

A novel 3D cell culture system could postpone primary hepatocytes dedifferentiation in vitro

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Aim: To investigate the superiority of a novel 3D culture system which can maintain the viability and differentiation of hepatocytes longer than the monolayer collagen cell culture system.

Methods: Primary mouse hepatocytes were isolated from 6-8 week old adult Kunming species mouse using a two-step collagenase perfusion procedure as described previously^[1] and cultured in the 3D Biotek (supplied by 3D Biotek, LLC Company, USA) cell culture system coated with collagen with the monolayer collagen cell culture system as a reference. Primary hepatocytes were maintained in WEM. The supernatant was collected for ELISA assay of Albumin, and quantitative fluorescence PCR for the detection of mRNA as a diagnostic test of hepatocyte differentiated status. Collagen purchased from BD Co Ltd. Albumin ELISA quantization kit was purchased from Bethyl Laboratories (Montgomery, TX)^[2-3].

Results: The 3D biotek cell culture system is a novel and superiority system for the culture of hepatocytes. This system may mimic in vivo tissue environments compared with monolayer system. (Fig.1) The result of Albumin demonstrated that the cells cultured in monolayer system experienced a rapid dedifferentiation from 5d and the quantity of the Albumin reached to 13.77ng/ml at 10d. But the cells cultured in 3D system dedifferentiated slowly and the concentration of Albumin is 350.19ng/ml at 9d and 348.74ng/ml at 10d. (Fig.2) Copy number of the albumin gene was higher in 3D culture compared to monolayer

condition at all time points. At the same time, the downtrend of the albumin gene and production of albumin protein was comparatively gentle in 3D culture condition compared to monolayer one.

Conclusion: This novel 3D biotek cultural system represents a tool to postpone the differentiation of primary hepatocyte sufficiently and is a useful approach for the primary hepatocyte culture and may be expanded to culture human primary hepatocyte for studying HBV infection and viral life cycle.

Key words: Hepatocytes, 3D cell culture system, Albumin, differentiation

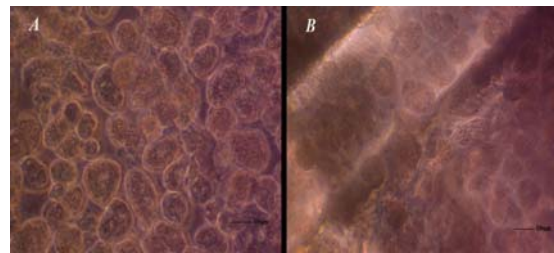


Fig.1. Primary hepatocytes cultured on monolayer cell culture system and 3D biotek cell culture system. (A) Hepatocytes on monolayer system after 2 days in culture. (B) Hepatocytes on 3D biotek system after 2 days in culture.

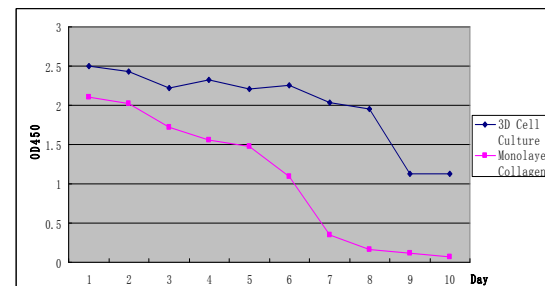


Fig.2. ELISA results for Albumin synthesis in hepatocytes cultured on monolayer cell culture system and 3D biotek cell culture system.

References:

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