OptiPrep™ Mini-Review MC08

Hepatic non-parenchymal (stellate, Kupffer and endothelial) cells – a short methodological survey

- This Mini-Review provides a review of the OptiPrep™density gradient methods for purification of the three main types of non-parenchymal cells (stellate, Kupffer and sinusoidal endothelial).
- The companion **Mini-Reviews MC09** and **MC10** provide a complete bibliography of publications that report the use of OptiPrep™. **MC09** is devoted entirely to stellate cells, while **MC10** provides similar information for both Kupffer and sinusoidal endothelial cells. **MC10** also covers, non-parenchymal epithelial cells, NK cells, oval cells and progenitor cells.

1. Total non-parenchymal cell fraction

An essential preliminary procedure in the isolation of the total non-parenchymal cells (NPC) from liver is the disaggregation of the liver tissue by enzymic perfusion. The aim of collagenase digestion (Method 1) of the liver is to release both NPC and parenchymal cells (PC) as intact cells. In a modified perfusion strategy, the liver is perfused with a mixture of collagenase and Pronase or *Clostridium perfringens* enterotoxin (Method 2), which destroys the PC selectively [1,2].

In Method 1 the bulk of the more rapidly sedimenting PC may then separated from the NPC by repeated differential pelleting at 50 *g* for 1-4 min. Although this method is simple, NPC yield is usually low. It is both more common and more effective to carry out the 50 *g* centrifugation once; to harvest all the cells from the supernatant by centrifugation at a higher *g*-force and then use a density barrier (prepared from OptiPrep™) to resolve the two types of cell. A common approach is to adjust the density of the cell suspension to approx 1.071 g/ml; this allows the NPC to float to the top and the PC and residual erythrocytes to pellet during the centrifugation [3,4]. In Method 2, the digest is often adjusted to a higher density (approx. 1.096 g/ml) to allow the NPC to float to the top. A recent detailed procedure described the use of equal volumes (20 ml) of 8.2% and 17.6% (w/v) iodixanol [5]. The NPCs are suspended in the denser layer. After centrifugation at 1400 *g* for 30 min, the top 15ml was discarded and the NPCs recovered in from the interface in the remaining low density solution.

- Detailed methods to purify a total NPC fraction are described in OptiPrep™ Application Sheet C24; this can be found on the OptiPrep™ Applications flash-drive or accessed via the following website www.axis-shield-density-gradient-media.com.
- A total NPC fraction is often a starting point for purification of Kupffer cells and/or endothelial cells by non-density gradient methods (adherence to a substratum or centrifugal elutriation).
- Stellate cells possess a distinctively low density and are successfully purified simply by modifying the format of the NPC flotation gradient (see Section 5).

2. Kupffer cells

The discontinuous gradient used to prepare the initial NPC fraction varies considerably; for example flotation through a 1.080-1.090 g/ml layer [6] or sedimentation on a two-layer gradient of 1.066 and 1.097 g/ml [7,8]. Further purification is then achieved by suspension of the interfacial cells in DMEM supplemented with 10% fetal bovine serum and 1% L-glutamine (plus the usual antibiotics) and adherence to culture dishes coated with coated with 2.5% glutaraldehyde-fixed BSA [9] or to non-collagen coated plates [10] or by elutriation [7]. The adherence method can provide a greater than 90% purity for Kupffer cells [11].

- More information about the density gradient systems may be obtained from OptiPrep™ Application Sheet C47; this can be found on the OptiPrep™ Applications CD or accessed via the following website www.axis-shield-density-gradient-media.com. Click on "Methodology" then "Mammalian and non-mammalian cells" and follow the links from the Index.

- A recent paper [12] reported the use of a more sophisticated gradient that avoided the need for the subsequent elutriation. In a five layer iodixanol gradient of 24%, 17%, 11.5%, 8.4% and 0% (w/v) iodixanol (total NPCs in the 24% layer) the Kupffer cells banded at the 8.4%/11.5% interface.

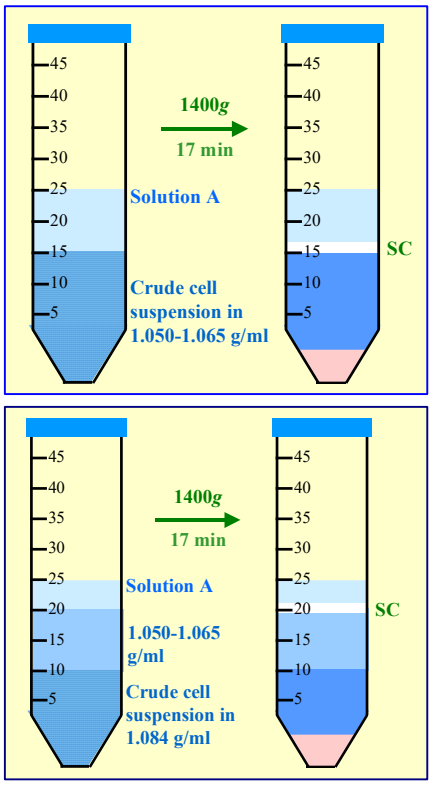
3. Sinusoidal endothelial cells

Generally the gradients used for the NPC isolation are similar to those described in Sections 1 and 2, Further purification is then achieved by suspension of the cells in DMEM supplemented with 10% fetal bovine serum and 1% L-glutamine (plus the usual antibiotics) and adherence to culture dishes coated with human fibronectin (1 mg/cm^2) [9], by elutriation [3,4], using magnetic beads [13] or by flow cytometry [14].

4 Progenitor cells

Grozdanov et al [15] harvested the NPC from the interface between a two-layer 1.063 and 1.079 g/ml gradient and progenitor cells were subsequently identified by flow cytometry.

- More information about the density gradient systems for the isolation of progenitor cells from a variety of tissues may be obtained from OptiPrep™ Application Sheets C23 and C48; this can be found on the OptiPrep™ Applications CD or accessed via the following website www.axis-shield-density-gradient-media.com. Click on "Methodology" then "Mammalian and non-mammalian cells" and follow the links from the Index.



5. Stellate cells

Stellate cells are perhaps the most widely studied type of hepatic cell, particularly with regard to their differentiation into myofibroblasts that occurs in liver fibrosis. Being the least dense of the NPC, these cells are regularly isolated by flotation from a dense medium. The method described by Borouwer et al [2] initially used Nycodenz®, but it was subsequently adapted to iodixanol. Of the two methods described in Figure 1, the format described in the lower panel has the advantage of banding the stellate cells at an interface separated from any unpelleted residual material originally present in the sample layer.

- For more information about the methods for the isolation of stellate cells from liver and pancreas may be obtained from OptiPrep™ Application Sheet C33; this can be found on the OptiPrep™ Applications CD or accessed via the following website www.axis-shield-density-gradient-media.com. Click on "Methodology" then "Mammalian and non-mammalian cells" and follow the links from the Index.

Figure 1 Separation of stellate cells by flotation
Solution A is the solution used to suspend the stellate cells (SC). See text for more information

6 References

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