Automatic Device for the Trypsinization of Animal Tissues

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Using a simple glass device, enzymatically released monodisperse cells can be separated and isolated from tissue fragments by means of a discontinuous fluid velocity gradient.

The enzymatic release of monodisperse cells from tissue fragments historically has its origin with the early work of Rous and Jones (9). Discontinuous batch methods were developed later (3, 4, 6, 12) and are still in widespread use. Because of the inherent problems in batch methods of trypsinization, continuous operating systems of varying degrees of sophistication have been developed (1, 2, 5, 8). The continuous methods of trypsinization obviate to some degree overdigestion due to prolonged mean residence time (MRT) of monodisperse cells in the enzyme solution. Those devices which rely upon a sintered-glass filter for cell sizing are easily clogged (1, 7, 8), whereas other devices do not minimize MRT (2) or are so complex mechanically that their construction is not feasible in most laboratories (5).

The device consists of a single Pyrex reactor which can be constructed with or without a water jacket (Fig. 1). The inner cylinder is 38 mm (OD), whereas the water jacket is 160 mm (OD). Reactor volume is approximately 75 ml. A 38-mm stainless-steel closure is used when the trypsin is dissolved in a N'-2-hydroxyethylpiperazine-N'-2'-ethane sulfonic acid (HEPES)-buffered salt solution (10). When a CO$_2$-bicarbonate-buffered salt base is used, a rubber stopper is substituted for the metal closure.

Operationally, three velocity patterns of fluid movement are involved. In the region below the flutes, the minced tissue fragments are suspended in a vortex by the action of the magnetic stir bar. In the fluted region, aggregates of cells are released by the combined action of the turbulence and the enzymatic digestion. Above the flutes, there is neither a vortex nor turbulence but only an upward component of velocity. Monodisperse cells occupy this upper region of the vessel prior to their egress from the chamber. It is the small volume (approximately 10 ml) and the upward velocity component which allows one to achieve a minimum MRT. A continuous velocity gradient vessel has been described in an earlier publication for the fractionation of a population of cells on the basis of differing cell diameters (11).

The unit is normally sterilized with inlet and outlet hoses attached and a magnetic stir bar in the inner cylinder. After sterilization the chamber is aseptically connected to a reservoir of trypsin and to a delivery vessel for

Fig. 1. Drawing of the automatic trypsinizing device. A, water jacket; B, inlet, water jacket; C, outlet, water jacket; D, trypsinizing chamber with four flutes; E, inlet, trypsinizing chamber (connected via regulating valve to trypsin reservoir); F, spillover, trypsinizing chamber.

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the collection of monodisperse cells. The receiving vessel contains a small amount of serum and is surrounded by crushed ice.

Tissue fragments prepared using a mincing tube and stainless-steel barber's shears are transferred in HEPES-buffered saline (10) to the trypsinizing chamber by means of a large orifice volumetric pipette (Bellco Glass, Inc., Vineland, N.J.). The magnetic stirring device is turned on and water at 37 C is circulated in the jacket. The flow of trypsin is now started from the reservoir. A Teflon stopcock at the reservoir is used to control flow rate. Alternatively, flow rate can be controlled by using a peristaltic pump. The speed of the stir bar and the flow rate of the trypsin must be empirically determined and are to a large extent dependent upon the type of tissue being trypsinized.

The automatic device described in this communication has been used with monkey kidney, rabbit kidney, puppy salivary gland, and chick embryo tissues. It has also been utilized to free secretory cells from human thyroid tissue. Cell viability, as measured by trypan blue dye exclusion, and total yield of cells were always as high and often higher than using discontinuous batch methods of trypsinization.

Although the trypsinization of tissue from different animal species requires somewhat different settings of enzyme flow rate and stirring speed, the device described in this note is capable of delivering an optimal monodisperse suspension of cells from a wide variety of animal tissues.

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