Medium Temperature Independent Cytotoxicity of Cell-adhesive Heat-generating Nanoparticles Named Magnetite Cationic Liposomes and its Therapeutic Use

TOMIO MORINO1*, HIROSHI TAKASE2, TOSHIKI ETANI1, TAKU NAIKI1, NORIYASU KAWAI1, AKIRA ITO3, TAKAHIRO YASUI1

1Department of Nephro-urology, 2Core Laboratory, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan
3Department of Chemical Systems Engineering, School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

Abstract: Cancer clinical research using heat-generating nanoparticles named magnetite cationic liposomes (MCL) has been conducted. Heat generation from intratumorally injected MCL particles was induced by alternating magnetic field (AMF) irradiation to kill cancer cells nearby located. Its feasibility and safety have been shown but efficacy was variable among tumors whose temperature rises were similarly achieved. To ensure efficacy heat dose divided by tumor volume ($J/cm^3$) has been proposed as candidate index to control clinical treatment. Purpose of this study is to investigate cytotoxicity of MCL particles upon AMF irradiation and discuss validity of the proposed index.

MCL particles were shown to adsorb human prostate cancer cells in vitro at 2 ng-MCL/cell, depending on positive zeta potential derived from a cationic lipid component. Optical and electron microscopic observations showed majority of MCL particles located on cell membrane and scarcely in cytosol. Heat generation activities of MCL particles were represented by specific absorption rate (SAR) with unit of $J/g$-MCL$\cdot$min and were shown variable due to irradiation conditions. Cytotoxicity of MCL particles upon AMF irradiation was found under a condition supplying heat dose of $1.2 \times 10^{-4} J$/cell with concomitant cellular morphological changes and membrane burst. Notably, temperature rise of culture medium was not observed under this condition. Cytotoxicity of MCL particles was considered to be caused by heat generated locally on cell membrane ($J$/cell) independently of medium temperature. These results would support the proposed heat dose index ($J/cm^3$ tumor volume) to control clinical treatment instead of tumor temperature.

Key Words: alternating magnetic field, cell membrane hyperthermia, clinical research, magnetic field heating, magnetites

1. Introduction

In 1957 magnetic particles able to generate heat by external magnetic field irradiation has been proposed.
to use for cancer therapy\(^1\). This idea has been studied in dogs for simultaneous treatment of metastasized lymph nodes where magnetic particles accumulated\(^2\). In 1979 magnetic particles in nano size were shown to incorporate into cancer cells and novel concept of intracellular hyperthermia has been shown\(^3\). In 1996 Shinkai *et al.* prepared nanoparticles composed of magnetites (Fe\(_3\)O\(_4\)), a cationic lipid and two neutral lipids, and named MCL particles\(^4\). Cellular uptake of MCL particles was shown with rat glioma cells by measuring Fe contents of trypsin treated cells and therapeutic concept was described as intracellular hyperthermia\(^4\). Protocol for solid cancer treatment was optimized and thrice AMF irradiations which induced MCL particles dispersion in tumor tissue were shown crucial for *in vivo* efficacy\(^5,6\). Clinical research was conducted against breast and other cancers with a criterion to keep tumor temperature at 43°C for 30 min\(^7\), as other heat-related therapy so far\(^1-6\). However, efficacy varied widely from complete regression to ineffective among tumors whose temperature rises were similarly achieved\(^8\).

In 2011 Creixell *et al.* has shown unique cytotoxicity of heat-generating nanoparticles loaded with anti-EGFR antibody\(^8\). They revealed that cytotoxicity did not coincide with perceptible temperature rise of culture medium and cellular death rates were correlative with heat dose divided by cell number (J/cell)\(^8\). In accordance with this report, data of clinical research using MCL particles\(^7\) was analyzed in details and efficacy was found to correlate with MCL dosage divided by tumor volume (mg-MCL/cm\(^3\))\(^9\). Then, heat dose divided by tumor volume (J/cm\(^3\)) was proposed as index to control treatment instead of tumor temperature\(^9\). In this study we studied cytotoxicity of MCL particles and discussed validity of the proposal. We also showed novel data on structure and functions of MCL particles and discussed its regulatory issue briefly.

2. Materials and Methods

2.1. Magnetites and lipids

Magnetites in about 10 nm (Granule type, Dai-ichi High Frequency Co., Ltd, Tokyo, Japan) were used because of its maxim heat generation activity under 100 kHz AMF irradiation\(^10\). Two neutral lipids of 1,2-dilauroyl-3-phosphatidylcholin and 1,2-dioleoyl-3-phosphatidylethanolamine were purchased from NOF Corporation, Tokyo, Japan. Cationic lipids of N-(α-trimethylammonioacetyl)-didodecyl-D-glutamate chloride (TMAG) and 1,2-dioleoyl-3-trimethylammonium-propane chloride (DOTAP) were purchased from Sogo Pharmaceutical Co., Ltd, Tokyo, Japan and Avanti Polar Lipids Inc., US, respectively.

2.2. Preparation of MCL particles

MCL particles were prepared by reported method\(^4\). Two neutral lipids and TMAG were solved in organic solvent and dried up to form film in a flask. Magnetites granules were suspended in sterile distilled water by sonication and applied to flask with lipids film. MCL particles were prepared by repeated sonication in the flask. MCL particles with DOTAP instead of TMAG were prepared for 16 hr cell exposure experiment. The particles without cationic lipid were prepared in the same weight ratio of magnetites and lipids\(^6\). The particles were prepared under aseptic condition and its sterility was confirmed before use.

2.3. AMF irradiator used

Irradiator generating 100 kHz AMF with three-phase power supply of 200 V (Hi-Heater type 5010, Dai-ichi High Frequency Co., Ltd, Tokyo, Japan) was used. Coil for generating AMF was designed to enrich
magnetic flux density outside by inserting ferrite core\(^\text{11}\)). The coil was built-in as irradiating upper direction and covered with surface board. Magnetic flux densities on the board are totally increased by high output power but decline far from the board (Document of irradiator specification).

2.4. Heat generation test of MCL particles

A flat bottom tube with 0.6 ml suspension of MCL particles was imbedded with Styrofoam and placed at a test distance from surface board of the irradiator. Tip of temperature sensor (Fiber optic thermometer FL-2000, Anritsu Meter Co., Ltd, Tokyo, Japan) was immersed in the suspension for temperature monitoring during AMF irradiation. Test conditions were adjusted to result in about 10 °C increase during 1 to 3 min irradiation. Heat generation activity was represented with SAR (\(J/g\)-MCL-min) value and calculated by reported equation\(^\text{10}\).

2.5. Cell adhesion and cytotoxicity tests of MCL particles

Human prostate cancer cells of LNCaP (ATCC CRL-1740) were cultured with 10% FCS RPMI-1640 medium (Gibco, ref. no. 22,400). Cells cultured for 40 hr were applied to MCL particles for 6 hr or 16 hr at more than 2 ng-MCL/cell. Adhesin of MCL particles was tested by optical microscope. Cytotoxicity of MCL particles was tested after 30 min irradiation of adsorbed cells under 3.5 mm distance from surface board and output power in 55 A. The cells were treated with trypsine for trypan blue exclusion assay or fixed with glutaraldehyde for electron microscopic study.

3. Results

3.1. Characterization of MCL particles

MCL particles were observed by electron microscopy by the reported method for liposome imaging\(^\text{12}\). Averagely 25 particles of 10 nm magnetites were observed to incorporate into about 100 nm MCL particles (Fig. 1). Dynamic laser scattering analysis (Otsuka Electronics Co., Ltd, Osaka, Japan) showed consistent average diameter of 107.1 nm with polydispersity index of 0.17. Simultaneously, zeta potential of MCL particles was shown positive in +48.9 mV due to a cationic lipid involved.

Heat generation activities of MCL particles were variable depending on irradiation conditions. Greater SAR values were obtained in closer distance from surface board and higher output power of irradiator, corresponding to magnetic flux density of test position. A SAR value under a representative condition of 3.5 mm
distance and output power in 55 A was calculated 2,020 J/g-MCL·min. MCL particles were visually more colloidal than original magnetites and showed about 1.5-fold higher SAR value as reported with other colloidal materials\textsuperscript{13).} Structural changes of MCL particles after AMF irradiation were not observed by electron microscopy (data not shown).

3.2. Cell adhesion activity of MCL particles

Live observation in optical microscope revealed that MCL particles moved quickly in culture medium and occasionally adsorbed to cells. Adhesion of MCL particles to human prostate cancer cells was completed by 6 hr as reported with rat glioma cells\textsuperscript{4).} Adsorbed MCL particles were not desorbed by PBS washing (Fig. 2A and B). Cationic lipid deficient particles with negative zeta potential of -21.2 mV showed less adhesion activity (Fig. 2C). Positive charge conferred by a cationic lipid was potent for adhesion to negatively charged cancer cell membrane as reported\textsuperscript{4,14).}

![Cell adhesion activity of MCL particles](image)

Fig. 2. Cell adhesion activity of MCL particles. Cells seeded in 3.5 mm dish (Thermo scientific, cat. no.153,066) were cultured for 40 hr and applied to MCL particles for 6 hr at more than 2 ng-MCL/cell. Cells were washed twice with PBS and applied to optical microscope. (A), Cells without MCL particles. (B), Cells applied to MCL particles. (C), Cells applied to cationic lipid deficient particles.

Cytotoxicity of MCL particles upon AMF irradiation was planned to test under high confluent cells condition. Adhesion of MCL particles was also confirmed with cells in about 70% confluence (Fig. 3A). Adhesion was observed in punctuated manner as cationic dextran\textsuperscript{14).} Amount of adsorbed MCL particles including possible cellular uptake was estimated 2 ng-MCL/cell under MCL saturation condition.

3.3. Cytotoxicity of MCL particles upon AMF irradiation and its heat dose

Cells adsorbed with MCL particles at 2 ng-MCL/cell (Fig. 3A) were applied to AMF irradiation. Irradiation time was fixed at 30 min as clinical treatment\textsuperscript{7).} Cellular morphological changes to rounded shape were observed under a irradiation condition of 3.5 mm distance from surface board and output power in 55 A (Fig. 3B), which gave a SAR of 2,020 J/g-MCL·min. Irradiated cells still preserved MCL particles on cell membrane and kept attaching to culture-well bottom (Fig. 3B). Cells were applied to trypan blue exclusion assay and presence of died cells was confirmed (Fig. 3Ca and b). Among died cells membrane bursted cells were observed (Fig. 3Cb bold arrow, Fig. 3Cc enlarged). Cellular death rate under this condition was shown as 43.8% with statistical significance versus sole treatment of MCL particles or AMF irradiation (Table 1, Experiment #3, 4, 5).
Heat dose of this cytotoxic condition was calculated with Creixell’s equation\(^8\) as follow; SAR value \((2,020 \text{ J/g-MCL} \times \text{min}) \times \text{amount of MCL (2 ng-MCL/cell)} \times \text{irradiation time (30 min)}\). Calculated heat dose of \(1.2 \times 10^{-4} \text{ J/cell}\) was comparable to reported cytotoxic heat dose of \(1.8 \times 10^{-5} \text{ J/cell}\) of other nanoparticles\(^8\).

3.4. No perceptible temperature rises under cytotoxic condition

During round trip to irradiator facility, cells cultured at 37°C in CO2 incubator had to be exposed to room temperature for about 50 min including 30 min irradiation. However, cell viability was not affected by round

![Fig. 3. Cytotoxicity of MCL particles upon AMF irradiation. Cells seeded at \(2 \times 10^5\) cells per a well of 12 wells plate (Thermo scientific, cat. no. 150,628) were cultured for 40 hr to obtain about 70% cell confluency. Cells were applied to MCL particles for 6 hr at 2 ng-MCL/cell. Four test wells in square position was set on center of surface board and irradiated simultaneously. (A), Cells before AMF irradiation. (B), Cells after AMF irradiation. (Ca and b), Trypan blue extrusion assay of irradiated cells. Cells indicated by arrows were counted as died cells. (Ce), Enlarged bursted cell indicated by bold arrow in Cb.](image)

| Experiment # | n | Death rate (%) | SD | t-test | Temperature range (°C) |
|--------------|---|---------------|----|--------|------------------------|
| 1. control   | 3 | 1.9           | 1.1| ------ | 37.0 constant          |
| 2. round trip| 3 | 3.3           | 1.3| No vs #1 | NT                     |
| 3. round trip, MCL | 3 | 8.1           | 2.6| No vs #2 | NT                     |
| 4. round trip, AMF | 3 | 5.3           | 2.7| No vs #2 | 28.2-25.2              |
| 5. round trip, MCL & AMF | 5 | 43.8          | 4.3| Yes vs #3,4 | 28.4-25.8             |

Cells in about 70% confluency were applied to MCL particles at 2 ng-MCL/cell for 6 hr and irradiated as described in Fig.3 legend. Cellular death rate of each experiment was tested several times (n) and average death rate (%) and standard deviation (SD) were shown. Statistical significance versus others was tested by Student’s t-test and shown as Yes when P-value was less than 0.001 (Experiment #3,4,5). Cells cultured at 37°C were exposed to room temperature for 50 min during round trip to irradiator facility but cell viability was not affected (Experiment #1,2). Medium temperature of test well was monitored by temperature sensor during 30 min irradiation and its representative range was shown (Temperature range). NT; not tested.
trip (Table I, Experiment #1 and 2). Medium temperature of test well was dropped to around 29°C at beginning of irradiation. Even during 30 min irradiation medium temperature continued to drop because heat loss from test well to ambient atmosphere was greater than heat generated in test well. Temperature ranges during 30 min irradiation were between 25.0°C and 29.0°C (Table I, Experiment #5, Temperature range), which were far lower than medium temperature reported to kill cancer cells\textsuperscript{15}).

3.5. Cell membrane distribution of MCL particles and death pattern

Cellular distribution of MCL particles was tested under 16 hr cell exposure condition in order to investigate cellular uptake after adhesion completion at 6 hr. Cell density in test dish was set low because punctuated adhesion was observed under 70% confluent cell condition (Fig. 3A). MCL particles with DOTAP was used since TMAG showed cytotoxicity in 16 hr exposure experiment. There was no significant difference between DOTAP and TMAG in 6 hr exposure experiment.

By electron microscopic observation magnetites in MCL particles were shown to locate abundantly on cell membrane but scarcely in cytosol as a few magnetites incorporated endosomes in a cell. After AMF irradiation, magnetites were again found massively on cell membrane in black but not found in cytosol (Fig. 4Ba-c). About 10 nm magnetites was confirmed on cell membrane at high magnification (Fig. 4Bd). Majority

![Fig. 4.](image-url)
of MCL particles was considered to distribute on cell membrane even under 16 hr cell exposure condition due to insignificant cellular uptake relatively.

Under this low cell density condition, almost all cells were confirmed to die by trypan blue exclusion assay and about half number of cells were observed as membrane bursted cells only in combination of MCL particles and AMF irradiation (Fig. 4Bb, c and e enlarged). The cells showed unusual monotonous staining of cytosol (Fig. 4Ba-c) which were clearly different from those incubated in high temperature medium (Fig. 4C) and untreated (Fig. 4A).

4. Discussion

4.1. Structure and functions of MCL particles

MCL particles prepared by the reported method\(^4\) showed around 100 nm diameter in electron microscopic observation and dynamic laser scattering analysis. The particles showed complexed structure of about 25 magnetites and lipids components, but typical lipids bilayer structure of liposome was not observed as intensified envelope\(^12\) (Fig. 1). Magnetites as active substance for heat generation to kill cancer cells were shown to locate on cell membrane and its release from MCL particles were not observed (Fig. 4Bd). Then, MCL particles are considered out of scope of liposome drug products defined by regulation to encapsulate active substance and release it for liposome activation\(^16\).

MCL particles showed cell adhesion activity against human prostate cancer cells as reported with rat glioma cells, depending on a cationic lipid involved\(^4\) (Fig. 2B and 3A). Charge dependent adhesion is widely available to cancer cells as cationic dextran\(^14\) and would be beneficial to reported

\textit{in vivo} activities against 9 animal tumor models\(^6\) including rat glioma\(^5\) and human prostate cancer\(^11\). Positive zeta potential is useful to ensure cell adhesion activity and SAR value is essential to specify heat generation activity of MCL particles.

4.2. Cell membrane targeting cytotoxicity of MCL particles

Majority of MCL particles was shown to locate cell membrane (Fig. 4Ba-c). Amount of adsorbed MCL particles due to electrostatic interaction was abundant and would exceed capacity of endosomal cellular uptake. Heat generated on cell membrane would cause reduction of cellular tension\(^17\) and resulted in morphological change (Fig. 3B). Extensive heating at a site of cell membrane would cause membrane burst (Fig. 4Be) presumably in thermodynamic fashion\(^18\). These features of cytotoxicity evoke novel concept of cell membrane hyperthermia in addition to intracellular hyperthermia\(^6,6\). However, it is unknown how slight heat dose such as \(1.2 \times 10^{-4} \text{ J/cell}\) induces cellular death. Precise elucidation of cytotoxic mechanism will be important for rational use of MCL particles.

4.3. Therapeutic use of MCL particles

So far heat-related therapy has been controlled by tumor temperature\(^1-6\) including clinical research using MCL particles\(^7\). Its rationale was inferred to originate from the cytotoxicity data obtained in high temperature medium such as 42.5°\(\text{C}\)\(^15\). However, in some cases of heat-generating nanoparticles, cytotoxicity did not always correlate with medium temperature as shown by Creixell \textit{et al.}\(^3\), Schaub \textit{et al.}\(^19\) and this study (Table I, Temperature range). In these cases, heat dose generated in limited cellular area where nanoparticles exist
is considered critical for cytotoxicity. In this study heat dose of $1.2 \times 10^{-4}$ J/cell on cell membrane was shown cytotoxic. These results on heat dose *in vitro* (J/cell) are not inconsistent with the proposed heat dose index *in vivo* (J/cm³ tumor volume) for control of clinical treatment⁹).

During development of hyperthermia devices, utility of thermal dose which could predict biological effects has been pointed out²⁰) and its importance was recently reminded for hyperthermia research in the future²¹). Heat dose *in vitro* (J/cell) would match concept of thermal dose to predict hyperthermia effect. Heat dose *in vivo* can be estimated before clinical treatment by the same equation described in this study. The heat dose needed for complete regression of animal tumor models and required performance of AMF irradiator will be discussed elsewhere (Morino T. et al., Manuscript in preparation).

Acknowledgements
We are thankful for assistance of the Research Equipment Sharing Center at the Nagoya City University.

Disclosure statement
The first author belongs to Dai-ichi High Frequency Co., Ltd. as corporate technical consultant.

References
1) Gilchrist R.K., Medal R., Shorey W.D., Hanselman R.C., Parrott J.C., Taylor C.B.: Selective inductive heating of lymph nodes. Ann Surg, 146: 596-606, 1957.
2) Gilchrist R.K., Shorey W.D., Russell C., Hanselman, Frederic A., DePeyster., Jehoon Y., Richard M.: Effects of electromagnetic heating on in viscer: A preliminary to the treatment of human tumors. Ann Surg, 161: 890-896, 1965.
3) Gordon R.T., Hines J.R., Gordon D.: Intracellular hyperthermia, a biophysical approach to cancer treatment *via* intracellular temperature and biophysical alteration. Med Hypothesis, 5: 83-102, 1979.
4) Shinkai M., Yanase M., Honda H., Wakabayashi T., Yoshida J., Kobayashi T.: Intracellular hyperthermia for cancer using magnetite cationic liposome: *In vitro* study. Jpn J Cancer Res, 87: 1179-1183, 1996.
5) Yanase M., Shinkai M., Honda H., Wakabayashi T., Yoshida J., Kobayashi T.: Intracellular hyperthermia for cancer using magnetite cationic liposomes: An *in vivo* study. Jpn J Cancer Res, 89: 463-469, 1998.
6) Ito A., Kobayashi T.: Intracellular hyperthermia using magnetic nanoparticles: a novel method for hyperthermia clinical applications. Thermal Med, 24: 113-129, 2008.
7) Kobayashi T., Kakimi K., Nakayama E., Jombow K.: Antitumor immunity by magnetic nanoparticle-mediated hyperthermia. Nanomedicine, 9: 1715-1726, 2014.
8) Creixell M., Bohórquez A.C., Torres-Lugo M., Rinaldi C.: EGFR-targeted magnetic nanoparticles heaters kill cells without a perceptible temperature rise. ACSNano, 5: 7124-7129, 2011.
9) Morino T., Etani T., Naiki T., Kawai N., Kikumori T., Nishida Y., Yamamoto N., Yasui T.: Outcome of cancer clinical researches using heat-generating nanoparticles and novel concept of its therapeutic use. Thermal Med, 35: 23-32, 2019.
10) Motoyama J., Hakata T., Kato R., Yamashita N., Morino T., Kobayashi T., Honda H.: Size dependent heat generation of magnetite nanoparticles under AC magnetic field for cancer therapy. BioMag Res Tech, 6: e4, 2008.
11) Kawai N., Ito A., Nakahara Y., Honda H., Kobayashi T., Futakuchi M., Shirai T., Tozawa K., Kohri K.: Complete regression of experimental prostate cancer in nude mice by repeated hyperthermia using magnetite cationic liposomes and a newly developed solenoid containing a ferrite core. The Prostate, 66: 718-727, 2006.
12) Lee R.J., Huang L.: Folate-targeted, anionic liposome-entrapped polylysine-condensed DNA for tumor cell-specific gene transfer. J Biol Chem, 271: 8481-8487, 1996.
13) Patil R.M., Thorat N.D., Shete P.B., Otari S.V., Tiwale B.M., Pawar S.H.: In vitro hyperthermia with improved colloidal stability and enhanced SAR of magnetic core/shell nanostructure. Mater Sci Eng Mater Bio Appl, 59: 702-709, 2016.

14) Marquez M., Nilsson S., Lennartsson L., Liu Z., Tammela T., Raitanen M., Holmberg A.R.: Charge-dependent targeting: Results in six tumor cell lines. Anticancer Res, 24: 1347-1352, 2004.

15) Dewey W.C., Hopwood L.E., Sapareto S.A., Gerweck L.E.: Cellular response to combination of hyperthermia and radiation. Radiology, 123: 463-474, 1977.

16) U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Liposome drug products chemistry, manufacturing, and controls; human pharmacokinetics and bioavailability; and labeling documentation. 2018; April. https://www.fda.gov/media/70837/download. Accessed Sept. 18, 2019.

17) Wolfenson H., Bershadsky A., Henis Y.I., Geiger B.: Actomyosin-generated tension controls the molecular kinetics of focal adhesions. J Cell Sci, 124: 1425-1432, 2010.

18) Diguet A., Yanagisawa M., Liu Y.J., Brun E., Abadie S., Rudiuk S., Baigl D.: UV-induced bursting of cell-sized multicomponent lipid vesicles in a photosensitive surfactant solution. J Am Chem Soc, 134: 4898-4904, 2012.

19) Schaub N.J., Rende D., Yuan Y., Gilbert R.J., Borca-Tasciuc D.A.: Reduced astrocyte viability at physiological temperatures from magnetically activated iron oxide nanoparticles. Chem Res Toxicol, 27: 2023-35, 2014.

20) Dewhirst W.M., Sim A.D.: The utility of thermal dose as a predictor of tumor and normal tissue responses to combined radiation and hyperthermia. Cancer Res (Suppl), 44: 4772s-4780s, 1984.

21) Abe M.: My path in hyperthermia research and expectations for the future. Thermal Med, 29: 69-78, 2013.
細胞吸着性と発熱性を有するマグネタイト正電荷脂質複合粒子の培養液温度非依存的な殺細胞活性とその臨床応用法

森野富夫1・高瀬弘嗣2・恵谷俊紀1・内木拓1
河合憲康1・井藤彰3・安井孝周1

1名古屋市立大学大学院医学研究科、腎・泌尿器科学分野
2名古屋市立大学大学院医学研究科、共同研究教育センター
3名古屋大学大学院工学研究科、化学システム工学専攻

要　旨: ナノサイズのマグネタイト正電荷脂質複合粒子（magnetite cationic liposomes, MCL）を腫瘍内に局所投与した後、体外からの交差磁場照射により発熱を誘導し、腫瘍を退縮させる治療法が本邦で開発された。既に、臨床研究が実施され、施術フィジビリティ並びに有害事象に関する大きな阻害要因は見出されないことが報告された。しかしながら、腫瘍温度は等しく上昇したにも関わらず、その有効性は広範囲（完全退縮～ほとんど無効）に亘ったため、更なる有効性の向上を目的に、腫瘍体積当たりの発熱量（J/cm³）を新たな施術コントロール指標とする提案がなされた。本研究の目的は、MCL粒子の殺細胞活性をインビトロで検討し、その妥当性を論じることにある。

検討の結果、MCL粒子は、ポジティブなゼータ電位に依存して、ヒト前立腺癌細胞に吸着し、その飽和量は2ng-MCL/cellであることが示された。更に、電子顕微鏡観察により、大多数のMCL粒子は細胞膜に局在することが確認された。一方、磁場照射によるMCL粒子の発熱活性は、比吸収率（J/g-MCL・min）で表記可能であり、各照射条件の磁束密度に応じて変動することが示された。MCL粒子が飽和吸着した細胞に対する殺細胞活性は、磁場照射により1.2×10⁴（J/cell）の発熱量を誘導した条件で認められ、その際の細胞膜の障害に起因すると考えられる細胞形態の変化と細胞膜のバースト現象が観察された。注目すべきことに、培養液の温度上昇は認められず、MCL粒子の殺細胞活性は、培養液温度とは無関係に細胞膜局所における発熱量（J/cell）に依存するものと考えられた。これらの結果は、臨床研究における測温指標の代替として、腫瘍体積当たりの発熱量（J/cm³）を施術コントロール指標とする先の提案を支持するものと考えられた。