Expert Commentary

# Microscale and Nanoscale Technologies in Low Temperature Biology and Biomedical Engineering

Microscale and nanoscale technology refers to the manipulation of matters with at least one dimension sized in the micro- and/or nano-meter range, typically from 10nm to 100μm. Recent advances in micro- and nanotechnologies have generated considerable interest and would likely make significant impact on all aspects of low temperature biology and biomedical engineering. The new technologies provide new capabilities and new directions of future research. Their applications can be extended not just to the micro-patterning and encapsulation of molecules and cells, cryopreservation of tissues and organs, cryotherapy, but also to medical diagnostics, drug discovery, precision medicine, tissue engineering and regenerative medicine.

Microtechnologies have promised for more precise control, manipulation and observation of cell responses upon various stresses with regard to low temperature biology, including cell dehydration and rehydration during the addition and removal of cryoprotective agents, as well as extra- and intra-cellular ice formation during the freeze-thaw cycle. Current microscale devices are advancing the cell-specific optimization of the loading/unloading of cryoprotectants and the freeze-thaw process. For example, microfluidic technique is playing a more and more important role in biopreservation by enabling the precise characterization of cell membrane permeability, thermodynamic and kinetic parameters of ice nucleation and crystal growth. Encapsulation at the microscale can effectively decrease the size of cell suspension droplets and make possible for vitrification at very low concentrations of cryoprotectants, which otherwise is not possible using the conventional cryopreservation method. The advance in micro-technology may also offer novel solution for the development of self-adaptive cryoprobes for the surgical ablation of irregular-shaped tumors.

The applications of nanotechnology in low temperature biology and biomedical engineering have seen rapid progress in recent years, with advanced capabilities to modulate the thermal properties of cryoprotectant solutions, cells and tissues, as well as transport properties of cell membrane, etc. It has been reported in several recent studies that hydraulic conductivity and cryoprotectant permeability of cell membrane and their activation energies may be altered by the incorporation of functional nanoparticles, and consequently the freeze-thaw cycle could be more easily manipulated. Exciting development also includes the use of biocompatible nano-capsules for successful intracellular delivery of impermeable cryoprotect molecules, the use functional nanoparticles to induce directional growth of ice crystals in the frozen region of the tumors and thus to improve the efficiency of tumor cell killing. Thus new nanotechnologies could potentially change our way of thinking and approach on biopreservation or destruction.

This issue of *CryoLetters* has published two reviews and seven original research articles in the subject of micro- and nanotechnologies, as well as their applications in cryobiology and cryopreservation.

The development of “off-the-shelf” cell-scaffold constructs for clinical uses has received an increasing interest in tissue engineering and regenerative medicine. However, the successful cryopreservation of cell-scaffold constructs remains a challenge for the industry. In their review article, Chen and Lv have provided an overview on recent progress of nanotechnology-based preservation of cell-scaffold constructs. The none-uniform temperature distribution, limited cooling and warming rates, and thermal stresses are three major hindering factors for successful vitreous cryopreservation of large bulk volume of therapeutic cells. In the second review article, Zheng and Zhao introduced the idea to integrate micro- and/or nano-scale (microfluidic) encapsulation technology with vitreous cryopreservation. The new technology could avoid a number of limitations of the traditional cryopreservation approach and opens up new ways for high efficiency encapsulation and vitrification at low protectant concentrations.

The selected original research articles fall into diverse topics, including the microscale measurement of oocyte membrane permeability (Han), the thermal conductivity measurement of tissues with a micron-scale thermal sensor (Jiang et al), the mechanical damage to sperm by microscale ice formation (Han and Critser), the effects of various nanoparticles on thermal properties of cryoprotectant solutions (Xu H et al, Xu Y et al), the effects of nanoparticles on survival and development of vitrified oocytes (Li et al), as well as 3D modelling on biodegradable nanoparticle-enhanced cryoablation of tumors (Xu S et al).

Applying microscale techniques to study cell membrane permeability and ice-induced cryoinjuries, Han has used a creative method that combines a microscale pulled glass capillary with a cryomicroscope stage for permeability measurement of mouse oocytes in supercooled cryoprotectant solutions. Capillaries of different sizes are assembled into interesting patterns to manipulate the size of extracellular ice and to permit the study on the mechanical damage of ice to rodent sperm.

The effect of nanoparticles on the thermal behaviours of cryoprotectant solutions has received considerable attention, even leading to the term "nano-cryoprotectants". Xu H et al have studied the influences of hydroxyapatite (HA) nanoparticles on glass transition, devitrification and ice-melting, while Xu Y et al have further investigated the effects of superparamagnetic nanoparticles on nucleation and crystal growth in vitrified VS55 solution during warming. The presence of nanoparticles indeed changes the kinetic stability of supercooled CPA solutions significantly, affecting nucleation, devitrification, and/or recrystallization. The studies inspire new thinking on the formulation of CPA solutions with the incorporation of nanoparticles. To examine experimentally the role of nanoparticles on cryopreservation by vitrification, Li et al have compared the toxicity of different nanoparticle concentration, type and size to the survival and subsequent development of porcine GV oocytes. Among four nanoparticle types (hydroxyapatite, silica dioxide, aluminum oxide, titanium dioxide), hydroxyapatite nanoparticles were the least toxic and could yield 100% development of GV-stage porcine oocytes if its concentration was less than 0.5%. Li et al have also demonstrated the limitation of nanoparticle technologies to formulate a better CPA solution.

The thermal conductivity of tissues and organs is unquestionably one of the most critical thermal properties used for planning and guiding surgical cryotherapy procedures, such as the treatment of liver tumors. There is insufficient data over the temperature range for cryotherapy and hyperthermia treatment. Jiang et al have explored a micron-sized hot probe with the porcine liver model and made comprehensive measurement of thermal conductivity in the temperature range for cryotherapy. The method is able to provide critical data for more accurate prediction for cryosurgery. In addition to microscale devices, nanotechnology may also play a role in cryotherapy. Nanoparticle-enhanced freezing has the potential for developing a conformal targeted cryoablation for tumors of irregular and complex shapes. At present, the safety and biocompatibility of nanoparticles are major concerns. Liu and his co-workers have developed biodegradable MgO nanoparticles to enhance the cryoablation of liver tumors. MgO nanoparticles are nontoxic, and have few side-effects to the human body. The innovation can improve the safety and biocompatibility of nanoparticles in clinical applications (Xu S et al).

We would like to thank all the authors for their contributions. This collection of reviews and original research articles represents some of the exciting recent advances of microscale and nanoscale technologies in the field of low temperature biology and biomedical engineering. We welcome your comment and criticism.

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