Developing the Cryomacroscope for Cryopreservation Applications

Justin S.G. Feig, Alexander C. Williams, Christopher C. Lin, and Yoed Rabin*

Biothermal Technology Laboratory
Department of Mechanical Engineering
Carnegie Mellon University
Pittsburgh, PA 15213, United States
*rabin@cmu.edu

***Abstract*—This study concerns the development of a new device prototype to address the unmet need for visualization of physical effects associated with large-scale cryopreservation--the preservation of tissues at very low temperatures. An early prototype of this device has been presented in 2005, and was termed the *cryomacroscope*. The goal in the current study is to develop an advanced-stage prototype of the cryomacroscope, with the following design objectives: (1) to visualize and record the process of cryopreservation *in situ*, (2) to design the device as an add-on unit for commercially available controlled-rate freezers, (3) to enable scanning of large samples, (4) to enable identification of physical effects such as ice formation and fracturing, and (5) to integrate data into a single movie, including time, temperature, and camera position. Results of this study demonstrate glass formation, crystallization, and fracture formation during a typical cryopreservation protocol.**

I. Introduction

Although cryopreservation represents the only method for long-term preservation of biomaterials, techniques for successful cryopreservation are only available for single cells and small tissue structures. While many factors affect the outcome of a cryopreservation protocol, controlling ice formation is key, being the cornerstone of cryoinjury. Additional hazardous effects are toxicity of the applied cryoprotective agents (CPAs), and structural damage as a result of thermo-mechanical stress [1].

One of the most promising techniques for large-scale cryopreservation is known as vitrification, where crystal formation is suppressed and the biological material is trapped in a glassy-like state (*vitreous* in Latin means *glassy*) [2]. Vitrification is achieved by rapidly cooling a high concentration CPA solution, which experiences an exponential increase in viscosity with the decreasing temperature. The minimum cooling rate needed to promote vitrification may vary depending upon the CPA composition, and is typically in the range of 2.5°C/min to 40°C/min [2].

Since crystallization is a kinetic effect, vitrification is a path-dependent process, dependent upon the specific thermal history. Crystallization may take place during cooling or rewarming. Furthermore, rewarming-phase crystallization (RPC) can take place as either growing ice nuclei which formed during cooling (recrystallization), or formation and growth of new ice crystals (devitrification). Another potentially devastating effect during vitrification is fracture formation.

The high cooling rates used during vitrification may result in large temperature gradients, leading to a non-uniform thermal strain distribution, which can potentially induce thermal stresses. If the stress exceeds the strength of the vitrified material, fracture formation will follow [1], where even a single fracture may prevent functional recovery of the specimen [3]. A non-uniform thermal strain distribution can also be driven by mismatches in thermal expansion coefficients between different materials, such as the container and the CPA, and between the biological material and the surrounding CPA.

II. Experimental Setup

The system configuration has been presented elsewhere [4], and is discussed here in brief for the completeness of the report. With reference to Fig. 1, the scanning cryomacroscope consists of a CCD camera attached to a

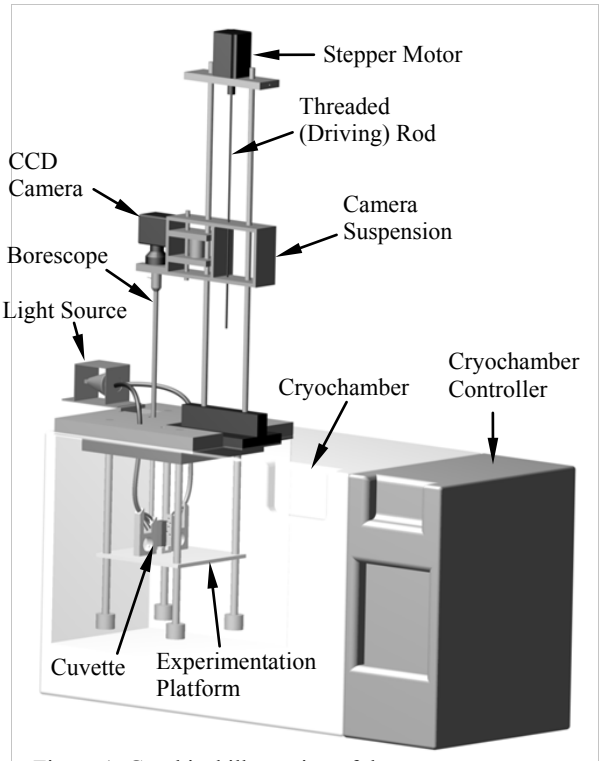
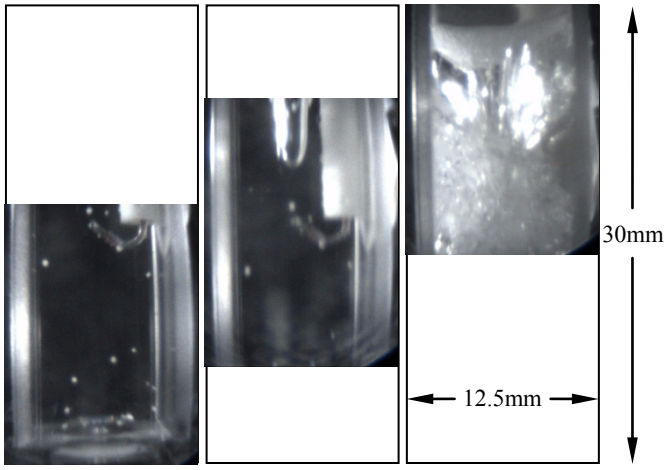


Figure 1: Graphical illustration of the cryomacroscope, integrated with a controlled-rate freezer [4].

vibration-damped carriage, and driven by a stepper motor. The image is transmitted to the camera via a rigid borescope. The sample is illuminated with fiber optic bundles, extending from an external light source. A data-acquisition device is used for temperature measurements using thermocouples. The sample container used in this study is a standard 4.5 mL cuvette, which is essentially a rectangular container having a viewing window of 12.5 mm × 12.5 mm × 30 mm; however, a large variety of containers can be used with the scanning cryomacroscope [2].

Beyond the achievement of gathering critical experimental data, the unique contribution of the current study is in the development of a specialized computer code designed to control scanning, record and compile video, post-process images, enable data acquisition, and provide an intuitive graphic user interface for the novice operator.



(a) 0h:14m:00s Tf = −155.9°C (b) 0h:14m:07s Tf = −156.1°C (c) 0h:14m:15s Tf = −156.0°C

Figure 3: Selected scanning results along a cuvette around the minimum temperature of a cryoprotocol during vitrification of DP6. Crystallization may develop as a volumetric effect—lower area in (a), or along surfaces—upper surface in (c). Fracture formation frequently appears at the onset of rewarming [1] and may occupy the entire volume (c); alternatively, it may be confined to specific areas (Fig. 4(b)).

III. Results and Discussion

Figure 3 displays typical scanning results on the CPA cocktail DP6, which has drawn significant attention in the cryobiology community. Figure 4 displays representative post-processed results from a cryoprotocol. Three typical effects are demonstrated in Figs. 3 and 4: (1) the CPA appears transparent well below the glass transition temperature (−119°C for DP6), indicating vitrification; (2) a large deformation at the CPA surface is observed, indicating the significant effects of thermal contraction; and, (3) even minor crystallization can be identified.

If the specimens shown in Figs. 3 and 4 were evaluated only at the storage temperature, which is common practice in cryopreservation, the sequence of the captured effects would be missed, which is critical in correlating tissue viability and functionality with the corresponding thermal history. Hence, the cryomacroscope represents a critical tool in evaluating the outcome of cryoprotocols.

Acknowledgements

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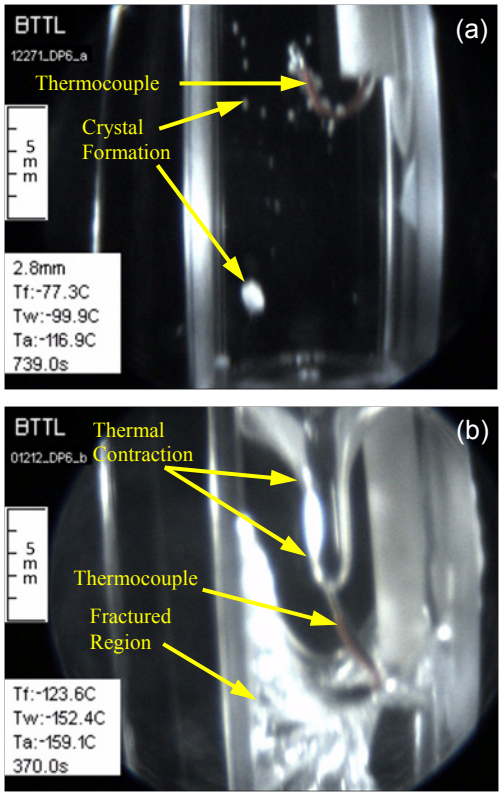

Figure 4: Post-processed results of vitrification for the cryoprotective cocktail DP6: (a) crystallization due to a sub-critical cooling rate, and (b) fractures in a vitrified specimen, which is 5°C below glass transition at the center (Tf) and 33°C below glass transition at the wall (Tw). The significant temperature gradient can drive thermal stresses, leading to fracture.