

Research on Microfluidic Technology in the Field of Organ Cryopreservation

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
Abstract

Breakthroughs in cryobiology have led to the development of quite a number of biomedical applications such as cell preservation. By using the single-cell control and high-throughput screening characteristics of microfluidic chips, combined with the optimization of microchannel and heat transfer structure, the flow characteristics of multiple protective agents and the cell heat transfer mechanism can be explored and the fluid temperature, concentration and flow rate of cell sites can be accurately controlled, which is difficult or impossible to achieve with current methods. Reasonable use of the advantages of microfluid will solve the problem of pain points in organ preservation. In this paper, the application of microfluidic chips in organ cryopreservation is discussed, the core challenges of organ cryopreservation are discussed and the future development prospects are prospected.

Keywords: Microfluidic chip; Organ freezing; Artificial organs

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Introduction

The important application of cryogenic technology in biomedicine is the cryogenic preservation of biological materials, that is, the cell or the whole tissue is reduced to a low subzero temperature, to reduce the metabolic rate and achieve the purpose of long-term preservation [1-3]. At low temperatures, all life activities and biochemical reactions tend to stop. At present, at the cellular level, cryopreservation has been very mature [4]. However, there are still many problems in the field of organ cryopreservation [5]. These problems lead to the slow popularization of organ preservation technology. Organ transplantation is an important and possible the only way to cure many diseases, so organ preservation is of great significance. Microfluidic technology has an important application prospect in the research of organ cryopreservation and can solve the above problems well. Microfluidic technology can control fluid flow and transfer materials by using the design of micro-channels [6]. In addition, microfluidic technology can also be used to introduce cryoprotectants and regulatory substances, such as sugars that change membrane permeability, cryoprotective solutions, antioxidants and anti-inflammatory substances, to explore the ability of organs to resist freezing damage and thermodynamic responses. In summary, based on microfluidic technology, it can provide a breakthrough point for the key organ cryopreservation technology to solve the shortage crisis of donor organs.

Current Situation of Technological Development

Application of microfluidic technology in the field of cell cryopreservation

In the field of cell preservation, trillions of cells worldwide are stored at low temperatures for daily clinical use. For example, cryopreservation of human oocytes preserves the future fertility of young women who may experience infertility [7]. In the process of low temperature storage of biological cells, the extracellular solution is often filled with protective solution, and the cell itself is also rich in water. With the decrease of temperature, the water in the extracellular solution freezes. The concentration of the extracellular solution increases,

resulting in osmotic pressure inside and outside the cell which dehydrates the cell. Accordingly, the protein damage of the cell and the instability of the cell membrane will be caused, resulting in "solute damage". At the same time, the formation of intracellular ice in the process of "cooling and freezing" and "rewarming" is the most important cause of cell damage and the research on the damage mechanism of intracellular ice formation is also a long-term hot spot in cryogenic biomedical research [8].

Based on the above problems, the current application of microfluidic chips in cell preservation focuses on the study of the transport characteristics of cell membranes [9], the study of CPA loading and unloading, and the study of freezing and rewarming [10]. The transport characteristics of cell membrane directly determine the transmembrane transport of water and CPA, which determines the competitive relationship between solute damage and ice crystal damage of cells. Proper regulation can achieve the optimal effect of preservation. Current studies on cell membranes tend to focus on the quantification of membrane permeability coefficient [9]. Through the precise temperature control of microfluidic chip, the thermodynamic response process of cells during freezing and rewarming can be studied.

Application analysis of microfluidic technology and artificial organs in the field of organ cryopreservation

In the field of organ cryopreservation, the mainstream direction is the new technology through low-temperature non-cryopreservation and *in vitro* machine perfusion. Uygun proposed an extended liver storage method combining supercooling and machine perfusion, in which rat livers cryopreserved for 4 days were transplanted into healthy rats, extending the feasible preservation time by two times [11]. The challenge of preserving liver tissue at sub-zero is due to the complex liver anatomy in which cells have different preservation properties and functions. Inspired by the high concentration of glucose produced by freeze-tolerant species as a cryoprotectants, the supercooling preservation of a non-metabolic glucose derivative (3-O-methyl-D-glucose, 3-OMG) was studied, which was the first study to test 3-OMG supercooling [12]. Heidi adjusted the preservation method in rats. The supercooling protocol that avoids freezing of the human liver by minimizing the gas-liquid interface allows it to be applied to larger organs, using which the human liver can be stored ice-free at -4 °C, which greatly extends the life of the organ *in vitro* [13]. Inspired by freeze-tolerant animals, Toner used ice nucleating agents to control ice and cryoprotectants to maintain the unfrozen liquid portion, proposing a protocol for freezing rat livers at -10 °C to -15 °C for up to 5 days in the presence of ice [14].

However, as two core difficulties in organ freezing, the problems of "Cell-interstitial interaction and mechanism of freezing damage in organs" and "Interaction between protective agents and cells and screening of optimal ratio" need to be further explored. The combination of microfluidic and organoid technology can well solve the above problems, and organoid technology has developed rapidly in recent years. As a 3D cell culture cultured *in vitro* from adult stem cells, it breaks through the existing limitations of traditional

in vivo and *in vitro* models and provides a new research idea for medical research [15,16]. With the rapid development of organoid technology, a platform for modeling biological and physicochemical characteristics and a transformation model for realizing high-throughput phenotypic drug screening have been established, which combined with microfluidic technology provides a new idea for the study of organ cryopreservation. CPA loading/unloading and freezing strategies are optimized on artificial organs cultured in microfluidic based on precisely determined membrane transport properties.

In the direction of screening cryoprotectants, the influencing factors of cryoprotectants are complex, including hydrogen bond modulation, influence on cell membrane characteristics, solute dilution effect and viscosity induction of cryoprotectants. The composition of protective agent and freezing strategy have important effects on water crystallization, membrane deformation inhibition, membrane permeability, and sugar transport characteristics, but the molecular mechanism of CPA-cell membrane interaction remains to be further revealed. In addition to artificial organs, the three-dimensional microenvironment of hydrogels is like that of extracellular matrix, allowing the diffusion of cellular nutrients and wastes, so 3D cell culture can be performed to explore the ice crystal diffusion mechanism and cryogenic preservation characteristics of extracellular matrix [17]. On this basis, high-throughput screening of protective agent components was performed and the screening results provided guidance for the ratio and loading strategy of protective agent components in organ perfusion and effectively extended the preservation time of organs.

Discussion and Conclusion

The analysis of cell thermal response in tissues is difficult and the concentration control of the environment of cells cannot be realized. The temperature sensor has a large detection area and the local site control of single cells cannot be accurately analyzed. Microfluidic chip design with single cell capture structure, microfluidic machining and local site temperature control can solve this problem and complete the loading of specific freezing curves. In addition, microfluidics can be used for microscale encapsulation of cells, allowing cryoprotection with low CPA concentrations. The application of microfluidic technology in the study of organ cryopreservation still has great development potential. With the continuous improvement of organ preservation requirements, microfluidic technology is expected to further improve the efficiency and effect of cryogenic preservation and extend the preservation time and survival rate of organs. In addition to artificial organs, microfluidic technology can be further combined with other emerging technologies, such as artificial intelligence and gene editing, to further promote research and application in the field of organ preservation. Although the application of microfluidic technology in organ cryopreservation research has a lot of potential, some technical difficulties and engineering challenges still need to be overcome. These challenges include the design and preparation of microfluidic chips, temperature gradient control, that is, accurate spatial-temporal temperature control and so on, to provide high-

precision cell-specific temperature control. Temperature and concentration control will directly determine the transmembrane transport, due to the membrane permeability coefficient and its correlation, optimize the CPA transmembrane transport. At the same time, the combination of hydrogels and artificial organs with microfluidic is also an important development direction of future organ cryogenic research, to promote the practical application and promotion of microfluidic technology in organ cryogenic preservation.

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