Fractionation of Hepatic Nonparenchymal Cells

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The majority of parenchymal cells from mammalian liver cells can be removed by very low speed centrifugation (50 g) but a simple low-density barrier (1.096 g/ml) is required to remove the remaining parenchymal cells from the 50-g supernatant which contains all of the lower density nonparenchymal cells. Continuous gradients of Nycodenz® can provide satisfactory resolution of Kupffer, stellate, and endothelial cells on an analytical basis but the separation of different cell types is not sufficient preparatively. Flotation through a low-density iodixanol barrier can, however, provide a satisfactory enrichment of the least dense nonparenchymal cell – the stellate cells.

KEY WORDS: liver cells, hepatocytes, parenchymal cells, nonparenchymal cells, stellate cells, Kupffer cells, OptiPrep™, iodixanol, density barrier

DOMAINS: cell biology, immunology, endocrinology, clinical medicine, medical research, methods and protocols

METHOD TYPE: extraction, isolation, purification and separation

SUB METHOD TYPE: centrifugation

INTRODUCTION

The fractionation of hepatic nonparenchymal (sinusoidal) cells (Kupffer cells, stellate cells, endothelial cells, etc.) on continuous metrizamide[1] and Nycodenz® gradients[2] has shown that the overlap in banding densities of these cells makes these gradients generally unsatisfactory as a stand-alone procedure to isolate the various cell populations.

Discontinuous gradients of iodinated media are widely used to provide a means of removing residual erythrocytes and parenchymal cell debris from nonparenchymal cell preparations and to prepare a stellate cell-rich fraction.

Preparation of Nonparenchymal Cells

Parenchymal cells are routinely prepared by collagenase digestion of the liver using a tissue perfusion system. These cells are then separated from the nonparenchymal cells by differential pelleting at 50 g for 1–4 min. Although the nonparenchymal cells can be isolated from the 50-g supernatant, the yields are usually low. The most widely used procedure is to perfuse the liver with a mixture of collagenase and Pronase or endotoxin to destroy the parenchymal cells selectively (see Refs. 1 and 2 for details).

Stellate Cells

These are the least dense of the nonparenchymal cells and can be floated effectively away from the rest of the cells. The low-density fraction may contain almost 80% stellate cells[2]. The actual density of the low-density layer used by different workers varies somewhat and some of these variations are presented in the Notes section

The following methods are adapted from Bøyum et al.[1] and from Brouwer et al.[2]. The advantage of using OptiPrep™ rather than Nycodenz® is that the density solutions can be made up by dilution of OptiPrep™ directly with Gey's Balanced Salt Solution (GBSS), while Nycodenz® must be diluted with GBSS minus the NaCl to keep the osmolality below 300 mOsm.

MATERIALS AND EQUIPMENT

- Gey's Balanced Salt Solution (GBSS): 7.0 g NaCl, 0.37 g KCl, 70 mg MgSO₄·7H₂O, 150 mg Na₂HPO₄·2H₂O, 220 mg CaCl₂·2H₂O, 2.27 g NaHCO₃, 30 mg KH₂PO₄, 210 mg MgCl₂·6H₂O, 1.0 g glucose dissolved in 1 l of water, gassed with 5% CO₂/air; the pH should be 7.4
- OptiPrep™ (60% w/v, iodixanol)
- 40% (w/v) Iodixanol Working Solution (WS): mix 4 vol of OptiPrep™ and 2 vol of GBSS
- Plastic conical centrifuge tubes (50 ml)
- Plastic Pasteur pipette for overlaying
- Low-speed (temperature-controlled) centrifuge with swinging-bucket rotor

METHODS

Preparation of Nonparenchymal Cells

1. Suspend the crude nonparenchymal cells in approx. 10 ml of GBSS ($1-4 \times 10^8$ cells).
2. Add WS to the cell suspension so that the final concentration of iodixanol is 17% (w/v) iodixanol solution ($\rho = 1.096$ g/ml).
3. Mix thoroughly but gently.
4. Layer approx. 2 ml of GBSS on top and centrifuge at 400 g for 15 min. at 20°C.
5. Allow the rotor to decelerate without the brake.
6. Collect the cells, which band at the interface between the GBSS and the 17% iodixanol.

FIGURE 1. Isolation of hepatic stellate cells by flotation. GBSS = Gey's Balanced Salt Solution.

STELLATE CELLS

1. Add WS to the cell suspension as described in steps 1–3 above.
2. Dilute W with GBSS to produce a solution containing 11.5% (w/v) iodixanol (see Note 1).
3. Layer 5 ml of this solution over the same volume of cell suspension (in 17% iodixanol); then layer 2 ml of GBSS on top (see Note 2).
4. Centrifuge at 1400 g for 17 min. at 20°C; allow the rotor to decelerate without the brake.
5. Collect the cells, which band at the interface between the GBSS and the 11.5% iodixanol (see Fig. 1).

NOTES

1. This concentration of iodinated density gradient medium is equivalent to approx 1.067 g/ml and has been used both with Nycodenz®[1,2] and iodixanol[3,4]. A lower-density concentration (1.053 g/ml) was used by Cassiman et al.[5]. This is equivalent to approx. 9% (w/v) iodixanol.
2. In the method as described by Brouwer et al.[2], the cell suspension was placed in the top layer ($\rho = 1.067$ g/ml) rather than the bottom layer ($\rho = 1.096$ g/ml) and this is an alternative strategy.

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