Isolations and Cultures of Primary Hepatocytes

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continues until adequate tissue digestion is achieved. The enzyme is temperature sensitive; the activity of the collagenase can be greatly influenced, leading to the decrease of the viability in isolated hepatocytes. The optimum temperature of enzyme solution is 37-38°C; this temperature is measured from the perfusion site (liver lobes) rather than solution container. The temperature of the solution ought to be controlled all the time based on this temperature to obtain the optimal efficiency. Besides, the solutions usually perfused hollow-fiber bioreactor to enrich O_2, to reduce the damages of the isolated hepatocytes.

Collagenase type IV was a classical and broad spectrum enzyme used in primary hepatocytes isolation in all species. With upgrade of commercial collagenase products such as Roche Liberase research grades and Serva collagenase NB grades, the isolations are more specific to the species, while the purity, viability, and yield of isolated primary hepatocytes are highly improved [4,18]. Except for the primary component, highly purified collagenaseand mixture, we found that the amount of neutral protease thermolysin also determines the viability and cell yield, and especially the further success of spheroid formation. If the thermolysin is too much, the spheroids usually are not round in shape; cells in them attach with each other, rather than aggregating, and are mostly covered by the blebs. N-acetylcysteine (NAC) is an antioxidant that acts through the replenishment of glutathione in the liver. It also has direct antioxidant properties and appears to have hepatoprotective efects against liver ischemia/reperfusion injury to improve the viability [9]. It has been proved to improve the viability and conserves the metabolic function of hepatocytes [4,18].

Iced medium containing Williams'-E can terminate enzyme digesting, help to maintain the cell activity, and remove the damage cells. Mechanical dissociation and fltering is necessary to remove connective tissue, and subsequent centrifugation is required to separate hepatocytes from both dead hepatocytes and non-

The function of isolated hepatocytes is more critical to be measured by assessing of ammonia degradation and ureagenesis. The study is performed after addition of 1 mM final concentration of ammonium such as NH_4Cl into the medium. In some continuous culture circumstance, even with high level of albumin production, the other functions like ureagenesis or cytochrome P450 would drop obviously. However, while hepatocytes demonstrate a higher ammonia clearance rate, the diazepam metabolism is more active, showed in our unpublished preliminary studies.

When come to clinical use in the future, commercial primary hepatocytes products will be available. Minimal criteria for defining release specifications should be established and standardized. Such criteria should not be confused with identifying criteria for research purposes. Clinical doctors should reach an agreement on the criteria to decide the usability of the hepatocytes.

Perspectives

New clinical perspectives of allogenic and xenogenic isolated hepatocyte utilization have recently been proposed, primarily for hepatocyte transplantation and as the basis of liver support systems to replace compromised liver function.

For hepatocyte transplantation, isolated liver cells have been used in a variety of configurations: suspended, matrix-attached and encapsulated, singularly or in small aggregates. Transplantation of isolated xenogenic hepatocytes into the peritoneal cavity, into the spleen or directly injected into the liver *via* the portal vein has been performed for the treatment of experimental acute liver failure [31]. Transplanted hepatocytes were shown to survive and function throughout the life-span of recipient small experimental animals. Several inherited metabolic disorders of the liver such as Nagasean albuminemic in naturally mutant animals have also been treated successfully by hepatocyte transplantation [32].

Isolated primary hepatocytes can be used to construct bioartificial liver support systems to treat patients with severe acute failure [10]. The rationale of such systems is to bridge the severely ill patient to the liver transplantation or recovery by supplement them with the essential liver functions. It has been assumed that $10\text{-}15 \times 10^9$ cells need to be replaced to support an acutely failing liver in adults [33]. Such large scale of hepatocytes is difficult to acquire, and the liver-specific function is another critical problem. The metabolic profiles of porcine hepatocytes are basically similar to those of humans, and freshly isolated primary hepatocytes retain most of their liver-specific functions; therefore, porcine hepatocytes are a reasonable alternative to human hepatocytes. Once a standardized method of isolating primary hepatocytes has been established, isolated liver cells will be easily accessible and more frequently available. One of the most important early issues has been solved in the initial approach to the construction of bioartificial liver devices.

Genetically altered hepatocyte use could represent the first choice for treatment of specific genetic defects of liver function. Defects of some genes, which are preferentially expressed in liver cells, can cause metabolic disorders and inherit to next generations. For example, hereditary tyrosinemia type 1 (HT_1) is an autosomal recessive inborn error of metabolism, caused by deficiency

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