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Journal of Stem Cells Research and Therapy

Editorial

Isolation of Bile Duct Progenitor Cells from Human Liver Samples

Beverly Norris*

Department of Stem Cell , University of Minnesota, Minneapolis, USA

INTRODUCTION

Samples of human liver tissue were obtained from the Liver Tissue Distribution System (LTCDS), a National Institutes of Health (NIH) service contract to provide human liver tissue from regional centers for distribution to scientific investigators throughout the United States. Each piece of human primary liver tissue obtained from LTCDS was minced well and digested with a buffer consisting of 1.67 mg/ml Collagenase Type II (Worthington), 1.33 mg/ml hyaluronidase (Sigma Aldrich) and 1.34 U/ml Dispase (BD Biosciences), dissolved in DMEM/ F12 (Gibco, CA), and supplemented with 0.5 mM EGTA, 5 mM glucose, 5 mM KCl, and 10 mM HEPES, pH 7.4. The suspension was then incubated in a 37°C water bath for about 45 min with intermittent shaking. The large hepatocytes were sedimented by centrifuging at 10 g for 3 min. The supernatant was then spun at 100 g for 5 min. followed by washing of the pellet with medium containing 10% serum followed by another spin at 100 g for 5 min. Nonparenchymal Cells (NPCs) including bile duct progenitor cells, were

medium. The medium was changed after two days and the cells were cultured in suspension culture for another 7-10 days in the culture medium previously described. They were next plated onto 6 well plates with or without hydrogel (ESI-Bio) coating according to manufacturer's instructions and grown for 2-3 weeks in adherent culture for maturation. Preliminary trials showed that a MOI of 25-100 gave maximum insulin+ cells with minimum

toxicity, so this range was adopted as the standard for the study. The Ad-PNM infection efficiency was obtained by counting all the cells in three fields of vision and taking an

average percentage of the cell population showing nuclear PDX1 immunostaining The NOD-SCID (Prkdcscid) mice from Jackson laboratories (three experimental and two controls) were initially made diabetic by injecting 120 mg/Kg of Streptozotocin (STZ) intraperitoneally for approximately 2 weeks. The mice were monitored until they reached a blood glucose level of 400 mg/dl. The clusters of insulin+ expressing cells were slightly trypsinized to detach from the plate without

included in the pellets. The bile duct progenitor cells expressing SOX9 were then plated in ultra-low attachment plates (Corning) in DMEM/F12 (Invitrogen) media containing 3% heat inactivated FBS, supplemented with 1XNEAA, and 20 ng/ml EGF (R&D Systems) for suspension culture. These cells formed small clusters of cells in suspension culture which were then placed in 6 well plates after 7 days and expanded in adherent culture in the same medium for 2-3 weeks with occasional splitting. An adeno viral vector (Ad- PNM), carrying mouse Pdx1, Ngn3 and MafA in a single polycistronic unit, separated by 2A sequences, was prepared as described previously [13]. The SOX9+ cell clusters were trypsinised to make a single cell suspension. They were then transduced with Ad-PNM at a 100 MOI (multiplicity of infection) in the ultra-low attachment plate. The cells were then incubated in a 37°C incubator for 48-72 hrs. The infection was done in absence of serum in

making them into a single cell suspension. Approximately 3-4 insulin+ clusters from each 6 well plate were injected into each of the three mice. The cells (after centrifugation) were mixed with fibrinogen-thrombin in 1:2 ratio in order to make a graft, each of which was slid under the kidney. The cells (after centrifugation) were mixed with fibrinogen-thrombin in 1:2 ratio in order to make a graft, each of which was slid under the kidney capsules of diabeticmice.

Correspondence: Beverly Norris, Stem Cell Institute, University of Minnesota, Minneapolis, USA, E-mail: BeverlyNorris45@hotmail.com

Received Date: May 03, 2021; **Accepted Date:** May 17, 2021; **Published Date:** May 24, 2021

Citation: Norris B (2021) Isolation of Bile Duct Progenitor Cells from Human Liver Samples. J Stem Cell Res Ther.11:e488.