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CRYOMACROSCOPY IN 3D: A DEVICE PROTOTYPE FOR THE STUDY OF CRYOPRESERVATION

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INTRODUCTION

This study presents a new device prototype for visualization of physical effects associated with large-scale cryopreservation—the preservation of tissues at very low temperatures. Cryopreservation represents the only method for long-term preservation of biomaterials. While techniques for cryopreservation of single cells and small tissue structures are well established, cryopreservation techniques for bulky tissues and organs are still at the developmental stage. Critical to the success of cryopreservation is the control of ice formation—the cornerstone of cryoinjury. One of the most promising techniques for large-scale cryopreservation is known as vitrification, where the crystal phase is suppressed, and the biological material is trapped in a glassy-like state (vitreous in *Latin* means glassy) [1].

Vitrification is achieved with the addition of a high concentration of cryoprotective agents (CPAs), which experience an exponential increase in viscosity with the decreasing temperature and, thereby, suppress crystallization. Hence, the likelihood of crystallization during vitrification is dependent upon the specific thermal history. Crystallization may initiate during cooling, may continue during rewarming—recrystallization, or even initiate during rewarming—devitrification. Another potentially devastating effect during vitrification is thermo-mechanical stress, caused by the high cooling and rewarming rates required to promote vitrification. When the developing stress exceeds the strength of the material, structural damage follows, with fracture formation as its most dramatic outcome [2].

The current study focuses on developing a device—the cryomacroscope—to visual physical effects along the path-dependent process of vitrification. This device is based on an early prototype developed to visual physical effects in thin vitrified specimens [3]. The innovation in the current device is in the use of a mechanism to scan bulky specimens relevant to large-scale cryopreservation. Design considerations for the new cryomacroscope prototype are derived from its potential versatile use: (i) as a basic research tool, (ii) as a tool to develop cryopreservation solutions and protocols, and (iii) as a quality assurance tool for mass production of cryopreservation products. The current study combines solid mechanics analysis to explain the onset of fracturing in selected cryopreservation experiments.

EXPERIMENTAL SETUP

Figure 1 displays a general view of the scanning cryomacroscope prototype, designed to be compatible with the commercial controlled-rate freezer, Kryo10-16 (Planer, Inc.., UK). Figure 2 displays an experimentation platform developed for basic research studies, used in the current study. The specimen container in this setup is a cuvette, rather than a vial or a bag, to improve the quality of imaging while minimizing optical distortion (Fig. 2).

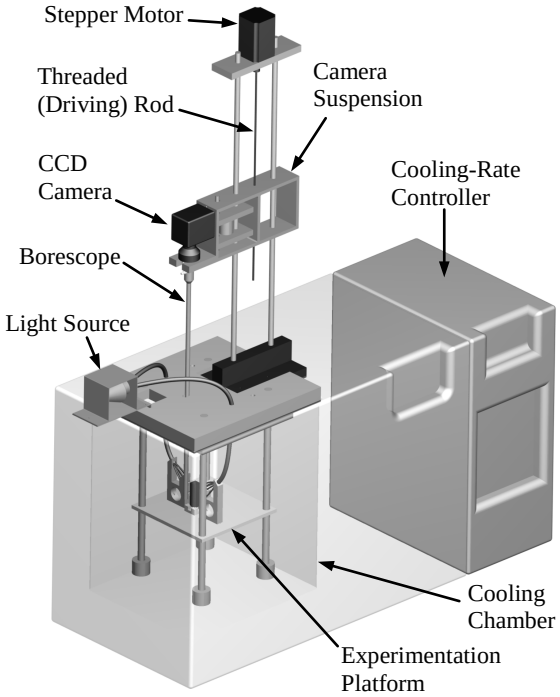

Figure 1. Schematic illustration of the scanning cryomacroscope assembly integrated into the lid of a cooling-rate freezer Kryo 10-16 (Planner, Inc.)

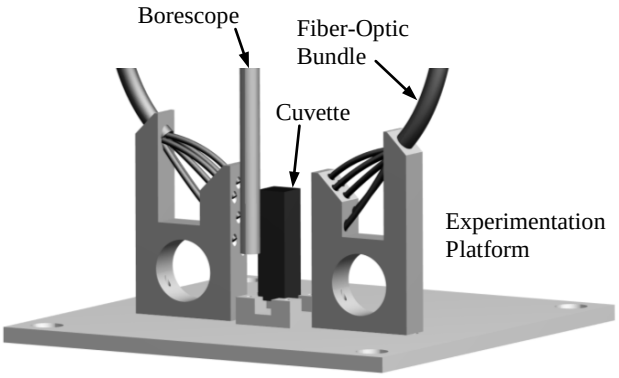

Figure 2. Schematic illustration of the experimentation platform; the cuvette is scanned through a 45° mirror, placed at the tip of the borescope, while the borescope is driven vertically by the stepper motor

RESULTS AND DISCUSSION

Figures 3 and 4 display representative results from cryopreservation protocols on the CPA cocktail DP6 and on 7.05M DMSO, respectively (the latter is a reference solution established for thermo-mechanical studies [3]). Four key effects are demonstrated in Figs. 3 and 4:

(i) The CPAs appear transparent well below the glass transition temperature (-119°C for DP6 and -132°C for 7.05M DMSO in the relevant cooling rates), indicating vitrification.

(ii) Large deformations are evident at the center of the specimen, where the CPA surface is curved inwards, pointed by yellow arrows on Fig. 4(a).

(iii) Contrary to common belief, but consistent with previous solid mechanics analyses [2,3], fracture formation frequently occurs at the rewarming stage of the cryopreservation protocol—either at the onset of rewarming, Fig. 3(b), or at an advanced stage, Fig. 4(b).

(iv) Qualitative results from preliminary thermo-mechanical analysis provide suggest reasons for the onset of fracturing, Fig. 4(a)-(c).

If the specimen would have been evaluated only at the storage temperature, which is common practice in cryobiology studies, the effects of fracture formation and surface deformation would have been missed. Such observations are critical in correlating tissue viability and functionality with the specific particular thermal history of the cryopreservation protocol. In conclusion, the new device represents a critical tool for the analysis of cryopreservation.

ACKNOWLEDGMENTS

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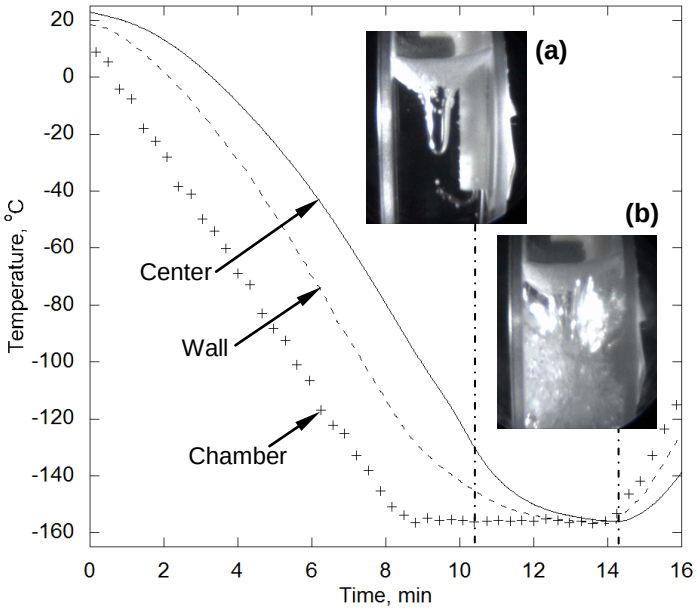

Figure 3. Typical results of vitrification for the CPA cocktail DP6: (a) a crystallized layer appears on the surface, while the remaining CPA is vitrified—the temperature at the center of the cuvette is -126°C, which is 5°C below Tg, and (b) fracture formation at the onset of rewarming—above T_{min}; the bottleneck formation at the center of the CPA is associated with thermal construction.

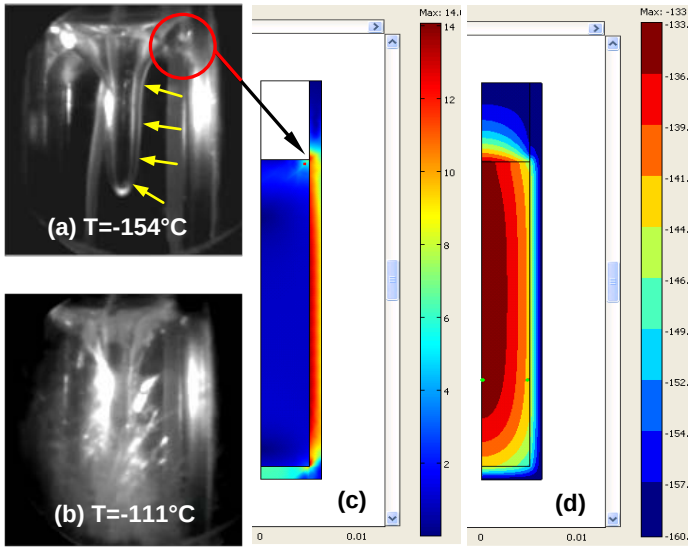

Figure 4. Representative results for 7.05M DMSO subject to 40°C/min cooling rate: (a) the vitrified (glassy) specimen appears transparent with a deformed surface due to thermal contraction, (b) fractures form at an advanced stage of rewarming, (c) results of mechanical stress analysis are consistent with the onset of fracturing, and (d) the corresponding temperature field.