

Unraveling the Biological Effects of Plasma-Activated Solutions: From Basic Science to Applications

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In recent years, research on the medical and agricultural applications of low-temperature plasma has been actively conducted. We discovered the anti-tumor effects of plasma-activated medium (PAM) [1] and further invented plasma-activated Ringer's lactate solution (PAL) [2]. Building on these findings, we have advanced the bioapplication research of various plasma-activated solutions.

We revealed that while PAM induces oxidative stress-dependent cell death in U251SP cells, PAL induces oxidative stress-independent cell death [3]. Furthermore, in addition to reactive oxygen and nitrogen species (RONS) such as hydrogen peroxide and nitrite ions, we identified various reaction products generated from interactions between plasma and lactate in PAL. Among these components, some, like 2,3-dimethyl tartrate, exhibit selective cytotoxicity toward cancer cells without affecting normal cells when used alone, while others, such as EA, GA, and TA, demonstrate selective cytotoxic effects when used in combination [4].

Furthermore, we found that PAL induces autophagy followed by ferroptosis in mesothelioma [5]. In addition, we identified specific components within PAL that are capable of inducing autophagy [6]. We have also developed a plasma-activated solution using L-arginine (L-Arg) [7].

In fiscal year 2023, a first-in-human investigator-initiated clinical study of PAL was launched, and a safety assessment on human skin was conducted.

We are also advancing research on the agricultural applications of plasma-activated solutions. Through years of study, we have discovered that PAL reduces the proportion of immature grains and increases the proportion of white-core grains, which are important for sake production. In addition, we have developed a smart agriculture system equipped with high-power LED panels capable of cultivating rice plants through to harvest [8].

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References

- [1] H. Tanaka, M. Mizuno, K. Ishikawa, K. Nakamura, H. Kajiyama, H. Kano, F. Kikkawa, M. Hori, Plasma-Activated Medium Selectively Kills Glioblastoma Brain Tumor Cells by Down-Regulating a Survival Signaling Molecule, AKT Kinase, *Plasma Medicine*, 1 (2013) 265-277.
- [2] H. Tanaka, K. Nakamura, M. Mizuno, K. Ishikawa, K. Takeda, H. Kajiyama, F. Utsumi, F. Kikkawa, M. Hori, Non-thermal atmospheric pressure plasma activates lactate in Ringer's solution for anti-tumor effects, *Sci Rep*, 6 (2016) 36282.
- [3] H. Tanaka, M. Mizuno, Y. Katsumata, K. Ishikawa, H. Kondo, H. Hashizume, Y. Okazaki, S. Toyokuni, K. Nakamura, N. Yoshikawa, H. Kajiyama, F. Kikkawa, M. Hori, Oxidative stress-dependent and -independent death of glioblastoma cells induced by non-thermal plasma-exposed solutions, *Sci Rep*, 9 (2019) 13657.
- [4] H. Tanaka, Y. Hosoi, K. Ishikawa, J. Yoshitake, T. Shibata, K. Uchida, H. Hashizume, M. Mizuno, Y. Okazaki, S. Toyokuni, K. Nakamura, H. Kajiyama, F. Kikkawa, M. Hori, Low temperature plasma irradiation products of sodium lactate solution that induce cell death on U251SP glioblastoma cells were identified, *Sci Rep*, 11 (2021) 18488.
- [5] L. Jiang, H. Zheng, Q. Lyu, S. Hayashi, K. Sato, Y. Sekido, K. Nakamura, H. Tanaka, K. Ishikawa, H. Kajiyama, M. Mizuno, M. Hori, S. Toyokuni, Lysosomal nitric oxide determines transition from autophagy to ferroptosis after exposure to plasma-activated Ringer's lactate, *Redox Biology*, 43 (2021) 101989.
- [6] T. Yamakawa, A. Tanaka, C. Miron, K. Nakamura, H. Kajiyama, S. Toyokuni, M. Mizuno, M. Hori, H. Tanaka, Effects of autophagy on the selective death of human breast cancer cells exposed to plasma-activated Ringer's lactate solution, *Free Radical Research*, 58 (2024) 758-769.
- [7] L. Du, C. Miron, T. Kondo, J. Kumagai, M. Mizuno, S. Toyokuni, H. Kajiyama, M. Hori, H. Tanaka, Selective Killing of Human Breast Cancer Cells by Plasma - Treated L - Arginine Solution, *Plasma Processes and Polymers*, 22 (2024).
- [8] H. Hashizume, H. Kitano, H. Mizuno, A. Abe, S.-N. Hsiao, G. Yuasa, S. Tohno, H. Tanaka, S. Matsumoto, H. Sakakibara, E. Kita, Y. Hirose, M. Maeshima, M. Mizuno, M. Hori, Direct plasma treatment of caryopses after flowering in brewer's rice cultivar Yamadanishiki enhanced those grain qualities through "Smart Agriculture System", *Scientific Reports*, 14 (2024).