OptiPrep™ Application Sheet C33

Preparation of stellate cells from liver and pancreas

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **The Optiprep™ Mini-Review MC08 "Hepatic non-parenchymal cells"** compares some of the methodologies for resolving stellate, Kupffer and endothelial cells
- **MC09 "Hepatic and pancreatic stellate cells – a bibliography"** provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™ for the isolation of these cells
- **To access these Mini-Reviews** return to the initial list of Folders and select "Mini-Reviews"
- **To access other Application Sheets** referred to in the text return to the Cell Index; key Ctrl "F" and type the C-Number in the Find Box

1. Background

Hepatic stellate cells represent up to 15% of the total liver cells and their ability to transdifferentiate into myofibroblast-like cells is regarded as a key event in hepatic fibrosis. There is therefore considerable clinical research interest in the isolation of these cells; after blood leukocytes and dendritic cells they are perhaps the most widely studied of any mammalian cell. Stellate (sometimes called fat-storing or Ito) cells are the least dense of the non-parenchymal cells (NPC) and unlike the other NPC (Kupffer cells and endothelial cells), they can be effectively purified to 90-95% purity using a simple density barrier or two-step discontinuous gradient. The latter is widely used in studies requiring purification of both stellate and Kupffer cells and is described **in "Hepatic cells" Application Sheet C24 – see index**. Pancreatic stellate cells also mediate fibrosis in chronic pancreatitis and are also the least dense cell in the tissue; very similar gradient strategies are therefore used in their isolation.

2. Preparation of the cell suspension

The detailed methodology in this Application Sheet is confined to the density barrier separation; methods for preparing the tissue cell digests, which are probably well established in the laboratory, are merely summarized below.

2a. Liver digestion

Parenchymal cells are routinely prepared by collagenase digestion of the liver using a tissue perfusion system. These cells are then separated from the non-parenchymal cells by differential pelleting at 50 *g* for 1-4min. Although the non-parenchymal 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 enterotoxin to destroy the parenchymal cells selectively [1,2].

2b. Pancreas digestion

The preparation of cell suspensions from pancreas follows more traditional lines of digesting the dissected and finely minced tissue with Pronase and collagenase at 37°C for 30 min before filtering through a nylon on stainless steel mesh [3].

2c. Cell suspension

The cells are normally pelleted from the crude suspension and maybe washed once or twice prior to density barrier separation in order to remove any residual enzymes and/or endotoxin. Deoxyribonuclease I may also be added to degrade any DNA released from damaged cells, which would otherwise cause aggregation of the cells. Cells are suspended in an isoosmotic salt solution; either a general-purpose medium such as Hanks Balanced Salt Solution (HBSS), which may be supplemented with Ca^{2+} if required, or a customized medium: Gey's Balanced Salt Solution (GBSS) is routinely used for hepatic cells.

3. Gradient selection

Nycodenz®

Since 1986 Nycodenz® has been widely used for the purification of hepatic stellate cells. One of the simplest methods, which was first described in 1987 by Schäfer et al [4] involved the addition of an isoosmotic solution of 28.7% (w/v) Nycodenz® to a suspension of NPC in order to raise its density to approx 1.072 g/ml (13.2% Nycodenz®). A layer of balanced salt solution is layered on top and after the centrifugation stellate cells are recovered from just above the interface. Subsequently Gressner and Zerbe [5] reduced the density to approx 1.049 g/ml (8.2% Nycodenz®) to improve the purity and the latter has been widely used (e.g. refs 6-9). The final concentration of Nycodenz® in the cell suspension may also be as high as 14.35% [10]. In some cases the NPC suspension is layered over a Nycodenz® cushion, which may be 13% [11], 9% [12] or 8.2% [13]. In few instances the crude cell suspension is layered on a discontinuous gradient of 8.2% and 15.6 % (w/v) Nycodenz® [14,15] but this gradient format is normally reserved for the purification of stellate cells and Kupffer cells.

The purification of pancreatic stellate cells using Nycodenz® is almost exclusively carried out according to the methods for hepatic cells [4,5] and was first described by Apte et al [16]. Generally the concentration to which the crude cell suspension is adjusted is approx. 11.4% (w/v) [e.g. refs 16-19] but there are variations: 12% [20], 13.2% [21] and 15.1% [22].

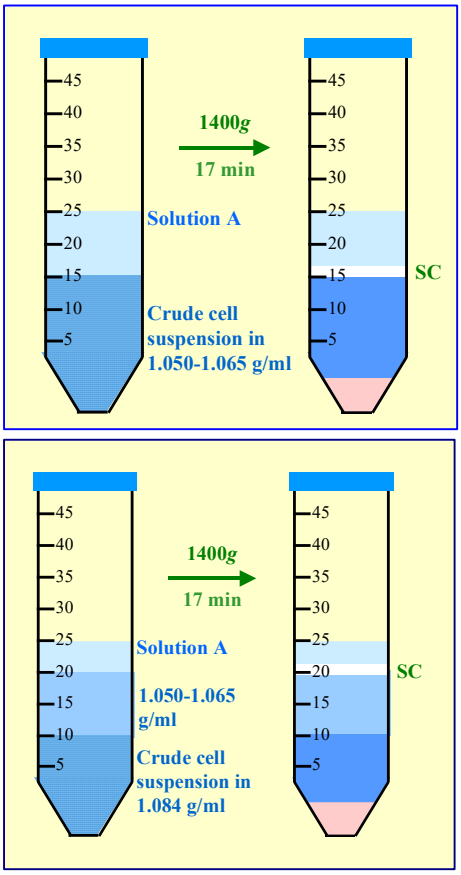

Figure 1 (top): Isolation of stellate cells by Stategy A
Figure 2: (bottom) Isolation of stellate cells by Strategy B

OptiPrep™

Since 1998 OptiPrep™ has also been used for the isolation of stellate cells from both liver and pancreas; Bachem et al [23] published the first paper in which the cell suspension was layered over a density barrier and Peterson and Rowden [24] used a multi-step discontinuous gradient of 5%, 10%, 20% and 25% iodixanol. The strategy of adjusting the density of the NPC suspension to a density just higher than that of the stellate cells (as with Nycodenz®) has also become popular. Also as with Nycodenz®, the final density of the liquid has tended to be reduced, examples as low as 1.045 g/ml (equivalent to an iodixanol concentration of 7.2% w/v) have been reported [3].

4. Methodological options

Because of the ease of use of OptiPrep™ the following methodology is based on the use of this medium. The preparation of a suitable Nycodenz® stock solution and its use are however given in Section 7. Only the flotation strategy is given, since this is well established as the method of choice in the isolation of the least dense particle from a mixture of predominantly denser particles. A variant of the regular flotation method (Strategy A) is given in Strategy B, in which the low-density resolving solution is layered upon the NPC suspension adjusted to 1.084 g/m/l (see Figures 1 and 2).

Strategy 2 is the preferred one since the stellate cells are separated from the sample by a “clean” resolving layer; they are thus completely divorced from the denser cells and any residual soluble components such as the digesting enzymes and any cytoplasmic material released from broken cells. This strategy is widely used in the purification of pancreatic islets and of dendritic cells from mouse spleen, thymus and lymph nodes.

The following methods are adapted from refs 2 and 3.

GBSS: Dissolve the following in 500 ml of water:
7.0 g NaCl,
0.37 g KCl,
70 mg $MgSO_4.7H_2O$
150 mg $Na_2HPO_4.2H_2O$
220 mg $CaCl_2.2H_2O$
2.27 g $NaHCO_3$
30 mg KH_2PO_4
210 mg $MgCl_2.6H_2O$
1.0 g glucose

Make up to 1 liter with water and gas with 5% CO_2/air, pH should be 7.4.

5. Solutions required

A. Gey's balanced salt solution (GBSS) or Hank's Balanced Salt Solution, with or without Ca^{2+}/Mg^{2+} (see Note 1).

B. OptiPrep™ (shake the bottle gently before use)

C. Iodixanol (40% (w/v)) working solution: mix 4 vol. of Solution B and 2 vol. of Solution A (see Note 2).

6. Protocol

Carry out all operations at 4°C.

6b. Strategy A

1. Mix Solution C with Solution A so that the final concentration of iodixanol is 8.0-11.5% (w/v) iodixanol solution (ρ = 1.050-1.065 g/ml) and use this to suspend the final washed cell pellet. Alternatively suspend the cells in Solution A and mix with Solution C to produce an 8.0-11.5% iodixanol suspension (see Note 3).

2. Transfer 10-20 ml to a centrifuge tube and layer 8-10 ml of Solution A on top (see Note 4).

3. Centrifuge at 1400 *g* for 15-20 min; allow the rotor to decelerate without the brake (see Note 5).

4. Collect the cells, which band at the interface between Solution A and the sample (see Figure 1).

6c. Strategy B

1. Mix Solution C with Solution A so that the final concentration of iodixanol is 15% (w/v) iodixanol solution (ρ = 1.084 g/ml) and use this to suspend the final washed cell pellet (see Notes 6 and 7).

2. Dilute Solution C with Solution A to produce a solution containing 8.0-11.5% (w/v) iodixanol (ρ = 1.050-1.065 g/ml) (see Note 3).

3. Layer 5-10 ml of this solution over the same volume of cell suspension (in 15% iodixanol); then layer approx 5 ml of Solution A on top (see Note 8).

4. Centrifuge at 1400 *g* for 15-20 min; allow the rotor to decelerate without the brake (see Note 5).

5. Collect the cells, which band at the interface between Solution A and the low-density barrier (see Figure 2).

7. Notes

1. Any medium, compatible with the cells, may be used. For more information about the preparation of density solutions for cells **see Application Sheet C01**.
2. Nycodenz® stock solution option: Make up Solution A **without the NaCl.** Place 50 ml of this in a 150 ml beaker on a heated magnetic stirrer set at approx. 50°C and add 28.7 g of Nycodenz® powder in small amounts until dissolved. Allow the solution to cool to room temperature and then make up to 100 ml with Solution A (minus NaCl). Filter sterilize if required. Dilute it with Solution A (complete) to produce solutions of the same concentration as the iodixanol solutions described in either Strategy A or B.
3. Use whichever concentration of iodixanol (or Nycodenz®) that provides the optimal recovery and purity of stellate cells.
4. Alternatively layer the cell suspension under Solution A using a syringe and metal cannula.
5. Use of the brake causes vortex formation in the liquid and mixing of the contents.

6. The actual density of this cell layer is not particularly critical, so long as it is dense enough to support the low-density solution.
7. In the two-layer method described by Brouwer et al [2] and others, the cell suspension was placed in the low-density layer.
8. Alternatively layer the cell suspension under the lower density solution using a syringe and metal cannula.

8. References

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