Chapter 3

An Optimised Method for Cryopreservation of Human Hepatocytes

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Abstract

Successful cryopreservation of hepatocytes is essential for their use in hepatocyte transplantation. Cryopreservation allows hepatocytes to be available for emergency treatment of acute liver failure and also for planned treatment of liver-based metabolic disorders. In addition, cryopreservation of human hepatocytes can facilitate their use in metabolism and toxicity studies. Cryopreservation can adversely affect the viability and function, especially reduce the attachment efficiency, of hepatocytes on thawing.

The cryopreservation process can be divided into steps so that improvements can be made on the ‘standard’ protocols that are followed in some laboratories. These steps are as follows: pre-incubation of cells; freezing solution, cryoprotectants and cytoprotectants; freezing process; storage; thawing; post-thawing culture. This chapter presents an optimised protocol for cryopreservation of human hepatocytes as developed at King’s College Hospital.

Key words: Human hepatocytes, cryopreservation, freezing, hepatocyte function, UW solution, glucose, fructose.

1. Introduction

Human hepatocyte preparations are limited by a lack of human tissue. Sources (from rejected or unused donor tissue or from liver resection tissue) are limited, erratic and unpredictable. However, when tissue is available, often large numbers of cells can be isolated. The problem is that usually not all the cells can be used immediately and hepatocytes do not proliferate in vitro (1). Therefore, a reliable method for preserving hepatocytes is essential. Currently, the only method for long-term preservation of cells is cryopreservation.

Hepatocyte cryopreservation was first fully investigated and published in the 1980s (2, 3). Since then cryopreservation protocols
have been published for hepatocytes from a variety of animal species, including rat (4, 5), pig (6, 7), mouse (8, 9), monkey (10, 11) and dog (12, 13). Optimised human hepatocyte cryopreservation protocols are fewer, presumably due to the limitation of human tissue to prepare hepatocytes for experiments, but there are still a large number of published human protocols (3, 14–22). Even with the best of these protocols, there is still a significant loss of function and this is related to the quality of the fresh cells and the type and nature of the liver tissue from which they were isolated (23). The state of the art of cryopreservation for hepatocyte transplantation has recently been reviewed (24).

2. Materials

2.1. Pre-incubation
1. William’s E Medium (WEM, Sigma-Aldrich Company Ltd., Gillingham, Dorset, UK) is prepared with the following additions: penicillin (50 U/ml, Life Technologies Ltd., Paisley, Scotland, UK) and streptomycin (50 µg/ml, Life Technolo-
gies Ltd.), L-glutamine (2 mM, Life Technologies Ltd.) and 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES, 100 µM, Sigma-Aldrich Company Ltd.).
2. Heat-inactivated foetal calf serum (FCS, Life Technologies Ltd.).
3. Falcon tubes – 50 ml sterile conical bottom (BD Biosciences, Cowley, Oxfordshire, UK).
4. Glucose, fructose, α-lipoic acid (Sigma-Aldrich Company Ltd.).

2.2. Freezing Solution
1. University of Wisconsin (UW) solution (Bristol-Myers Squibb Pharma Ltd., Hounslow, UK).
2. Dimethyl sulphoxide (DMSO, Sigma-Aldrich Company Ltd.).

2.3. Cryopreservation Process
1. Kryo 10 Controlled Rate Freezer (CRF), Series III (Planer Products Ltd., Middlesex, UK).
2. Cryotubes (5 ml, Nunc Nalgene, Hereford, UK).

2.4. Storage
1. –140°C freezer (Lab Impex Research Ltd., East Sussex, UK).

2.5. Thawing
1. Waterbath (Model JB2, Grant Instruments (Cambridge) Ltd., Royston, Hertfordshire, UK).

2.6. Culture and In Vitro Cell Assays
1. Trypan blue solution (0.4%, Sigma-Aldrich Company Ltd.).
2. Collagen-coated (Biocoat™) flat-bottom 96-well culture plates (BD Biosciences).
3. Culture media consists of phenol red-free WEM with the additions in Section 2.1, Point 1, and 5% (v/v) FCS.