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Synergistic Development of Biochips and Cell Preservation Methodologies: a Tale of Converging Technologies

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Abstract
Purpose of the Review Over the past several decades, cryopreservation has been widely used to preserve cells during long-term storage, but advances in stem cell therapies, regenerative medicine, and miniaturized cell-based diagnostics and sensors are providing new targets of opportunity for advancing preservation methodologies. The advent of microfluidic-based devices is an interesting case in which the technology has been used to improve preservation processing, but as the devices have evolved to also include cells, tissues, and simulated organs as part of the architecture, the biochip itself is a desirable target for preservation. In this review, we will focus on the synergistic co-development of preservation methods and biochip technologies while identifying where the challenges and opportunities lie in developing methods to place on-chip biologics on the shelf, ready for use.
Recent Findings Emerging studies are demonstrating that the cost of some biochips have been reduced to the extent that they will have high utility in point-of-care settings, especially in low resource environments where diagnostic capabilities are limited. Ice-free low temperature vitrification and anhydrous vitrification technologies will likely emerge as the preferred strategy for long-term preservation of bio-chips.
Summary The development of preservation methodologies for partially or fully assembled biochips would enable the widespread distribution of these technologies and enhance their application.

Keywords Cryopreservation · CPA · Lab-on-a-chip · Organ-on-a-chip · Dry preservation · Biochips · Microfluidics

Introduction

Cryopreservation, the use of low temperatures to reduce biological activity in order to achieve suspended animation, is widely used to preserve living cells and simple tissues and is of great interest for preserving many types of cells that are used either therapeutically or as source materials for engineered tissues and organs [1–3]. Although cell cryopreservation is a mature field and many individual cell types have been successfully banked for decades [2, 4], advances in stem cell therapies, regenerative medicine, and miniaturized cell-based diagnostics and sensors are providing both new challenges and new targets of opportunity.

The existence of an "optimal cooling rate" for preserving cells was suggested by Mazur in 1960 [5]. The premise of this theory is that the use of an intermediate cooling rate can avoid the cell injury that is caused by excessive osmotic dehydration at low cooling rates and lethal intracellular ice formation at very high cooling rates (Fig. 1). This intermediate rate is specific to a given cell type, with differences between cell types largely attributed to differences in the ability of a given cell to transport water across the cell membrane [6]. This conceptual framework allowed for significant advances in understanding and predicting the variable response of different cell types to low temperatures and spurred the development of new instrumentation, such as diffusion chambers and cryomicroscopes, which enabled better quantification of the underlying biophysical characteristics of cells, especially the membranes, which delineated the response of cells to low temperatures [7–9].

This article is part of the Topical Collection on *Artificial Tissues*

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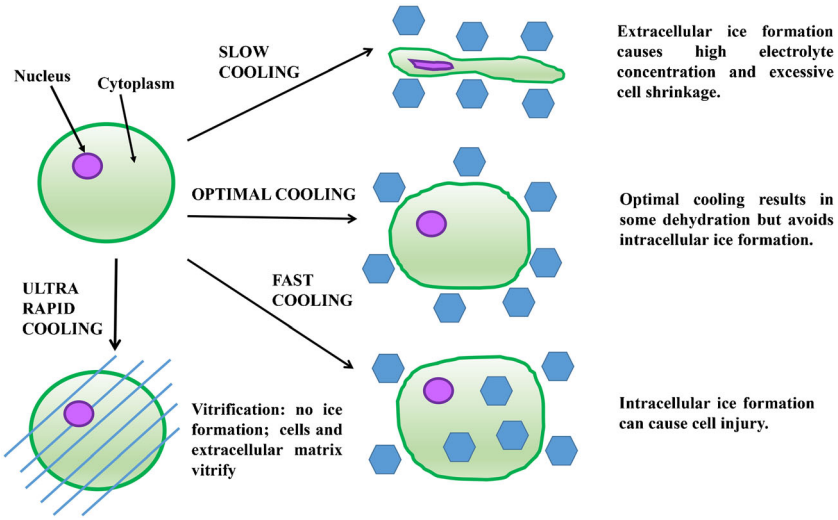

Fig. 1 Schematic of physical events in cells during freezing with different cooling rates. *Blue hexagons* = ice crystals

Although early devices to monitor ice formation and cell volume changes during microscopic imaging might be considered crude by today's standards, they did enable considerable advances in both theory [10] and the development of practical protocols for preserving cells, and they laid the groundwork for modern-day microfluidic-based tools for manipulating chemical and thermal profiles of cells during imaging experiments.

Lab-on-a-chip (LOC) devices, which integrate different technologies such as microfluidics, chemistry, and molecular biology onto a single chip, enable the completion, on a small scale, of analyses that are typically done at bench scale in a laboratory. These devices have significantly impacted the optimization efficiency of preservation protocol development. With the advent of microfluidic technologies, it became easier to deploy precise concentration gradients of solutions that simulated the osmotic stresses of freezing [11, 12] as well as exposure to cryoprotectant agents at various temperatures [13]. This has led to the development of advanced preservation protocols, as well as "preservation on chip" approaches. Organ-on-a-chip (OOC), a multi-channel 3D microfluidic cell culture chip that simulates the activities, mechanics, and physiological response of entire organs and organ systems, also has the potential to serve as an advanced screening tool for the development of new compositions that support preservation. In general, on-chip technologies containing biologics (biochips) have been increasing in potential and benefits in a variety of biological applications, including point-of-care diagnostics, genomic and proteomic research, cell biology, analytical chemistry, drug delivery, environmental monitoring, and biohazard detection. As these tools pervade the marketplace, preservation methods for biochips provide an opportunity to extend and enhance these applications, especially to low resource settings (Fig. 2). In this review, we will mainly focus on the synergistic co-development between preservation methods and biochip technologies while identifying where the challenges and opportunities lie in developing methods to place biochips on the shelf, ready for use.

Cell Preservation Fundamentals

Cell preservation for extended periods of time is a critical requirement for maintaining cell cultures, enabling cell transplantation, and supporting the entire biomanufacturing and distribution process for engineered tissues and organs. In this latter case, autologous patient cells, biological scaffolds, and cell-seeded matrices can be targeted by various preservation methods to improve the efficiency of the manufacturing process and enhance on-demand availability for transplantation. Although the current state of the art preservation methods for cell-based biologics utilize low temperature storage, the development of dry preservation methodologies would transform this industry, essentially eliminating the cost and complexity of refrigerated transport.

The numerous and varied chemical reactions that underpin metabolic life processes are temperature dependent. Most enzymes show a 1.5- to 2-fold decrease in metabolic activity for every 10 °C decrease in temperature [14]. For example, a decrease in temperature from 37 to 0 °C will decrease cellular metabolism over 12-fold [14]. Consequently, one of the most ideal methods for cell preservation is the reduction of temperature. However, as stated earlier, the best practices for cell preservation are dependent on cell type and can vary widely, with success sometimes also dependent on the age of the cell and organism [15]. As such, preservation protocols for each cell type must be optimized based on the cell source and the membrane transport characteristics for that cell type.

Generally, there are four methods to preserve cells by lowering temperatures, namely, hypothermic preservation, conventional cryopreservation (controlled ice crystal formation), vitrification (solidification without ice crystal formation), and lyophilization (controlled ice crystal formation followed by removal of ice). For a comprehensive overview, readers are directed to several excellent books and/or review articles that have been written on these topics [16, 17]. For the purposes of

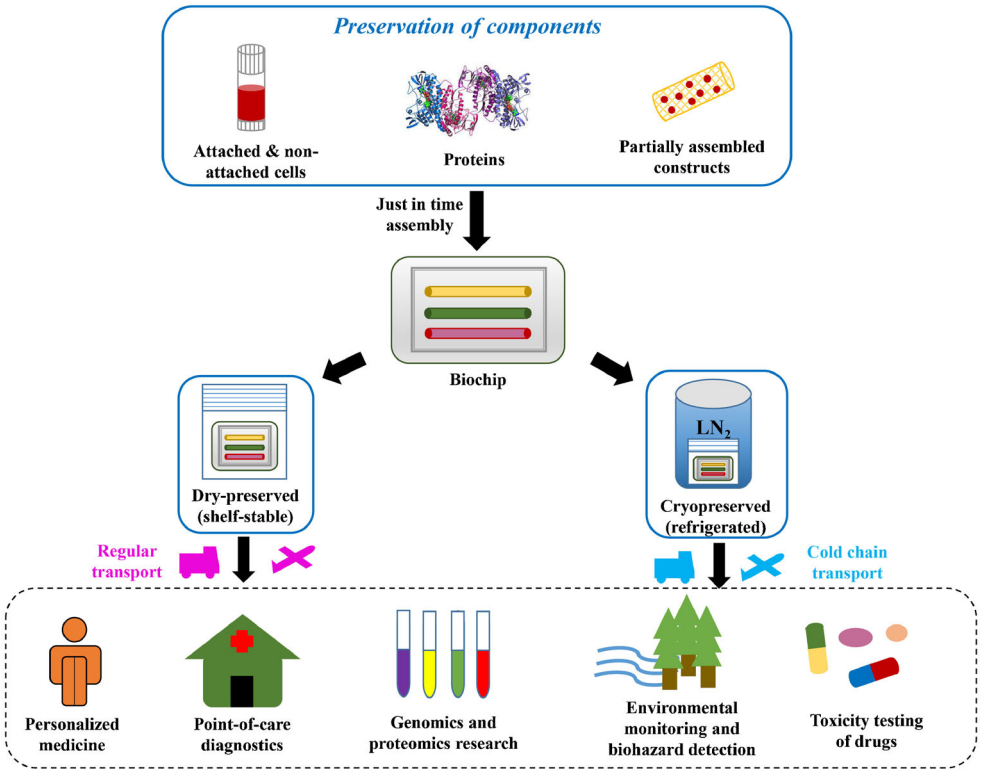

Fig. 2 The application of portable biochips is enhanced with preservation technologies

the current review, these topics will be briefly covered in order to provide adequate background and context.

Hypothermic preservation has been the most commonly used approach for organ preservation for transplantation, such as preservation of an entire heart [18]. Due to the fact that no ice forms during the process, the effects of cooling are often reversible. Hypothermia can be also used for short-term cell preservation on the order of hours to days [19–21]. However, long-term preservation of cells has not been achieved using this approach. Metabolism does not cease at temperatures above 0 °C and not all chemical reactions are slowed to the same degree. Consequently, interrelated metabolic pathways may be "dislocated" by cooling [19], but the duration of time that cells can be stored at temperatures above 0 °C is limited.

Slow cooling cryopreservation involves cooling to very low (cryogenic) temperatures in a manner that mitigates the damaging effects of ice by controlling its extent and location. During the process of cooling, when the temperature becomes lower than the freezing point of either the intracellular or extracellular solution, freezing may occur. To form ice crystals, water molecules, via random movement, must assemble into an ice-like structure with a critical size, a nucleus, in a process termed ice nucleation. The probability of ice nucleation is a function of the volume of the solution. Therefore, ice formation usually occurs first in the extensive extracellular space compared to the much smaller volume of intracellular solution. As pure water comes out of solution in the form of ice, extracellular ice formation consequently results in a high solute concentration surrounding the living cells, which causes water to leave the cells by osmosis (Fig. 1). If the cooling rate is too slow, significant dehydration can occur, leading to a harmful high electrolyte concentration both inside and outside of cells. If the cooling rate is too fast, intracellular ice nuclei and/or ice crystals can form. If the ice nuclei are small enough to be innocuous during cooling, they may still pose a danger to cell survival as they can become sites of recrystallization of large ice crystals later during the thawing process. Intracellular ice formation is typically lethal to cells [22–24]. An optimized cooling rate, as defined by Mazur, is expected to minimize the cell injury caused by both of these phenomena [5, 6]. The use of penetrating cryoprotective agents (CPAs), such as dimethylsulfoxide (DMSO) and glycerol, can further ameliorate preservation-related injury by reducing the probability of intracellular ice formation, the volume of ice formed, and the degree of osmotic dehydration. Non-penetrating CPAs (polyethylene glycol, sucrose, etc.) can also help by modulating the osmotic imbalances that occur during thawing and during the washing steps that are required to remove CPAs before cell use.

Recent studies for the improvement of cryopreservation of stem cells have focused primarily on two areas: modification of the freezing medium and freezing/storage protocols. DMSO-based freezing medium has traditionally been used to cryopreserve hematopoietic stem cells (HSCs) [25, 26], mesenchymal stem cells [27, 28], and human embryonic stem cells [29, 30]. However, DMSO is associated with adverse effects upon infusion and has an epigenetic effect on cells. For example, Iwatani and coworkers found that DMSO can upregulate expression of

Dnmt3as and affect the DNA methylation status that epigenetically controls mammalian development and cellular differentiation [31]. As a result, alternative methods that do not involve DMSO have recently been a focus of development efforts. For example, a DMSO-free CPA solution based on ethylene glycol, 1,2-propylene glycol, and sucrose, supplemented with polyvinyl alcohol as an additive, has recently been used for the cryopreservation of umbilical cord blood-derived mesenchymal stem cells [32]. It has also been suggested that ectoin has the potential to replace DMSO as a cryoprotectant in serum-free cryomedium to preserve human mesenchymal stem cells [33]. Du and his colleagues demonstrated that the DMSO-free solution based on trehalose is an efficient cryoprotectant for cryopreservation of the whole sheep ovary [34]. Toward the goal of improving the practicality of preservation methodology, the effects of cooling rate and storage temperature on stem cell recovery have also been investigated [35–37]. For example, standard practice for preserving HSCs employs a freezing medium with 10% DMSO and a controlled cooling rate of 1 °C/min, followed by storage in liquid nitrogen. Many studies have investigated the use of −80 °C mechanical freezers for cryopreservation of HSCs and achieved cell viabilities better than that obtained with the standard method [35, 36].

Vitrification, as an alternative cryopreservation technology, has been widely used for the long-term storage of living cells, especially small volumes of reproductive cells, including embryos [38–40]. Vitrification is a process of solidification that avoids the crystallization of ice during cooling and rewarming. Using fast cooling rates, the viscosity of the solution is rapidly increased with decreasing temperature until an amorphous "glassy" solid forms. This phenomenon relies on a delicate balance of extreme cooling rates and high concentrations of cryoprotectant solutions. In order to accomplish the vitrification of living cells or tissues, such as oocytes or embryos, the most common strategy is to minimize the volume of the sample (vitrification solution + cells), which not only offers the benefit of increasing both cooling and warming rates but also decreasing the probability of ice crystal nucleation in the small sample [41]. Higher concentrations of cryoprotectants are required for larger samples where the cooling rates become constrained by heat diffusion limitations.

A technique that combines multiple physical processes, lyophilization, or freeze-drying is widely used to preserve proteins at non-cryogenic temperatures, and lyophilization methods have also been under development for various blood cell types [42–45]. During freeze-drying, aqueous samples are first frozen to *induce* the formation of ice crystals in the preservation matrix. The vapor pressure is then reduced in the sample environment in order to remove ice by sublimation, a process known as "primary drying." The remaining components in solution may crystallize or form an amorphous or glassy phase portion, depending on the nature of the composition. The final finishing step is desorption of unfrozen water during "secondary drying", which is accomplished by controlled rewarming at low pressure. Dried samples can then be stored refrigerated (above 0 °C) or at ambient conditions without loss of viability. In the case of proteins, denaturation is often avoided by using compositions containing sugars and polysaccharides that form a glassy matrix in the freeze-concentrated phase, which serves to immobilize and protect the protein. The glassy phase can help prevent protein unfolding and aggregation by spatial separation of the protein molecules [46, 47]. It has been also suggested by Bruni and Leopold that the glassy state may assure quiescence and stability in a living system for lengthy periods [48].

A glassy state can also be obtained at ambient temperatures by direct drying, and nature has provided many examples of this strategy in the form of anhydrobiotes. These anhydryobiotic organisms, which include fungal spores, yeast cells, and artemia, are able to persist without water for decades or centuries. When triggered by dehydration events, such organisms often produce large quantities of sugars and sugar alcohols that can replace the water around polar residues in membrane phospholipids and proteins, thereby maintaining their integrity in the absence of water (known as the water replacement hypothesis) [47]. When water again becomes available, they rapidly swell and resume active metabolism. It is hypothesized that the sugars are involved in stabilizing anhydrobiotic organisms in part due to their ability to form glasses [47]. Glasses can be readily diluted by the addition of water, thus restoring conditions permissive for normal metabolism. The glass transition temperature, T_g, the temperature at which the sample changes behavior from a glassy mechanical solid to a state with decreased viscosity, is strongly affected by plasticizers such as water; thus, an important aspect of this preservation methodology is precise control over water content. As will be discussed shortly, some of these preservation techniques lend themselves more readily to implementation with biochips than others.

Microfluidics and the Advent of Lab-on-a-Chip and Organ-on-a-Chip

Microfluidics is the science and technology of controlling and manipulating liquids at a scale in the range of microliters to picoliters. Microfluidic chips employ a network of microchannels that have been specially designed and molded for a given application and/or analytical technique. Some also employ living functioning cells and cell-based constructs such as a blood-brain barrier for analysis [49, 50]. A varied number of inlet and outlet ports allow fluids to pass through different channels of different diameter, in order to sustain the biological functioning of the cells, or to deliver analytes for testing purposes. Microfluidic chips have been applied in many areas such as medicine, biology, chemistry, and physics. Because of its small size, low volume requirement for samples, and convenience for rapid analysis, portable LOC devices are now

beginning to be used for diagnostic tests in developing countries. For example, a wide range of diseases are characterized by changes in the protein concentrations in a patient's physiological fluids [51, 52]. LOC devices have the potential to transpose antigen-antibody assays into assay formats that are much less demanding in terms of infrastructure. However, one of the important hurdles in the processes of miniaturization and automation is the storage of multiple reagents. Unlike controlled research environments, LOC devices are likely to be used under a variety of environmental conditions. The challenge for storage of reagents inside the microfluidic chip must be addressed in the context of fluctuations in temperature as well as physical shocks.

Although LOC devices do not necessarily contain biologics, in contrast, OOC technologies are microfluidic devices that utilize living cells in continuously perfused, micrometer-sized chambers in order to simulate tissue- and organ-level physiology [53]. Over the past decade, researchers have fabricated chips for the study of the liver [54], kidney [55, 56], lung [57], heart [58], bone marrow [59], skin [60], and blood vessels [61], among others. Many of these devices have recapitulated the complex functions of living cells. For example, bone marrow-on-a-chip, the first method to reproduce cellular, functional, and structural bone marrow in the laboratory, has enabled a new method to test the effects of toxic agents and new drugs on bone marrow to prevent lethal radiation poisoning and the dangerous side effects of cancer therapies, all without animal testing [62].

The Use of Microfluidic Technologies for Cell Preservation Processing

Recently, microfluidic concepts have been used to elicit fine control over the chemical environment during processing for preservation, enabling an improved understanding of chemical injury, and also a new technology for cell processing. A typical cryopreservation process includes loading CPAs, freezing with an optimal cooling rate, thawing with an appropriate warming rate, followed by wash-out of the CPA. Each step can induce damage to cells. Osmotic shock occurring during loading and unloading of CPA is one of the major causes of cell damage during the cryopreservation process [63, 64]. A microfluidic approach to minimize osmotic shock to cells during cryopreservation has been introduced by Song and colleagues [11]. In their studies, they used a three-channel microfluidic device to load and unload CPA into and out of cells in order to minimize the exposure time to high chemical gradients. Cells were injected into the middle channel while CPAs simultaneously flowed into the microfluidic channel from the two sides. Cells experienced changes in CPA concentrations progressively, thus minimizing osmotic shock. After performing the freezing and thawing steps using conventional technology, the same microfluidic concept was used to unload CPAs from the thawed cells, using a PBS wash. This microfluidic approach improved post-thaw cell survivability by 25% on average over conventional cryopreservation protocols with one-step loading and 10% higher viability than a stepwise approach.

Biochips on Demand

Since the general process of cell preparation, including cell revival from storage in LN_2, cell culture in flasks, then seeding onto chips, can take several days to 2 weeks before the cells cultured on chip are ready for use, Li and his colleagues proposed that directly freezing and thawing cells on a PDMS-glass chip would be beneficial for lab-on-a-chip technology and microchip-based life science [65••]. They firstly suspended cells in the freezing medium containing cryoprotectants and then injected the cell suspension into the microchannel. The chip was then put into a 50-ml centrifuge tube filled with isopropyl alcohol and placed horizontally in the −80 °C freezer (achieving a nominal cooling rate of 1 °C/min). Samples were thawed in 37 °C sterile water and the chip then returned to a 37 °C, 5% CO_2 incubator. By using this simple protocol, they demonstrated that 3T3 cells could be successfully recovered and grown in the microchannels after 4 months of storage. HUVECs were successfully cryopreserved for 1 year. However, the number of adhering cells after thawing decreased with the extent of the frozen storage period. After 4 months of cryopreservation, the number of living 3T3 cells dropped to less 100/mm^2 from the 250/mm^2 observed for 20 days of cryopreservation. The same trend was found for HUVECs suggesting that the storage temperature was not low enough for long-term storage. On-chip cryopreservation, including both CPA loading and freezing, has also been successfully demonstrated by Li and his coworkers by using a microfabricated chip with an incubation microchamber and microfluidic channels [66]. Compared to only 27% survival rate of yeast cells obtained by directly plunging the chip into LN_2 vapor, a two-step, temperature-controlled (by microheaters), on-chip cryopreservation process yielded a 74% post-thaw survival rate. The advantages of using this on-chip cryopreservation system include more uniform temperature distributions in the microchamber because of the reduced volume and fast and local temperature control via microheaters that can be manually adjusted to keep the temperature inside the chamber at intermediate values between −20 and −40 °C.

In general, microfluidic technologies require very low volumes of samples and reagents. The low volume requirement is suitable for use with vitrification methods using ultra-rapid cooling rates. Zou and his coworkers studied the ultra-rapid cryopreservation of human spermatozoa using a PDMS-based microfluidic chip [67•]. Compared to conventional cryopreservation, no cryoprotectant is needed for this new method, because the fluid volume in the chip is minimized to ~5×10^{-3} μL, much smaller than other carriers designed for conventional

cryopreservation. Thus, this method avoids the use of toxic CPAs and the time-consuming processing steps for their removal. However, in contrast to Thomson's study using slow, controlled-rate freezing [68], the amount of spermatozoa DNA damage was increased in the post-thaw spermatozoa samples, suggesting the susceptibility of spermatozoa to damage in the freeze-thaw process. In addition, for the evaluation of acrosome integrity, frozen-thawed spermatozoa cryopreserved in designed channels with the height of 50 and 100 μm displayed a lower proportion of intact acrosome compared to that of post-thawed spermatozoa by conventional freezing.

Dry preservation is an emerging alternative method for the long-term storage of biological samples [69–72], especially those where only biomolecules are intended for recovery, such as in the case of dried blood spots [73, 74]. Cross-contaminants or airborne contaminants occurring during drying or exposure of blood spots to the environment after drying are challenges for this approach. Therefore, an approach that offers reliable preservation, avoids contamination, and can be executed by minimally trained users is desirable. Begolo and his coworkers described a device that meets these requirements and can be used with commercially available sample preservation matrices [75•]. The device, based on SlipChip technology, a microfluidic device designed to perform multiplexed microfluidic reactions without pumps or valves [76], incorporates commercially available chemical stabilization matrices (Ex. RNAStable from Biomatrica, Inc.), and consists of three layers. The middle layer is the moving part of the device and can be slipped to connected upper and lower layers. The device can be placed in three positions that respectively correspond to "Loading" (injecting samples into the device), "Drying" (exposing samples to the desiccant that has been pre-loaded into the bottom portion), and "Recovery" (connecting to through-holes for rehydration and collection) functions. After sample collection, the device can be slipped back to the "Drying" position for further storage. Although ultimately intended to be used to preserve RNA in whole blood, the concept was demonstrated using control RNA spiked with deactivated HIV-1 RNA.

Dry preservation was also recently explored by Asghar and colleagues, using trehalose to preserve a multilayered surface on a microfluidic device [77••]. Such microfluidic devices used to test for various diseases and conditions, including HIV and cancer, need to be stored at low temperatures (4 to 8 °C) to prevent the degradation of the capture antibodies that are contained in one of the layers. The geometry of the layers, including the surface functional groups, is essential for proper functioning of the device. Refrigerated transportation of these biomaterials is expensive and increases the assay costs. Because of the difficulty of dehydration in small channels they employed both vacuum drying and heating in order to dry the products within 4 h. They then vacuum sealed the dry-preserved device in plastic and assessed the effects of humidity and simulated extreme weather conditions to test its functionality and shelf-life. The results revealed that they were able to preserve the microfluidic devices for 6 months at room temperature, yielding 90% specificity. These stabilized microfluidic devices were reactivated and used with complementary metal-oxide semiconductor (CMOS) lens less imaging technology (the same imaging sensor found in cell phone cameras) to count CD4 T cells rapidly at a cost less than $1. The captured CD4 T cells were counted rapidly and automatically from unprocessed whole blood.

While the ultimate goal would be to preserve the functionality of cells and tissues on biochips, dry preservation of nucleated cells for ambient storage is not yet a mature technology. Success with the dry preservation of sperm used with in vitro fertilization techniques has been reported [78], and we have also demonstrated that progress with drying of the germinal vesicle within feline oocytes [79] and sperm (unpublished data), but full functionality of dried nucleated cells following long-term storage remains elusive. The preservation of biomolecules and complex biomaterials on chips demonstrates how the convergence of emerging technologies could yield a shelf-ready product in the near future.

Remaining Hurdles

The low manufacturing cost of microfluidic devices is a big advantage for many applications [80]. For example, a point-of-care conventional analytical device such as glucose meter costs in the range of $20 to $150. A microfluidic equivalent could bring the cost down to less than $1. Also, a plastic (acrylic-based) microfluidic device can be used to detect contamination in drinking water and cost only $0.52, whereas colorimetry- and spectroscopy-based devices that achieve the same end, cost on the order of $10,000 [81]. As emerging microfluidic platforms incorporate increasingly complex biological constructs, the cost of preservation should be minimized to ensure that the packaged shelf-ready product can meet global demand for low-cost diagnostics.

Stabilizing cells and tissues within microfluidic devices presents two significant challenges: (1) preservation of cells or tissues for long-term shelf life and (2) stabilization of the functional performance of the microfluidic devices. Although microfluidic devices can facilitate cryopreservation of cells, optimization is still required to improve survival rates. Comprehensive studies to optimize cooling profiles and holding temperatures with respect to various cell types and temperature control during the thawing process are necessary for the improvement of on-chip cryopreservation techniques. It is well-known that the choice of cell preservation methodology largely depends on cell type, and the development of optimal preservation protocols will depend on membrane properties and other cell variations. Because current research is focused on the generation of multi-organ chips which combines at least two culture spaces for multi-organ applications [82], the challenge of meeting the processing demands of the various cell types may preclude using slow cooling

methods. Organs-on-chips that include multiple cell types or even multi-tissue co-culture might be more suited to preservation using vitrification approaches.

Also, the complexity introduced by interfacing multiple materials is a challenge that must be faced when preserving biochips. In addition to the possibility of damage to the chip from thermo-mechanical stresses associated with the differential expansion of materials, the interface between animate and inanimate materials must be considered. Cell survival on a fixed substrate has been observed to be lower than if the same cells are cryopreserved in suspension [83–85]. In particular, cell-cell and cell-substrate junctions have been shown to render cells more sensitive to cryoinjury compared to isolated cells in suspension. Directly cryopreserving cells on chips dramatically decreases the number of cells that remain adhered [65••]. Furthermore, the extreme temperature changes of PDMS-based chips experienced during cryopreservation and rewarming can cause significant distortion of the PDMS geometry, changing the channel geometry, which could lead to delamination of cells from the chip. Depending on the nature of this detachment, cell death may be triggered via apoptosis or direct injury to the cell may occur. Therefore, if maintaining cell adhesion on chips is an important requirement during chip storage, this could pose challenges during cryopreservation. With current technologies, cryopreservation of cells suspended in channels might be a preferred approach for single-cell constructs, but vitrification is likely to be necessary as the biological complexity of the chip increases. Vitrification methods that yield stable products without requiring refrigeration would clearly be preferred due to the significantly lower cost and complexity to ship and store the final product.

Conclusions

In this review, we summarized the synergistic development of biochips and cell preservation methodologies and illustrated the strategies that could be employed for preservation of biologics on chip. Given the recent trend toward DMSO-free cell preservation approaches, preservation strategies that utilize materials inspired by nature are a logical starting point for the development of biochip storage strategies. Although “on-chip” cryopreservation of cells by slow cooling has been reported, as the number of cell types and the necessity of maintaining cell junctions and tissue structure increases, ice-free low temperature vitrification technologies will likely be the best strategy for long-term preservation. Although not yet mature for whole cells and tissues, anhydrous vitrification methodologies could facilitate the storage and transport of biochips in a dried state, thereby avoiding the need for a refrigeration “cold-chain” and enabling their use in settings where electricity and refrigeration are in short supply.

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Compliance with Ethical Standards

Conflict of Interest Shangping Wang and Gloria D. Elliott declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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