and others at the National Cancer Institute (NCI/NIH) in 2011, is attracting attention as a new cancer treatment technology **(Figure 2)**. This treatment method is expected to be the “fifth cancer treatment” following surgery, radiation, chemotherapy, and cancer immunotherapy, as it can target and destroy cancer cells in a different way, and it was the first in the world to be approved for insurance coverage in Japan in September 2020 for recurrent and previously treated head and neck cancer with high EGFR※2 expression. However, at the time of the start of Phase III trials, the mechanism of cell death caused by NIR-PIT had not been elucidated, and this was a major hurdle to the clinical approval of NIR-PIT.

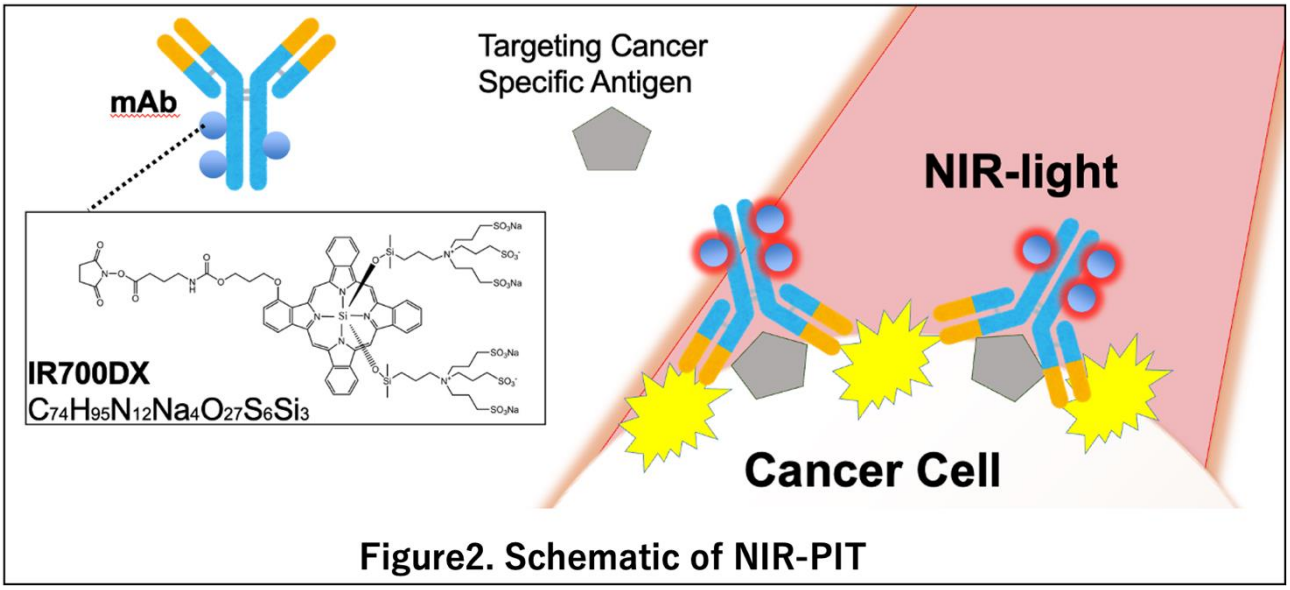


Figure2. Schematic of NIR-PIT

The first author and corresponding author, Dr. Kazuhide Sato, identified the substance that triggers cell death with this treatment as the starting point of cell death by NIR-PIT in the 2018 ACS Central Science journal. Specifically, when the complex is irradiated with near-infrared light, the hydrophilic side chain axial ligand ($C_{14}H_{34}NO_{10}S_3Si$; silanol) of IR700 (silica phthalocyanine (SiPc)) dissociates from the complex through photochemical ligand reactions dissociated from the complex, and the remaining structure, including the antibody, rapidly became hydrophobic and aggregated **(Figure 3)**(Sato K, et al, ACS Central Science, 2018, Nov 28;4(11):1559-1569.) This photochemical reaction causes antibodies to the surface antigens on the tumor cell membrane to aggregate, inducing cell destruction, and it was clear that the photochemical reaction was the starting point for the initiation of cell death by NIR-PIT. However, the effect of this reaction on the target cell and how it leads to cell rupture has not yet been elucidated, and the details of cell death by NIR-PIT are still unknown.

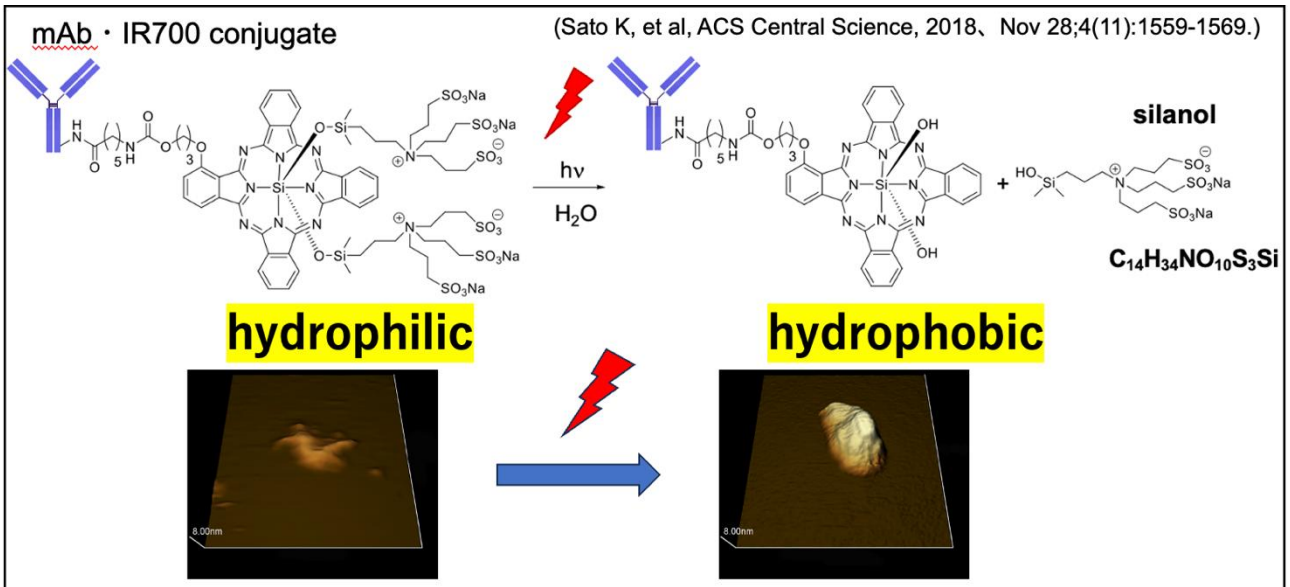


Figure3. Photochemical reaction of NIR-PIT

Research Results

When the extracellular fluid was made hypertonic using a 20% sucrose solution, the cell death rate of NIR-PIT decreased. Also, even when cell function was stopped at 4°C, there was no change in the effect of NIR-PIT cell death compared to room temperature. From this, it was found that cell death is related to the rupture of the cell membrane and the influx of water from outside the cell. When an actin polymerization inhibitor was used, NIR-PIT cell death was suppressed. Analysis using SE-ADM showed that black aggregated particles were formed just below the cell membrane, and these particles decreased when an actin polymerization inhibitor was used. Biochemical analysis of actin proteins showed that NIR-PIT causes actin proteins to aggregate. In addition, real-time observation showed that actin on the cell membrane of cells stably expressing actin GFP loses fluorescence (structural change due to aggregation) immediately after NIR-PIT near-infrared irradiation. This reaction also occurred in tumors *in vivo*. With the protein chemistry analysis in the tube, the antibody-IR700 complex (trastzumab-IR700), the target antigen (HER2), and actin all aggregated when irradiated with near-infrared light.

From the above experimental results, it can be seen that the two silanol groups of IR700 undergo photodissociation when exposed to NIR-light, and the remaining silicon phthalocyanine structure aggregates. This rapid change from hydrophilic to hydrophobic not only causes receptor binding, but also causes aggregation of the cortical actin meshwork structure that supports the cell membrane and receptor proteins. The destruction of the cytoskeletal actin directly beneath the cell membrane causes the cell membrane to lose its ability to maintain its structure, and water flows into the cell due to osmotic pressure, causing the cell to be ruptured. This series of events is the mechanism of cell death in NIR-PIT, and is completely different from the conventional mechanisms of cell death (apoptosis and

necrosis).

Overall, the mechanism of cell death in NIR-PIT can be explained by the **“photochemical necrosis”** mechanism, in which the rupture of actin filaments (actin network directly below the cell membrane) below the cell membrane prevents the maintenance of cell membrane structure, and water flows in due to the osmotic pressure difference between inside and outside the cell, leading to cell death (Figure 4).

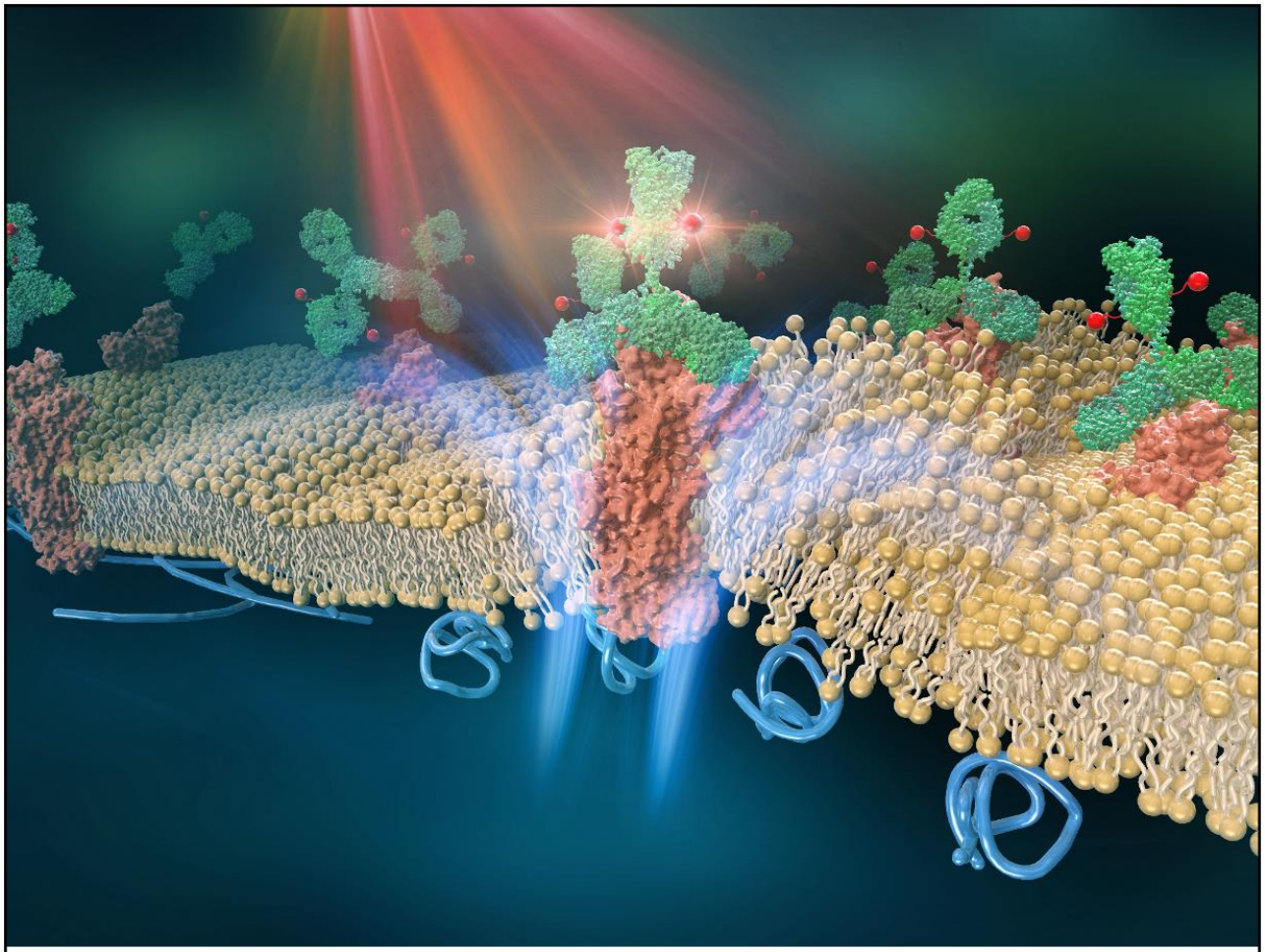


Figure4. Schematic of Photochemosis

Research Summary and Future Perspective

Due to this unique cell death **“photochemosis”**, NIR-PIT is attracting attention as a “fifth cancer treatment technology” that is completely different from existing anticancer drugs, radiation therapy, and surgery, and it is expected to be incorporated into standard treatment along with its therapeutic effects. With the scientific support of elucidating this mechanism, NIR-PIT is expected to be used in combination with various other cancer treatment methods, and it is expected to be applied to multidisciplinary cancer treatment. In particular, the combination of NIR-PIT and cancer immunotherapy (such as immune checkpoint inhibitors) is expected to have a significant effect, and in the future, it may become a treatment option for many patients.

We will continue to conduct further research to elucidate the details of the changes in NIR-PIT treated cancer cells and to apply it to cancers for which there are few treatment options.

Publication

Photoinduced Actin Aggregation Involves Cell Death: A Mechanism of Cancer Cell Cytotoxicity after Near-Infrared Photoimmunotherapy

ACS nano,
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Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Acs_250219.pdf