Developing polymeric delivery system for the entrapment of insulin-producing stem and liver cells.

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INTRODUCTION AND OBJECTIVE

Microencapsulation of cells is a promising approach for the continuous delivery of drugs and proteins. Moreover, encapsulation of living cells in a protective, biocompatible and semi-permeable polymeric membrane has been proven to be an effective method of immunoprotection of the cells (Goren 2010). Extracellular matrix (ECM) signaling influences cellular differentiation, maintenance, and function. In mature tissues, cell–matrix interactions can activate intracellular signaling pathways that regulate cell proliferation, survival, and many other cell processes (Weber 2008). Hence, addition of ECM to the encapsulation system regulates the activities of adherent cells, including proliferation, differentiation and cellular secretion level of insulin by activating the desired cell signaling via integrin-ligand-bonds and subsequently stimulating the gene expression level. We propose to encapsulate human liver cells (Hum-Hep) or human mesenchymal stem cells (hMSC) after transduction with pancreatic and duodenal homebox gene-1 (PDX-1), which induced trans-differentiation into functional insulin-producing cells, as a possible diabetic therapy application. The rationale for using Hum Hep has physiologic, developmental and molecular reasons (Meivar-Levy 2006), while hMSC are known to differentiate to different type of cell and are hypo-immunogenic (Goren 2010).

The main goal of this research is to develop a polymeric system which encapsulates PDX-1genetically engineered Hum Hep cells or hMSCs for diabetic therapy. The encapsulated cells are implanted near the organ target, in a way that the entrapped cells produce and secret insulin as needed mimicking the natural pattern of insulin secretion by a healthy pancreas.

MATERIALS AND METHODS

Hum Hep cells kindly given by Prof Sarah Ferber and hMSCs were transduced using recombinant adenovirus containing PDX-1 gene. Once transduction was established, cells were encapsulated in alginate-Poly-l-lysine (PLL). Transduced cells were also encapsulated in alginate-PLL mixed with ECM isolated from pancreatic tissue.

Decellularization of native pork pancreas protocol includes a combination of physical, chemical and enzymatic cleaning. ECM liquefaction and application will be discussed.

Encapsulated cells viability and morphology were evaluated using AlamarBlue assay, Hoechst and Fluorescein Diacetate (FDA) cell viability assay in which living cells are stained fluorescently. Histological cross section of acellularized pancreatic ECM was characterized using Hematoxylin & Eosin (H&E) staining. Tamra staining was used in order to study the distribution of the ECM inside the capsule.

The effect of encapsulation parameters on the biological activity of the encapsulated cells, meaning insulin secretion, was followed using C-peptide secretion measured by RIA kit radioimmunoassay.

RESULTS AND DISCUSSION

Characterization of the encapsulated cells

Encapsulated cells viability was determined using the Alamar Blue assay (Fig. 1-A). It was found that both kinds of cells were viable for more than 120 days (data not shown for hMSC). Fluorescent micrographs of encapsulated cells stained with FDA were taken on day 10 and 108 post encapsulation (Fig. 1-B,C). Results show that cells grow and spread in their surrounding with no visible cell aggregate formation.

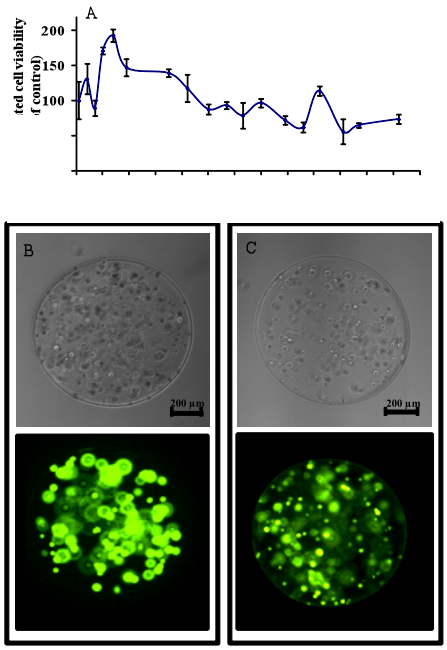

Figure 1: Viability of encapsulated human liver cells in alginate-PLL microcapsules [A]. Microcapsules stained with FDA 10 days [B] and 108 days [C] post encapsulation.

Characterization of the decellularized pancreatic ECM and ECM-cell encapsulation

Sections of decellularized pancreas were stained with H&E staining in order to assure that all cellular components which can provoke immune response are removed. As can be seen in figure 2-C, no traces or residues of cellular or nuclear components could be detected in the decellularized pancreas. HR-SEM image (Fig.2-D) exhibited typical morphology of collagen fibers.

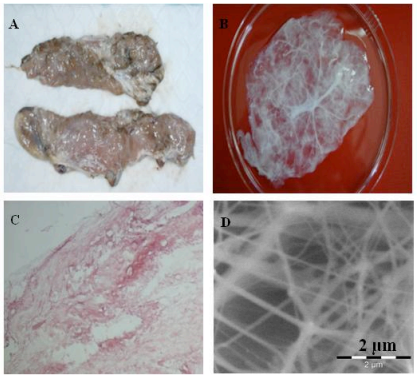

Figure 2: Native pancreas [A]. Acellularized pancreatic ECM [B]. Histological cross section of acellularized ECM [C]. HR-SEM microscopy of ECM [D].

Preliminary results show that ECM is spread all over the capsule (Fig.3-D) while cells are gathered around micro pieces of ECM inside the capsule (Fig.3-B,C).

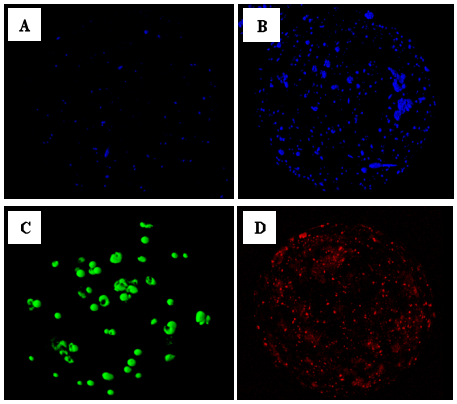

Figure 3: Florescence staining of ECM-cell encapsulation. Cells were stained with Hoechst (nucleus) [B] and FDA [C]. Collagen fibers were stained with Tamra [D]. Control without cells [A].

Therapeutic efficacy of encapsulated Hum Hep cells and hMSC secreting insulin

Cells were transduced with PDX-1 adenovirus and then encapsulated. Transduced Hum Hep cells and hMSC, non-transduced encapsulated cells and transduced encapsulated cells were exposed to high glucose concentration (17.5 mM) for 15 min (Fig 4). C-peptide was measured 5 days after the initial exposure to the viral treatment. As can be seen in figure 4-A, secretion of C-peptide from non transduced encapsulated Hum Hep cells is similar to the secretion from transduced cells and transduced encapsulated cells. the results emphasized our hypothesis that the microeviromet inside the capsule permit a native environment that encourage the bioactivity of these cells as a glucose dependent cells even without viral transfection. On the other hand, C-peptide secreted from transduced encapsulated hMSCs (Fig.4-B) is significantly higher than the levels secreted from encapsulated cells ($p<0.01$), transduced cells ($p<0.01$) and from C-peptide obtained from Hum Hep experiments. Moreover, C-peptide secreted from encapsulated hMSCs is significantly higher than the levels secreted from transduced cells ($p<0.05$). These results indicate that the microenvironment within the microcapsule is not only permissive for hMSC transdifferentiated into β-cell survival, but may actually contribute to the insulin secretion.

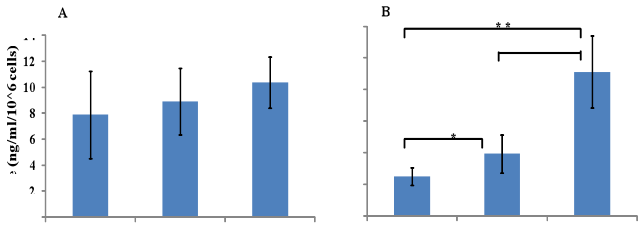

Figure 4: Secretion of C-peptide from Hum Hep cells [A] and hMSC [B] 5 days post viral transduction. n=7.

CONCLUSION

Our findings demonstrate that the microenvironment within the alginate-PLL microcapsule is permissive for cell survival and insulin secretion. Therefore, entrapped insulin producing cells can be considered as a platform for diabetes cell therapy.

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ACKNOLEGMENTS

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