

Extracellular Matrix Proteinsh an Effect on Cell Adhesion and Proliferation but No Significant Differentiation of Adult Rat Pancreatic Duct Epithelial Cells (ARIP))

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INTRODUCTION: Research suggests that adult pancreatic stem cells/ progenitor cells could differentiate into insulin-producing cells or cultivated islet-like clusters. ARIP cells, an adult rat pancreatic ductal epithelial cell line, were used as a model to explore the possibility of insulin-producing cell differentiation.

METHODS: ARIP cells were from ATCC and maintained in F12K medium supplemented with 10% FCS. Tissue culture (TC) surface was coated with Collagen Type I (CN), Fibronectin (FN), Laminin (LN) and Vitronectin (VN) respectively. 3000 cells/cm² of cell suspension was cultured for 6 hours. Cells were stained with 2uM of Calcein AM. Fluorescent images were captured from 4 random areas surrounding each centre of a well. Individual cell features were analysed using a Leica Qwin program.

Insulin content was estimated using an Ultra Sensitive Rat Insulin ELISA Kit. DNA content was measured using Hoechst 33258 assay. Gene expression was analysed using RT-PCR.

RESULTS: Four types of extracellular matrix proteins enhanced cell adhesion of ARIP cells. The individual cell area and perimeter of ARIP cells were increased by extracellular matrix proteins, suggesting the enhance of cell adhesion. Examination of the surface coverage of ARIP cells in the presence of ECM protein coated surfaces and defined serum-free medium showed that laminin significantly enhanced cell surface coverage after 96 hours' culture. Insulin content (ng/mg DNA) studies were subsequently carried out using GLP-1, ECM proteins in defined serum free medium conditions. Results using these constituents singly were inconclusive; in combination, however, results showed that the insulin content was only enhanced in the presence of collagen. The insulin gene, however, did not show distinct expression corresponding to the above insulin content.

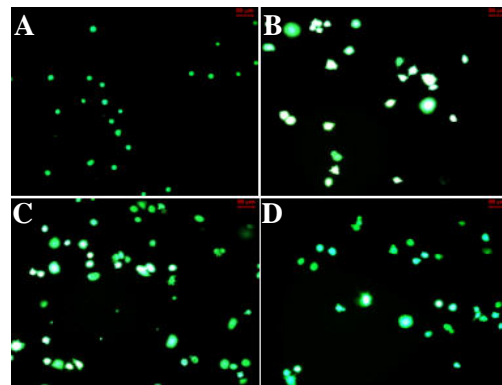


FIG.1: ARIP cells cultured on different surfaces for 6 hours. A. Control. B. 10µg/cm² CN surface. C. 10µg/cm² FN surface. D. 10µg/cm² LN surface.

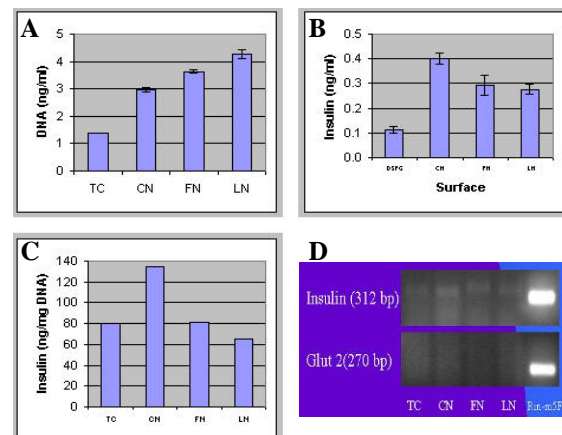


Fig.2: Insulin content and gene expression. ARIP cells were cultured for 48 hours in F12K medium supplemented with ITS, BSA nicotinamide and GLP-1. Each value=mean±STD from triple tests. A. DNA content. B. Insulin content. C. Insulin content/DNA content. D Gene expression.

CONCLUSIONS: Cell morphology, adhesion and proliferation of ARIP cells were affected by ECM proteins. The insulin-producing cell differentiation of ARIP cells is however not affected by ECM and/or GLP-1 significantly.