OptiPrep™ Mini-Review MC11

Isolation of pancreatic stellate cells

- This Mini-Review primarily provides a complete bibliography of publications that report the use of OptiPrep™ for the purification of pancreatic stellate cells.
- **OptiPrep™ Application Sheet (C33)** describes the methods available for the purification these cells. It may be accessed from the OptiPrep™ Applications flash-drive or from the following website: www.axis-shield-density-gradient-media.com
- This Mini-Review provides short methodological review with a full bibliography.

Pancreatic tissue has been obtained principally from rats, but also from mice and human sources. In the latter case tissue was obtained from patients undergoing resection for malignancy or during the course of surgical treatment for chronic pancreatitis [1]. In its simplest form the disaggregation procedure involves incubation of the finely minced tissue with collagenase, sometimes with pronase, sometimes additionally DNase I is included or added subsequently. With rat tissue, a solution of collagenase (1 mg/5 ml) may first be infused into the pancreas *in situ* via the biliopancreatic duct or thoracic aorta. Subsequently the tissue may be removed and shaken at 37°C for 15 min, prior to further treatment. With or without the infusion, the excised pancreas is often finely minced with scissors and then treated, with collagenase P; for mouse concentrations of 0.03-0.05%, for rat 0.025-0.05%. Concentrations of pronase, if used, are 0.02-0.05% for rat and 0.1% for mouse. DNase I concentrations vary from 0.025% to 0.1% which may be included in the incubation with collagenase or added after a filtration step. Filtration is normally carried out through a nylon mesh. The enzymic digestions are regularly executed at 37°C. For more information on these procedures see refs 1-14.

Occasionally a more elaborate pre-gradient treatment is employed. Kaku et al [15] digested the washed pancreas with 0.12% collagenase for 20 min with shaking; subsequently the digested pancreas was suspended in 10 ml of medium supplemented with 1% BSA and 2 mM EDTA and agitated for 3 min at 37°C; this was repeated three times. A second digestion was carried out with collagenase, α-chymotrypsin, hyaluronidase and DNase I.

2. Density gradient

Occasionally the cells are simply layered on top of a density barrier and the cells recovered from the interface [1-3]. The most commonly used method however is to suspend the cells in culture medium or balanced salt solution (e.g. HBSS) containing 12% (v/v) OptiPrep™; a small volume of medium or HBSS layered on top and then centrifuged at approx. 1,000-1,400 *g* for 10-30 min [4-13]; stellate cells are recovered from the interface. Sometimes they are suspended in 15% or 17% OptiPrep™ and overlaid with 11.5 or 12.0% OptiPrep™ [14-15], see Figure 1. In the simpler of the two strategies, i.e. suspending the cells in 12% (v/v) OptiPrep™ beneath HBSS (upper part), the stellate cells that float to the interface are essentially adjacent to the original cell suspension layer. This will contain residual disaggregating enzymes, partially broken cells and other pancreatic cells, which do not sediment. In the alternative strategy in which the cells are adjusted to 17% OptiPrep™ and 12% OptiPrep™ layered on top, the stellate cells are separated from the load zone by a clean layer of 12% OptiPrep™ (lower part of Figure 1). It is thus easier to aspirate the stellate cells free from extraneous material by using this two-layer gradient format.

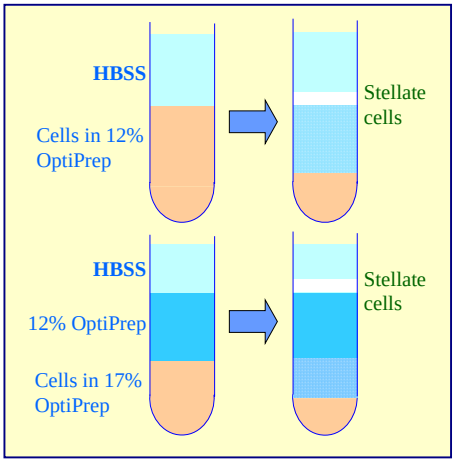

Figure 1: Two flotation strategies for purification of pancreatic stellate cells (for details see text).

3. References

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