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Hepatocytes isolation from adult rats for liver recellularization

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Abstract

Background: Currently hepatocytes obtaining is prerequisite to create the necessary conditions for medical research, because it is an important tool in developing of new strategies in tissue engineering domain, which represents obtaining functional organs in laboratory conditions.
Material and methods: The study was made on adult Wistar rats liver with body weight 274.66± 2.52 g (n=3) which were used for hepatocytes extraction by perfusion through the upper cave vein with combination of type II collagenase and type I dispase and Hank's 0.9 mM MgCl2, 0.5 mM EDTA and 25 mM HEPES (HiMedia, India).
Results: The cells were counted with trypan blue 0.25% in hemocytometer and cultured in William's E medium (HiMedia, India) with 2 mM L-glutamine, 5% fetal bovine serum (Lonza, Belgium), antibiotic antimycotic solution (HiMedia, India), 100 nM dexamethasone and 100 nM insulin, with 2.5 x 10^5 cells per well in 12-well plates. After isolation were obtained 324, 48 ± 1, 25 x 10^6 hepatocytes, with a viability of 94.7 ± 0.9 % which indicates a high yield of cells viability.
Conclusions: The hepatocyte isolation method by liver perfusion with the combination of collagenase-dispase is feasible for obtaining a large amount of functional hepatocytes intended for the recellularization *in vitro* of decellularized liver scaffolds. The yield and viability of hepatic cells could be increased by enzymatic digestion of liver tissue using combination of collagenase/dispase solution due to the less cytotoxic effect.

Key words: hepatocytes, cell separation, cell survival, collagenases, dispase, in vitro techniques.