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Analytical model for residual stresses in polymeric containers during cryogenic storage of hematopoietic stem cells

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Abstract

Hematopoietic stem cell (HSC) therapy can significantly lower instances of infection in chemotherapy patients by accelerating the recovery of white blood cells in the body. However, therapy requires that HSCs be stored at cryogenic temperatures to retain the cells' ability to proliferate. Currently, cells are stored in polymeric blood bags that are subject to fracture at the extremely low storage temperatures, which leads to cell contamination, thereby reducing their effectiveness. Therefore, we have developed an analytical model to predict the accumulation of stresses that ultimately lead to crack initiation and bag fracture during cryogenic storage. Our model gives explicit relationships between stress state in the container and thermoelastic properties of the container material, container geometry, and environmental factors that include temperature of the system and pressure induced by excess gas evolving from the stored medium. Predictions based on the model are consistent with experimental observations of bag failures that occurred during cryogenic storage applications. Finally, the model can provide guidance in material selection and bag design to fabricate bags that will be less susceptible to fracture.

Keywords: Blood bags; Hematopoietic stem cells (HSC); Cryogenic storage; Failure model; Residual stresses; Thermal expansion mismatch; Gas evolution

1. Introduction

During chemotherapy, the number of white blood cells in the patient's body is dramatically reduced, which can lead to infection and even death. However, hematopoietic stem cell (HSC) therapy can significantly lower these adverse instances by promoting the recovery of a patient's white blood cell count [1]. During HSC therapy, the stem cells are extracted from the patient prior to the chemotherapy treatment and stored until after the treatment is completed. The cells are then reintroduced into the patient, and about a month later, the patient's white blood cell count returns to normal, significantly reducing the risk of infection.

One of the major problems with HSC therapy is the contamination of the cells, which can limit their effectiveness. Cell contamination occurs primarily during the storage phase of the therapy due to failure of the storage container. Currently, HSCs are stored in blood bags that have been developed, tested, and cleared by the FDA for use in storing cells with no nuclei such as red blood cells and platelets at 193 K [2]. However, previous studies have shown that nucleated cells such as HSCs have less ability to proliferate after thawing if stored above 113 K [3]. Therefore, hospitals began using blood bags to store HSCs at liquid nitrogen temperatures (~77 K), significantly below the approved storage temperature of the bags. The practice

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of using blood bags for HSC storage at these extremely low temperatures has resulted in numerous reports of blood bag fracture, resulting in contamination of the contents [4,5]. Depending on the bag material, failure rates of up to about 10% have been reported [5]. Of these failures, 42% ultimately resulted in cell contamination.

Blood bag failure at liquid nitrogen temperatures is typically characterized by brittle fracture [6,7]. At temperatures below the glass transition temperature (T_g), polymeric materials are stiff and have little ability to dissipate applied strain through viscous relaxation [4,8,9]. Thus, the application of strain to the polymer can result in a considerable accumulation of stress in the material. Stress gives rise to elastic strain energy that provides the driving force for the nucleation and propagation of cracks that lead to failure of the blood bag. Further, the magnitude of the accumulated stresses is sensitive to the geometry of the blood bag. Therefore, to formulate a predictive model for the failure of blood bags during the cryogenic storage of stem cells, it is critical to elucidate the origins of these stresses and to quantify the influence of bag material and geometry on the accumulated stresses and, thereby, fracture.

Two potential strain mechanisms that can give rise to residual stresses in these systems have been identified and are illustrated in Fig. 1. The first originates due to a thermal expansion mismatch between the frozen stem cell medium and its polymeric container. The thermal expansion coefficients of polymeric materials at cryogenic temperatures typically range from 3×10^{-5} to $8\times10^{-5}\,K^{-1}$ [9–11]. The frozen cell medium, however, will, on average, have a lower thermal expansion coefficient of approximately $2.5\times10^{-5}\,K^{-1}$ over the same temperature range, assuming the medium's behavior is analogous to that of ice [12]. Therefore, as the system is cooled, the blood bag will contract at a faster rate than the medium. Because the medium is elastically stiff, it does not yield to this contraction and significant residual stresses can accumulate in the blood bag. This is shown schematically in Fig. 1a.

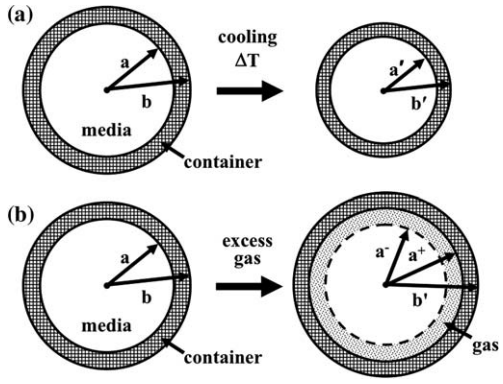

Fig. 1. Schematic representation of two potential mechanisms that can induce strain, and thereby residual stresses, in the polymeric container. These mechanisms are: (a) a temperature change when there is a thermal expansion mismatch between the container and medium and (b) excess gas evolving from the medium.

The second potential mechanism of residual stress generation derives from the presence of gases in the medium (private communication, Cullis HM, American Fluoroseal). The premise is that as the medium solidifies, dissolved gases, such as CO_2, segregate out of solution and form gas bubbles, which are trapped in the frozen medium. As the system is cooled to 77 K, these gases will solidify and leave evacuated voids within the frozen medium. During storage at 77 K, nitrogen diffuses through the polymer and the frozen medium into these voids. As the system is thawed, the condensed gases will return to a gaseous state, so the voids will contain the original amount of gas plus the nitrogen that migrated in during storage. The increased pressure within the voids will drive the nitrogen out of the frozen medium, and the "evolved" gas will build up a gas layer between the medium and the polymeric blood bag (see Fig. 1b), which is still brittle (i.e., below the glass transition temperature). This phenomenon will result in an increased pressure against the wall of the blood bag that gives rise to residual stresses.

It is not clear how these two mechanisms (contraction during freezing and gas expansion during thawing), individually or in combination, contribute to the overall stress state in blood bags during the cryogenic storage of stem cells. Therefore, it is the goal of this work to derive an analytical model for the stress state that evolves in blood bags based on these two mechanisms. The model will allow us to determine the relative effects of each mechanism and their variation with the type of blood bag material and geometric design.

In this paper, we describe the derivation and application of an analytical model for the stress state of blood bags filled with HSCs at cryogenic temperatures based on contributions from thermal expansion mismatch and evolved gases. In the next section, we give a detailed outline of the model and its derivation. The subsequent section describes results of the application of the model to elucidate the effects of strain mechanism, material, and bag geometry on the stress state. This is followed by a discussion of the results, and finally, a brief summary.

2. Materials and methods

2.1. Overview

The objective of this work was to develop an analytical model of the stress state in polymeric blood bags that arises due to strains induced from thermal expansion mismatch during freezing and/or excess gas evolution during thawing. During freezing and thawing of blood bags containing cell medium, the largest stresses, and therefore strain energies, accumulate at temperatures below the T_g of the polymer. At these temperatures, viscous stress relaxation mechanisms in the polymer are negligible. Further, above their T_g the polymer materials (elastomers) that comprise the bags are elastically soft and can accommodate very large strains (~300%) before yielding. Therefore, although