NOVEL PEPTIDE-FUNCTIONALIZED HYDROGELS FOR PANCREATIC ISLET ENCAPSULATION

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INTRODUCTION

Pancreatic islet transplantation is a very attractive method to restore glycemic control in type I diabetic patients. Encapsulation of islets in semi-permeable hydrogels may enable islet transplantation in the absence of immuno-suppression because the capsules can protect islet tissue from the immunoreactive cells and antibodies. Hydrogel systems applied so far for islet encapsulation have limited success in clinics due to lack of biocompatibility and inefficient immunoprotection from cytotoxic factors of low molecular weight (MW < 50 KD) that permeate through the hydrogel capsules.[1] The aim of our study is to develop a biocompatible hydrogel that not only provides mechanical isolation of pancreatic islets from host immune machinery, but also improves islet survival and function by inhibiting the toxicity of low MW pro-inflammatory cytokines that cause early graft failure.

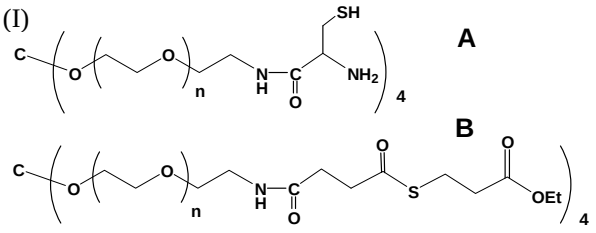
EXPERIMENTAL

4-armed PEG terminated with a N-terminal cysteine (PEG-Cys,) or a C-terminal thioester (PEG-ThE), maleimide-terminated peptides for hydrogel functionalization were all manually synthesized and purified by RP-HPLC. For cell encapsulation, cultured mouse islet β-cell (MIN6) were suspended in DMEM media. Such cell suspension was mixed with a solution of PEG-Cys and a solution of PEG-ThE in DMEM (pH 7.5). Final concentration of each PEG molecule is 5% w/v. The mixture was incubated at 37°C for 15 minutes and the gels were then washed with DMEM. To form hydrogels presenting functional peptides, maleimide-terminated peptides were added to the solution of PEG-Cys at a molar ratio of 1:25 (MA-peptide : PEG-Cys) prior to mixing cells with PEG-Cys and PEG-ThE. *In vitro* viability assays of cells entrapped in hydrogels were performed using a live/dead cell assay kit from Invitrogen. Glucose-sensitive insulin secretion analysis was carried out using the mouse insulin ELISA kit from Mercodia AB.

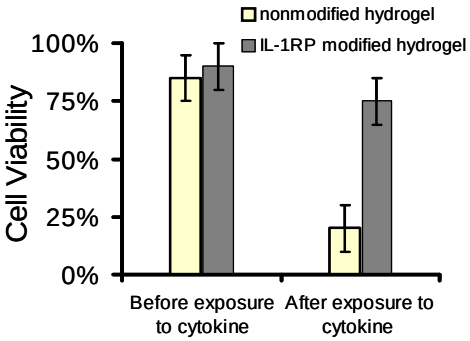
RESULTS

Mouse islet-derived MIN 6 cells were encapsulated in hydrogels *in-situ* formed by native chemical ligation (NCL)[2] between PEG-containing macromers (Figure (I) **A** and **B**) . Presented on such biocompatible hydrogels are peptides GRGDSPG and FEWTPGWYQPY (IL-1RP) that were conjugated to **A** by Michael-type addition. The latter is an antagonist peptide of cell surface receptor for IL-1β,[3] a cytokine that plays an critical role in causing early graft failure after islet transplantation. The hydrogel formed by NCL was able to protect encapsulated MIN6 islet cells from cytotoxic T-lymphocytes. Furthermore, when IL-1RP peptide was presented on the capsule, cytokine-induced death of encapsulated cells was significantly inhibited (Figure (II)). The encapsulated β-cells in hydrogels also maintained their glucose-stimulated insulin secretion function.

(I)



(II)



Our work demonstrated our peptide-functionalized hydrogel could improve the survival and function of islet cells by providing the protection from both immunoreactive cells and low MW toxic cytokines. In particular, hydrogels functionalized with an IL-1 receptor antagonist peptide can help islet cells to survive and function properly in the presence of activated lymphocytes and inflammatory factors. Future work will focus on *in vivo* tests using animal models. Our work suggests encapsulating tissues with hydrogels presenting anti-inflammatory factors may represent a generally efficient solution to improve tissue graft function in transplantation and tissue engineering applications.

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