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Review

An update on methods for cryopreservation and thawing of hemopoietic stem cells

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ABSTRACT

The aim of this article is to review a number of variables that may affect the cryopreservation of minimally manipulated products containing allogeneic or autologous hemopoietic progenitor cells (HPC) used for transplantation, with particular reference to processing, type and addition of cryoprotectant, cell concentration, volume, freezing procedure, cooling rate, storage, thawing, and quality management. After defining final product's requirements in compliance with norms, laws and regulations, it is crucial to define the critical control points of the process.

New approaches of processing were developed in the last few years such as automatic devices for volume reduction and high cell concentration in the frozen product. DMSO at 10% final concentration is still the most used cryoprotectant for HPC cryopreservation. Although controlled rate freezing is the recommended method for HPC cryopreservation, alternative methods may be used. Last generation vapor storage vessels ensure temperature stability better than older tanks. Their use may reduce risks of cross-contamination.

Finally we review advantages and disadvantages of thawing procedures that may be carried out in the laboratory or at the patient's bedside.

Contents

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1. Introduction

Transplantation of allogeneic or autologous hemopoietic progenitor cells (HPC) is a consolidated procedure to reconstitute bone marrow after myeloablative chemotherapy in many hematological diseases. HPC for clinical use can be collected either from bone marrow [HPC(M)], from peripheral blood after mobilization [HPC(A)], or from umbilical cord blood [HPC(CB)] [1,2]. In the allogeneic setting, the collection of HPC (M) and HPC(A) can be scheduled in order to nearly coincide with their use, so that cryopreservation is infrequently required. On the contrary, in the autologous setting and for unrelated HPC(CB), products must be cryopreserved to allow therapeutic use several weeks, months or years after collection [3]. The aim of cryopreservation is to preserve the therapeutic properties of HPC, but the freezing and thawing procedures determine an alteration of the cellular osmosis which can cause cell injury [4,5].

In spite of long experience of HPC processing facilities, consensus is lacking on a universally accepted method for HPC cryopreservation and on maximum time of cryopreserved storage [6,7]. Accordingly, each facility must develop its own protocol and implement validated processes under a quality system compliant with pertinent standards, norms and regulations developed by professional organizations and competent authorities.

In this article we review a number of variables that may affect the cryopreservation of minimally manipulated products containing HPC, as defined in the NetCord-FACT International Standards for Cord Blood Collection, Banking, and Release for Administration [8], and in the FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing and Administration [9], with particular reference to processing, type and addition of cryoprotectant, cell concentration, volume, freezing procedure, cooling rate, storage, thawing, and quality management.

1.1. Regulations and standards

Each processing facility is responsible to determine which norms, laws and regulations are applicable. A large number of regulations were developed in the last 15 years. Good Manufacturing Practices (GMP), a well established quality assurance system used since a long time by pharmaceutical manufacturers, must now be used also in the manufacture of HPC for clinical use. The US Food and Drug Administration (FDA) included the GMP into the Good Tissue Practice under the Federal Regulations' codes. Europe incorporated GMP in EU Directive 2003/94/EC and daughter norms. In the US, HPC processing requires an IND (Investigational New Drug) or a BLA (Biologics License Application). Cord Blood Banks operating outside the US need to qualify under an IND to export cord blood units to the US or to affiliate with a US organization covered by an existing IND. The US National Marrow Donor Program (NMDP) qualifies the cord blood banks willing to operate under its IND. Cord Blood Banks must renew annually a registration with the FDA, and cord blood unit shipment to the US is authorized only if a US agent has been identified.

A large number of countries developed national regulations on minimally manipulated HPC. In the European Union (EU), each member state may develop additional regulations based on those developed at the EU level. A GMP license is required for the production in countries where HPC are classified as a medicinal product. Relevant governmental authorities include the following: in the US, the Office of Human Research Protection under the Department of Health and Human Services (HHS) and/or the FDA; in Canada, Health Canada; and in Australia the Therapeutic Goods Administration. Table 1 reports a non comprehensive list of current regulations for the management of minimally manipulated HPC. Specific standards and accreditation programs have been implemented by professional organizations such as the AABB (currently named Advancing Transfusion and Cellular Therapies Worldwide, formerly named American Association of Blood Banks) and the Foundation for Accreditation of Cellular Therapy (FACT). With regard to the issues discussed in this review, we have mainly focused on the NetCord-FACT International Standards for Cord Blood Collection, Banking, and Release for Administration [8], and the FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing and Administration [9].

1.2. Process design

The implementation of the cryopreservation process requires knowledge of freezing principles and of the characteristics of the products to be frozen, definition of predetermined final product's requirements, definition of steps starting from collection to infusion, identification of critical control points, control and review of the process, and analysis of results for continuing process improvement. Furthermore, knowledge of the scientific literature and of local, national and international norms and rules is of utmost importance [3,8–12]. HPC donor's requirements, required tests and results have been identified for each type of HPC and are used to determine safety, purity, potency, and identity of the product [8,9].

Fig. 1 describes the steps of the cryopreservation process for HPC(A), HPC(CB), and HPC(M) under minimal manipulation conditions. It is crucial to define the critical control points of the process, as the effectiveness and safety of the products may be compromised. Each processing facility and/or cord blood bank should identify the critical control points of its process.

Table 2 reports the main critical points and the corresponding controls that we developed for HPC(CB).

Those developed for HPC(A) and HPC(M) are as follows: 1) one product at a time is processed both inside and outside the biological safety cabinet to prevent mix up; 2) the