Controlling Cell Behavior in a Hydrogel Environment by Genetic Modification

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Introduction

Cell encapsulation represents one of the current leading methodologies aimed at the delivery of biological products to patients for the treatment of multiple diseases. An ideal encapsulation system acts as a barrier to allow passage of nutrients and waste products, while excluding immune cells. Despite concerted efforts of numerous groups, a viable encapsulation system leading to prolonged function is elusive. Common problems that lead to the stresses that cells have to undergo, include insufficient diffusion of nutrients, oxygen resulting in hypoxia, lack of cell-cell and cell-ECM interaction leading to anoikis [1]. Therefore, one of the challenges associated with this field is controlling the behavior of the cells when they are no longer receiving the nutrients and signals that are present under physiological conditions.

Encapsulation of pancreatic islets has long been a strategy for treatment of diabetes. Islets in their native environment are highly vascularized. Once isolated from the pancreatic tissue they are found in variable sizes and on placing it in a capsule creates new stresses, which can lead to loss of function. This study seeks to observe the effect on cell survival and function with different sizes of cell aggregates in the polymeric environment and then to test the importance of cell-ECM interactions by genetic modification.

Methods: The study uses MIN6 cells, a murine ß-cell line which exhibits similar physiological characteristics of pancreatic ß-cells including glucose-dependent insulin secretion [2]. To study the effect of variable cell aggregates on cell function and survival, MIN6 cells were cultured and placed in untreated 6 well plates at different concentration in order to prepare different sizes of aggregates of 50µm, 200µm and 500µm. They were incubated at 37°C and 20% oxygen for 24 hours on an orbital shaker. The cell density of 300,000 cells is maintained for each size aggregate in a gel. The cell pellet is resuspended in 40 µl of PEG solution without affecting the aggregates followed by exposure to UV light for 8 minutes, encapsulating the cells in the matrix. These photoencapsulated cells in PEG have been shown to survive the exposure to UV light [3]. To Test the importance of cell-ECM interactions, MIN6 cells were genetically modified by mILK. ILK is an integrin ß-1 binding protein that enhances phosphorylation of protein kinase B (PKB/Akt). Activated PKB/Akt plays a critical role in regulation of adhesion-mediated cell survival signals [4]. We hypothesized that ILK overexpression would prevent ß-cell anoikis caused by the lack of physiological cell-ECM interactions within the PEG capsule. The MIN6 aggregates of same sizes are infected to overexpress mILK and Cre protein is used as a control. They were encapsulated after 24 hours of infection. The cells were monitored for a time period of 2 weeks. For functional studies, encapsulated cells were stimulated daily with 16.7mM glucose solution for an hour. To quantify the amount of insulin secreted, Insulin ELISA tests were performed on the samples. Survival studies were performed using Alamar Blue, the samples were tested for absorbance for the same time period and the data was correlated with the insulin release studies.

Results: The PEG capsule with three different cells aggregate sizes having a constant cell density of 300,000 cells were monitored for functionality and survival for a time period of 14 days. Initially, Insulin release studies of unmodified MIN6 aggregate cells were conducted. Aggregates of size 500µm displayed high release of insulin on the initial days, with a progressive reduction of release by two-thirds of the initial in the first week, which reduced to no release after 2 weeks. Gels with aggregates size 200µm too showed a high release in the initial days, with slow decrease in release and managed to release one-fifth on day 14. For the 50µm aggregates, like the 500µm, had a similar pattern of loss of function. With a high release initially, the release dropped below the limits of detection by 2 weeks. The survival study was also performed on these cells; the absorbance value correlated with the dip in the functionality. Similar studies were performed for genetically modified MIN6 cells. It was observed, that the effect of ILK was pronounced mainly on the cells with aggregate size of 50µm. They performed better in terms of viability and function when compared to the control, with more than two-thirds of the insulin release on day 14 as shown in fig.1. The survival study was also carried out for modified cells and the value of the absorbance explains the pattern of functionality.

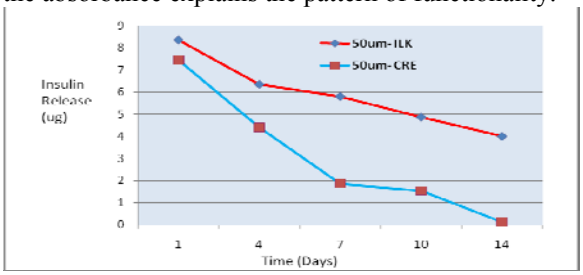

Figure 1. Insulin release profile for genetically modified 50µm Aggregates.

Conclusions: These studies show loss of function and viability for the big and the small cell aggregates encapsulated in a hydrogel, which could be due to insufficient oxygen supply to the core and a lack of proper cell-ECM interactions respectively. Targeting the signal transduction pathway of ILK seems to be a promising target for extending function and viability for small aggregates and helping in eliminating anoikis. It is hoped that continuous efforts and combinatorial approach that seeks to address the range of stresses placed on the encapsulated tissue leads to a long-term function.

References:[1] (De Vos P. Diabetologia. 1997; 40, 262)
[2] (Ishihara H. Diabetologia. 1993; 36, 1139-45)
[3] (Anseth KS. Biomaterials. 2002; 23,4315-4323)
[4] (Yoganathan. Pharmacol Ther. 2002; 93: 233–242)