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Letter to the Editor

Electric and magnetic fields in cryopreservation

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ABSTRACT

Electromagnetic warming has a long history in cryobiology as a preferred method for recovering large tissue masses from cryopreservation, especially from cryopreservation by vitrification. It is less well-known that electromagnetic fields may be able to influence ice formation during cryopreservation by non-thermal mechanisms. Both theory and published data suggest that static and oscillating electric fields can respectively promote or inhibit ice formation under certain conditions. Evidence is less persuasive for magnetic fields. Recent claims that static magnetic fields smaller than 1 mT can improve cryopreservation by freezing are specifically questioned.

There have been several recent articles in the scientific literature and popular press concerning cryobiology applications of CAS (“Cell Alive System”) freezers from ABI Corporation Ltd. (Abiko, Japan). In 2010, Cryobiology reported findings by Kaku et al. in which a CAS freezer was used to produce very weak magnetic fields during freezing of periodontal ligament (PDL) cells and tissues in 10% dimethyl sulfoxide [9]. Superior cell viability and tissue histology was observed compared to control cells cryopreserved without a magnetic field. A follow-up study by the same group in 2011 in Cryobiology reported favorable comparisons between frozen-thawed PDL tissue vs. unfrozen controls, and clinical results after transplantation of teeth cryopreserved by the CAS process [1], again using a very weak magnetic field (0.01 mT). Other papers suggested that ice crystal damage might be reduced by “slightly magnetizing” a whole ovary during freezing without cryoprotectant [24,14]. Forbes magazine reported in 2008 that 47 researchers were experimenting with ABI Corporation freezing technology to preserve human organs [11].

The proposed use of magnetic fields to improve cryopreservation by freezing raises many questions. Before asking them, a brief review of what is known about effects of electric and magnetic fields on ice formation follows.

Electric fields and ice formation

Water molecules have an intrinsic electric dipole moment, making water a dielectric material. Water molecules rotate in response to an applied electric field, which is the mechanism by which oscillating electric fields heat pure water (dielectric heating). Electric fields oscillating at radio or microwave frequencies are a preferred method for achieving rapid and uniform warming of cryopreserved materials [13,15,32].

It’s been known for a long time that static electric fields can nucleate ice formation in supercooled water [3,21–23]. The effect has been beneficially used in cryobiology to reduce supercooling during freezing to prevent intracellular ice formation [20]. Such electrofreezing is typically accomplished by applying kilovoltage to electrodes in direct contact with supercooled water.

The physical mechanism of electrofreezing is still poorly understood. Surface interactions are involved, and there is a dependence upon electrode composition [6]. Uniform electric fields applied using electrodes external to water samples are relatively ineffective. A uniform field of 100 kV/m only raised the freezing point of 1 mL samples by 1.6 °C [30], and no increase in the homogenous nucleation temperature of water droplets was found at this field strength [25]. Molecular dynamics simulations suggest that an electric field strength on the order of 5×10^9 V/m is necessary to nucleate bulk supercooled water into cubic ice [28].

Although it is difficult to electrically align water molecules to cause freezing, it may be easier to electrically disturb water molecules to alter or prevent freezing. There is evidence that oscillating electric fields can suppress ice formation and enhance supercooling [5,7,8,4]. Jackson et al. showed that 2.45 GHz microwave radiation could reduce the amount of ice formed during attempted vitrification of ethylene glycol solutions [7,8,4]. Sun et al. studied freezing in the presence of electric fields oscillating at frequencies between 1 and 200 kHz, and found ice crystal domain size to be minimized at a frequency of 50 kHz [26].

Magnetic fields and ice formation

Water has no intrinsic magnetic dipole moment. Water is diamagnetic, which means that it develops a magnetic dipole moment in response to an applied magnetic field. Since a magnetic field is required to both induce a magnetic moment, and exert a

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force on the magnetic moment, magnetic forces on water molecules vary as the square of the applied magnetic field strength. This means that weak magnetic fields have little effect on water, while gradients of strong magnetic fields (>10 Tesla) can exert enough force to levitate water against gravity. This is in contrast to the force of electric fields on water molecules, which varies linearly with field strength as the electric field acts on a constant dipole moment.

There is little published research on effects of magnetic fields on ice formation [31]. One paper reported that strong static magnetic fields nucleated ice formation in 0.5 mL samples of distilled water, with a field strength of 0.5 Tesla causing equilibrium freezing at 0 °C [2]. Another paper observed that containerless 6 mm globules of water levitated in an 18 Tesla magnetic field supercooled to −10 °C before freezing [29]. This is an unremarkable degree of supercooling for such a sample size, suggesting that there is no obvious enhancement or inhibition of freezing by static magnetic fields in bulk water.

In the laboratory of this letter author, a 1.08 Tesla neodymium-iron-boron magnet (Radio Shack #64-1895) was observed to nucleate ice formation on its surface when immersed in a 10 g 50% w/w ethylene glycol solution cooled in a scintillation vial held above liquid nitrogen. However the effect vanished when the magnet was covered by aluminum foil. The same magnet was observed to cause no change in the devitrification tendency of a vitrified 10 g 57% w/w ethylene glycol solution during warming in room air. These observations are also consistent with there being no effect of static magnetic fields on freezing of bulk water away from surfaces.

It is possible that oscillating magnetic fields may influence ice formation. However effects attributed to oscillating magnetic fields could be caused by oscillating electric fields that accompany oscillating magnetic fields according to the Maxwell Faraday equation. Determining whether pure magnetic fields influence ice formation would require performing tests in regions where induced electric fields are small, such as along the central axis of a current loop or loops producing an oscillating magnetic field. Mochimaru et al. reported improved porcine ovarian tissue cryopreservation in the presence of an alternating magnetic field of unspecified strength or frequency, but no difference in the freezing point of distilled water or 1.5 M dimethyl sulfoxide in PBS in the presence of the magnetic field [16].

Recently a study was performed to scientifically investigate claims of better food preservation using commercial freezers with a magnetic field generator [27]. No significant difference was said to be found between food frozen with a 0.0005 Tesla magnetic field and control experiments. This is not surprising because 0.0005 T (0.5 mT) is a very weak magnetic field.

Questions about CAS freezer findings

The paper by Kaku et al. in Cryobiology in 2010 reported studies of periodontal ligament (PDL) cell cryopreservation using a CAS freezer with magnetic field strengths ranging from 0 to 0.15 mT [9]. Fig. 1B showed that the proportion of living thawed cells surviving after 48 h of culture rose from 40% at zero magnetic field to above 70% for 0.005, 0.01, and 0.15 mT magnetic fields. All other evidence presented for a magnetic field benefit was based on comparisons between PDL tissue frozen in a CAS freezer with a 0.01 mT magnetic field and PDL tissue frozen in a "normal programmed freezer". The scientific question of whether magnetic fields had a beneficial effect would have been more clearly answered if the control experiments used the CAS freezer with no magnetic field rather than a normal freezer. Is it possible that there were differences between the CAS freezer and the normal freezer other than the applied magnetic field?

In the above 2010 Cryobiology paper, Fig. 1E obtained at a field strength of 0.01 mT is identical to Fig. 1A published in a paper in the journal Biomedical Research [12]. The papers respectively identify 0.01 mT and 75 mA as the optimum magnetic field and field-generating current for PDL cell freezing. Fig. 2B of the Biomedical Research paper shows high cell survival after thawing and 48-h culture for all field-generating currents tested, ranging from 5 to 150 mA. If 75 mA current produces a magnetic field of 0.01 mT in a CAS freezer, this means that high PDL cell survival (65–75%) was found at magnetic field strengths ranging from 0.00067 to 0.15 mT. How small must a magnetic field be for the beneficial CAS effect to disappear?

For reference, the strength of Earth's natural magnetic field present in the laboratory is between 0.025 mT near the magnetic equator to 0.06 mT near the poles [10]. Fig. 1B of the 2010 Cryobiology paper reported that cryopreserved PDL cell survival increased from 40% survival to 70% survival when the CAS field strength was raised from 0 to 0.005 mT. Did this field include or not include the 0.04 mT field naturally present from the Earth?

Neither the Biomedical Research paper [12] nor two Cryobiology papers [1,9] about CAS freezing make any reference to a time dependence of the magnetic field. However all these papers refer to magnetic fields causing water molecules to "vibrate" and prevent water clusters from forming. The Mochimaru poster studying porcine ovarian tissue freezing using the CAS freezer specifically refers to inhibiting formation of ice crystals by an "alternating magnetic field" [16]. A 2008 Forbes article quoted the inventor of the CAS freezer as saying that the freezers use strong magnetic fields and "other kinds of energy" [11]. ABI Corporation patents disclose static magnetic fields, alternating magnetic fields, oscillating electric fields, and even acoustic energy [17–19]. Patent data tables show utilization of radio frequency electric fields of 150 V/cm in combination with static magnetic fields of 1 mT (10 Gauss) and 50 Hz oscillating magnetic fields of 0.5 mT [18].

Were the physical parameters of the PDL cell and tissue freezing experiments fully disclosed in the 2010 and 2011 papers in Cryobiology [9,1]? In particular, was the applied magnetic field only a static magnetic field at the stated weak strength, or was an alternating magnetic field also used? Was an oscillating electric field also used? Surely the only field used could not have been a static magnetic field weaker than Earth's own field.

Interesting results are apparently being obtained with CAS freezers. That electromagnetic fields can influence ice formation by non-thermal mechanisms is an important observation. It is necessary to know all physical conditions that lead to such observations so that they can be replicated and studied in other laboratories, even using different equipment.

References

[1] S. Abedini, M. Kaku, T. Kawata, H. Koseki, S. Kojima, H. Sumi, M. Motokawa, T. Fujita, J. Ohtani, N. Ohwada, K. Tanne, Effects of cryopreservation with a newly-developed magnetic field programmed freezer on periodontal ligament cells and pulp tissues, Cryobiology 62 (2011) 181–187.

[2] V.D. Aleksandrov, A.A. Barannikov, N.V. Dobritsa, Effect of magnetic field on the supercooling of water drops, Inorganic Materials 36 (2000) 895–898.

[3] L. Dufour, U¨ ber das gefrieren des wassers und u¨ber die bildung des hagels, Poggendorfs Ann. Physik 114 (1861) 530–554.

[4] D.Y. Gao, T. Jackson, W. Zhang, Development of a novel microwave cavity to vitrify biological tissues for use in surgical transplantation, ASME Bioengineering Division 37 (1997) 185–188.

[5] Y. Hanyu, M. Ichikawa, G. Matsumoto, An improved cryofixation method: cryoquenching of small tissue blocks during microwave irradiation, Journal of Microscopy 165 (1992) 225–235.

[6] T. Hozumi, A. Sato, S. Okawa, K. Watanabe, Effects of electrode materials on freezing of supercooled water in electric freeze control, International Journal of Refrigeration 26 (2003) 537–542.

[7] T.H. Jackson, A. Ungan, J.K. Critser, D. Gao, Novel microwave technology for cryopreservation of biomaterials by suppression of apparent ice formation, Cryobiology 34 (1997) 363–372.

[8] T.H. Jackson, A. Ungan, D.Y. Gao, J.K. Critser, On the effects of microwave irradiation during cryopreservation, ASME Heat Transfer Division 322 (1995) 77–83.
[9] M. Kaku, H. Kamada, T. Kawata, H. Koseki, S. Abedini, S. Kojima, M. Motokawa, T. Fujita, J. Ohtani, N. Tsuka, Y. Matsuda, H. Sunagawa, R.A.M. Hernandes, N. Ohwada, K. Tanne, Cryopreservation of periodontal ligament cells with magnetic field for teeth banking, Cryobiology 61 (2010) 73–78.
[10] M.C. Kelley, The earth's ionosphere: plasma physics and electrodynamics, Academic Press, Burlington, MA, 2009. p. 16.
[11] T. Kelly, Mr. Freeze, Norio Owada's freezing method can keep milk fresh for months. Livers, too, Forbes, June 2 (2008) 76.
[12] T. Kawata, M. Kaku, T. Fujita, J. Ohtani, M. Motokawa, K. Tanne, Water molecule movement by a magnetic field in freezing for tooth banking, Biomedical Research 21 (2010) 351–354.
[13] F.D. Ketterer, H.I. Holst, H.B. Lehr, Improved viability of kidneys with microwave thawing, Cryobiology 8 (1971) 395.
[14] K. Kyono, M. Hatori, F. Sultana, C. Nishinaka, T. Kyoya, H. Uto, S. Kanto, M. Kuchiki, Y. Nakajo, K. Fujii, N. Owada, T. Sankai, Cryopreservation of the entire ovary of cynomolgus monkey in a magnetic field environment without using cryoprotectant. The 19th Annual Meeting of The European Society of Human Reproduction and Embryology. Human Reproduction 23 (Suppl. 1) (2008) P-493, 198.
[15] T.P. Marsland, S. Evans, D.E. Pegg, Dielectric measurements of the design of an electromagnetic rewarming system, Cryobiology 24 (1987) 311–323.
[16] Y. Mochimaru, N. Kuji, M. Yamada, T. Hamatani, Y. Yoshimura, T. Sankai, K. Kyono, M. Mihara, C. Suzukamo, N. Kashiwazaki, Effect of magnetic field supplementation during the freezing process for porcine ovarian tissue cryopreservation. The 19th Annual Meeting of The European Society of Human Reproduction and Embryology. Human Reproduction 23 (Suppl. 1) (2008) P-361, 146.
[17] N. Owada, Highly-Efficient Freezing Apparatus and Highly-Efficient Freezing Method, United States Patent 7237,400 (2007).
[18] N. Owada, S. Kurita, Super-Quick Freezing Method and Apparatus Therefor, United States Patent 6250,087 (2001).
[19] N. Owada, S. Saito, Quick Freezing Apparatus and Quick Freezing Method, United States Patent 7810,340 (2010).
[20] A. Petersen, H. Schneider, G. Rau, B. Glasmacher, A new approach for freezing of aqueous solutions under active control of the nucleation temperature, Cryobiology 53 (2006) 248–257.
[21] H.R. Pruppacher, The effect of an external electric field on the supercooling of water drops, Journal of Geophysical Research 68 (1963) 4463–4474.
[22] W. Rau, Eiskeimbildungdurchdielektrischepolarisation, Z. Naturforschg 6a (1951) 649–657.
[23] R.W. Salt, Effect of an electric field on the freezing of supercooled water and insects, Science 133 (1961) 1480–1483.
[24] T. Sankai, N. Owada, K. Kyono, Cryopreservation of the Ovary, Journal of Mammalian Ova Research 27 (2010) 101–105.
[25] C.A. Stan, S.K.Y. Tang, K.J.M. Bishop, G.M. Whitesides, externally applied electric fields up to 1.6×10^5 V/m do not affect the homogenous nucleation of ice in supercooled water, Journal of Physical Chemistry B 115 (2011) 1089–1097.
[26] W. Sun, X. Xu, W. Sun, L. Ying, C. Xu, Effect of alternated electric field on the ice formation during freezing process of 0.9% K_2MnO_4 water, Proceedings of the IEEE International Conference on Properties and Applications of Dielectric Materials (2007) 774–777.
[27] T. Suzuki, Y. Takeuchi, K. Masuda, M. Watanabe, R. Shirakashi, Y. Fukuda, T. Tsuruta, K. Yamamoto, N. Koga, N. Hiruma, J. Ichioka, K. Takai, Experimental Investigation of Effectiveness of Magnetic Field on Food Freezing Process, Transactions of the Japan Society of Refrigerating and Air Conditioning Engineers 26 (2009) 371–386.
[28] I.M. Svishchev, P.G. Kusalik, Crystallization of liquid water in a molecular dynamics simulation, Physical Review Letters 73 (1994) 975–978.
[29] M. Tagami, M. Hamai, I. Mogi, K. Watanabe, M. Motokawa, Solidification of levitating water in a gradient strong magnetic field, Journal of Crystal Growth 203 (1999) 594–598.
[30] S. Wei, X. Xiaobin, Z. Hong, X. Chuanxiang, Effects of dipole polarization of water molecules on ice formation under an electrostatic field, Cryobiology 56 (2008) 93–99.
[31] M.W. Woo, A.S. Mujumdar, Effects of electric and magnetic field on freezing and possible relevance in freeze drying, Drying Technology 28 (2010) 433–443.
[32] M. Wusteman, M. Robinson, D. Pegg, Vitrification of large tissues with dielectric warming: biological problems of some approaches to their solution, Cryobiology 48 (2004) 179–189.

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