

# Synthesis of Immunoisolation Barriers That Provide Localized Immunosuppression for Encapsulated Pancreatic Islets

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Pancreatic islet encapsulation into synthetic, passive material matrixes can provide protection for transplanted islets from destruction via cell-contacted mediated interactions with autoreactive immune cells for treatment of Type I diabetes mellitus. However, one of the fundamental deficiencies with current encapsulation technology is that passive material barriers cannot protect islets from exposure to cytokines and other small, diffusible cytotoxic molecules produced by activated immune cells, subsequently leading to  $\beta$ -cell destruction. Preparation of material matrixes that can actively provide localized immunosuppression of autoreactive immune cells may prolong the viability, and hence function, of encapsulated islet grafts. We have demonstrated the ability to conjugate apoptosis-inducing anti-Fas monoclonal antibodies (MAbs) to the surfaces of poly(ethylene glycol)-modified hydrogels, providing a surface that actively attempts to locally down-regulate the autoimmune response by destroying autoreactive T cells against pancreatic islet cells. We have conjugated anti-Fas MAbs to a high degree to the surface of these hydrogels, with retention of anti-Fas recognition of the Fas antigen as shown by ELISA testing. Apoptosis induction of Fas-sensitive Jurkat T cells was enhanced in the presence of anti-Fas conjugated hydrogels. In addition, this apoptosis induction was specific to anti-Fas MAbs, with no apoptosis induction with control antibodies or with Fas-insensitive T cells. These experiments promote the concept that surface-conjugated hydrogel constructs can provide localized immunosuppression for encapsulated grafted tissue.

## INTRODUCTION

Pancreatic islet transplantation represents an attractive treatment option for Type I diabetes mellitus but requires lifelong systemic immunosuppressive therapies to be successful. Encapsulation of islets within a cell impermeant polymer matrix provides a passive physical barrier that allows for small molecule diffusion through the barrier but prevents T-cell infiltration and cell contact-mediated destruction of grafted islets. Most of the current research has focused on forming polyionic complexes using sodium alginate mixed with different polycationic compounds to form polymeric barriers for immunoisolation of islets (1). The first and most extensively investigated microencapsulation system of individual islets was with a polyelectrolyte complex of alginate and polylysine (2). Over the past 20 years, this system has been continually optimized and has seen limited success in a number of animal models. Islets encapsulated in alginate matrixes have survived *in vivo* for a significant duration of time while maintaining euglycemia, up to 6 months (3), but biocompatibility issues with these types of matrixes still persist, especially with the choice of polycation used to form these membrane barriers (4).

Poly(ethylene glycol) (PEG) represents an alternative biomaterial that holds potential promise for encapsulation of pancreatic islets. PEG is a biocompatible polymer that has gained FDA approved when incorporated into different drug formulations (5). Cruise et al. microencapsulated islets by photopolymerizing PEG diacrylate (PEGDA) to form their passive membrane barriers (6). Islets encapsulated in PEGDA were able to maintain normoglycemia in streptozotocin-induced mice for up to 4 months (7). Investigations into the biocompatibility of PEG has shown that *in vitro* PEG itself does not activate immune cells, such as splenic lymphocytes and

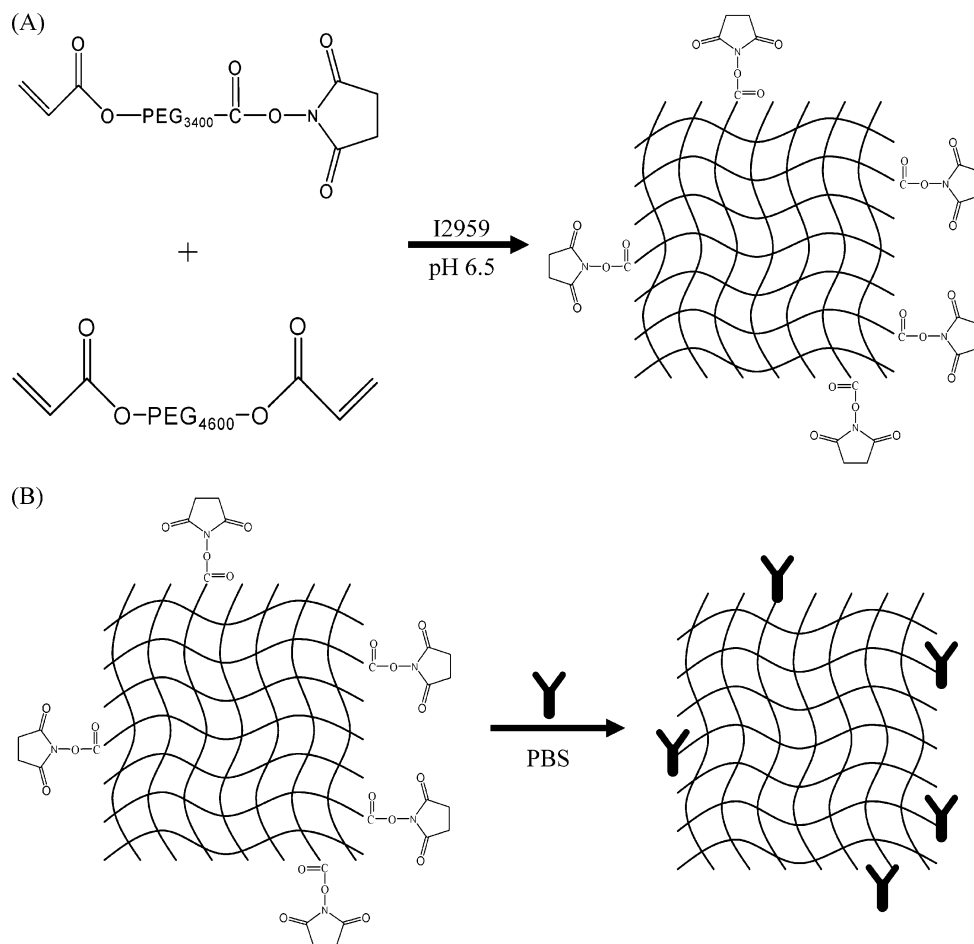
macrophages; however, PEG cannot actively protect islets from cytotoxic molecules produced by preactivated immune cells (8). This example illustrates one of the fundamental deficiencies with current encapsulation technology, that cytokines and other effector molecules produced by immune cells can still diffuse through these passive barrier matrixes to inactivate or destroy transplanted  $\beta$ -cells within these islets (9–11).

Encapsulation of grafted islets with passive materials may prevent destruction of  $\beta$ -cells initiated by cell–cell contact with autoreactive T cells; however, such passive barriers cannot protect islets from exposure to cytokines and other effector molecules, such as IL-1, produced by activated immune cells that can subsequently lead to  $\beta$ -cell destruction (9, 12). Preparing encapsulation matrixes that can actively suppress the autoimmune response will therefore be beneficial in prolonging the survivability and function of islet grafts. One method of activating these polymeric barriers is to conjugate monoclonal antibodies to the surface of these hydrogel constructs that can actively induce destruction of T cells, thereby providing localized immunosuppression for transplanted tissue grafts.

## EXPERIMENTAL PROCEDURES

**Preparation and Characterization of Mouse Anti-Human IgG Conjugated Hydrogels.** Poly(ethylene glycol diacrylate) (PEGDA, MW = 4600 g/mol) was polymerized with *N*-hydroxysuccinimide-PEG-acrylate (NPA, MW = 3400 g/mol) by UV-initiated photopolymerization to form PEGDA-*co*-NPA hydrogels with PEGDA-to-NPA ratios ranging from 95/5 to 50/50 (wt) using a 0.05% (wt) UV photoinitiator, 1-[4-(2-hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propan-1-one (I2959, Ciba Geigy, Newport, DE). Hydrogel constituents were dissolved in 0.15 M sodium phosphate buffer, pH 6.5, and hydrogels were prepared by photopolymerization for 15 min at 365 nm. Mouse anti-human IgG (MAH IgG, Fitzgerald Industries International, Concord, MA) was then conjugated to these

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**Figure 1.** (A) Schematic representation of photopolymerization of PEG<sub>4600</sub> diacrylate and NPA, forming hydrogel networks with pendant NHS moieties on the hydrogel surface, and (B) conjugation of MAH IgG MAb to the surface of these hydrogels.

hydrogels at concentrations ranging from 0.05 to 1 mg/mL for 1 h in PBS. Hydrogels were then incubated with 50 mM glycine in PBS to quench the reactivity of any unreacted NHS groups remaining in the gels.

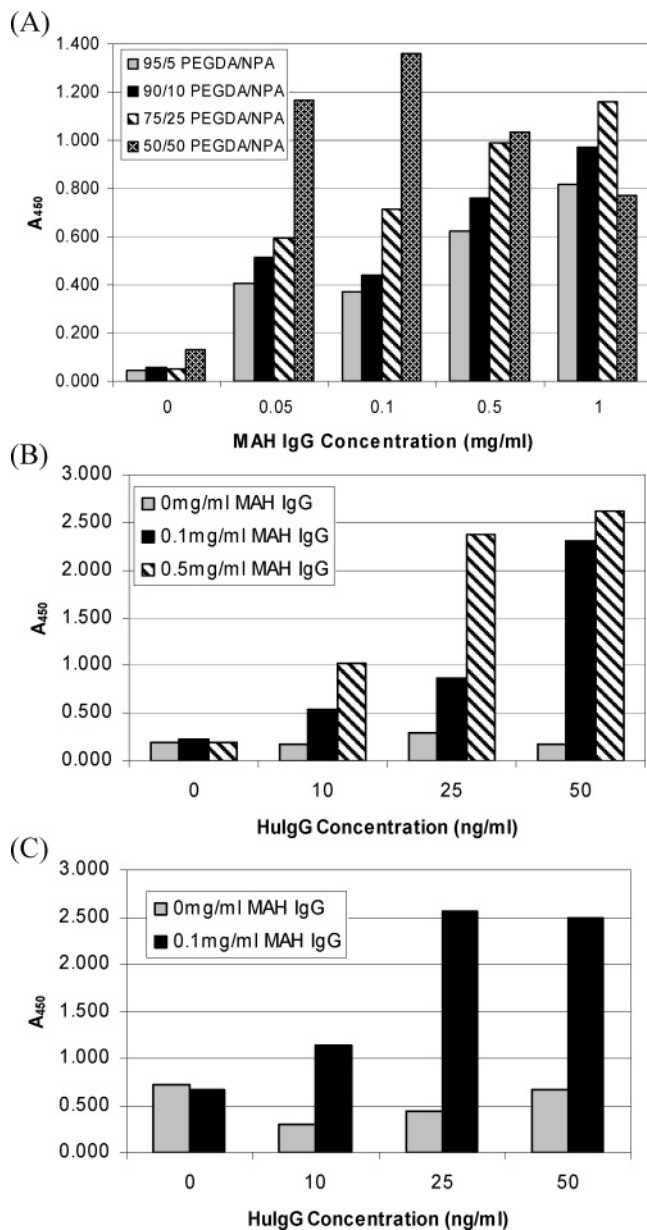
The level of MAH IgG conjugated to the surface of the hydrogels was assessed by a modified indirect ELISA using goat anti-mouse horseradish peroxidase (GAM HRP) (Jackson Immunoresearch, West Grove, PA) to detect surface-conjugated MAH IgG. A modified sandwich ELISA was prepared to determine the extent to which MAH IgG retained the ability to recognize its antigen, human IgG, after conjugation. 75/25 or 50/50 PEGDA-*co*-NPA hydrogels conjugated with MAH IgG were exposed to human IgG at concentrations ranging from 0 to 50 ng/mL, followed by incubation with goat anti-human HRP. Hydrogels in both assays were incubated with Ultra-TMB (Pierce Biotechnology, Rockford, IL), and quantitative absorbance measurements were taken at 450 nm.

**Immunostaining and Cryosectioning of Hydrogels.** 75/25 PEGDA-*co*-NPA hydrogels were polymerized and conjugated with 0–0.5 mg/mL MAH IgG, as described in the previous section. Hydrogels were then soaked in Cryo-Gel (Instrumedics, Hackensack, NJ), snap-frozen in liquid N<sub>2</sub>, and sectioned into 50 μm sections with a Leica CM 1850 cryostat (Leica, Bannockburn, IL) and placed onto glass slides. The sections were then soaked three times in PBS for 5 min and blocked with 1% BSA in PBS for 1 h. After an additional PBS wash, the sections were then immunostained with Alexa 488-conjugated goat anti-mouse F(ab')<sub>2</sub> antibody fragments for 1 h. The sections were washed three times with PBS and then mounted with Biomedal Gel/Mount (Biomedal, Foster City, CA) supplemented with 1,4-diazabicyclo[2.2.2]octane and cover-

slipped. Fluorescence images were taken with a Zeiss Axioplan 2 confocal microscope (Zeiss, Thornwood, NY).

**Preparation and Characterization of Anti-Fas MAb Conjugated Hydrogels.** PEGDA-*co*-NPA hydrogels were prepared at 100/0 to 50/50 PEGDA/NPA ratios as described in the previous section. These hydrogels were then conjugated with 0–0.25 mg/mL of anti-Fas MAb (DX2 clone, R&D Systems, Minneapolis, MN) using the same reaction conditions as described previously for MAH IgG. Following conjugation to DX2 antibodies, 50 mM glycine methyl ester (Sigma-Aldrich, St. Louis, MO) was then added to the gels to quench any unreacted NHS groups within the gels. Both modified indirect and sandwich ELISAs were performed as described previously. The degree of DX2 conjugation was assessed by indirect ELISAs using goat anti-mouse HRP. Sandwich ELISAs to verify DX2 recognition and binding of recombinant human Fas (Peprotech, Rocky Hill, NJ) was performed utilizing goat anti-Fas PAb (R&D Systems) for the sandwich antibody and donkey anti-goat HRP for secondary detection (Jackson Immunoresearch).

**Apoptosis Assessment by Annexin V Assay.** Jurkat T cell lymphoma cells were a kind gift from Dr. Peter Mariner (Department of Chemistry & Biochemistry, University of Colorado). Jurkat cells were cultured with RPMI 1640 supplemented with 100 units/mL penicillin/streptomycin and fungizone and 10% fetal bovine serum (FBS). For apoptosis induction experiments, 75/25 PEGDA/NPA hydrogels were polymerized in 96-well TCPS under aseptic conditions. These gels were soaked in sterile-PBS for 5 min and then conjugated to 0.25 mg/mL MAH IgG, or 0.25 mg/mL DX2 anti-Fas MAb in PBS. After washing and blocking remaining reactive sites on hydro-



**Figure 2.** (A) Indirect ELISA for detection of MAH IgG conjugated to PEGDA-co-NPA hydrogels. PEGDA-co-NPA hydrogels were photopolymerized at 100/0, 90/10, 75/25, and 50/50 (wt) ratios of PEGDA to NPA. Hydrogels were conjugated to MAH IgG at concentrations ranging from 0 to 1 mg/mL antibody. MAH IgG conjugated to the surface of hydrogels was detected using GAM HRP. Sandwich ELISAs were formed for (B) 75/25 and (C) 50/50 PEGDA-co-NPA hydrogels conjugated with MAH IgG. Hydrogels were conjugated with 0–0.5 mg/mL MAH IgG and then exposed to Human IgG antigen. Sandwiches were completed using goat anti-human HRP.

gels using 50 mM glycine methyl ester, 5000 or 10000 Jurkat cells were added to the gels and the cells were allowed to incubate suspended atop these hydrogels for 2 days at 37 °C. Jurkat cells were then removed and placed into fresh wells following the incubation period. The Annexin Apoptosis Assay (Invitrogen, Carlsbad, CA) was then used to determine the extent of apoptosis. Cells were stained with annexin V-FITC conjugate and then counterstained with propidium iodide (PI), which allows for the differentiation between necrotic and apoptotic cells. The cells were resuspended in RPMI without phenol red and supplemented with 10% FBS, and images were taken with a Nikon Eclipse TE300 fluorescent microscope (Nikon, Lewisville, TX). The fraction of apoptotic and necrotic cells within each condition tested was quantitated by counting cells within

four random fields of view. Approximately 150–400 cells were counted per field of view.

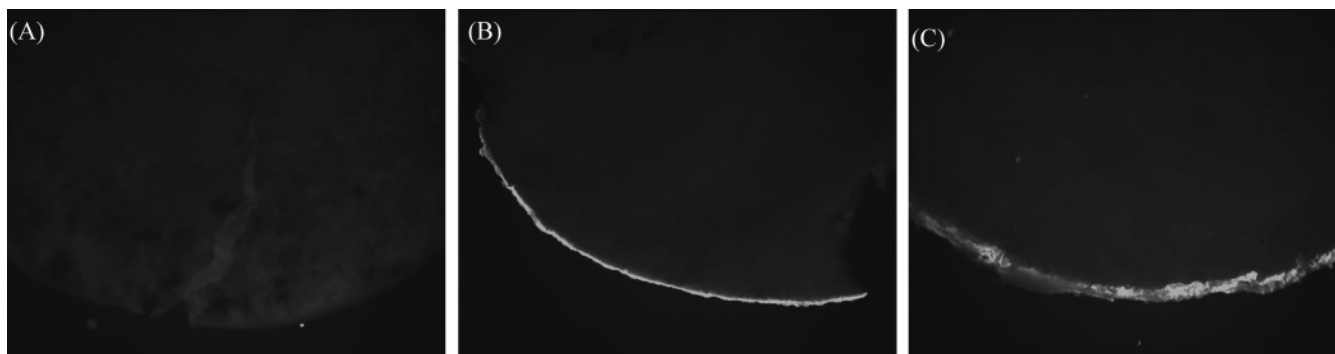
Additional apoptosis studies compared the ability of DX2-conjugated hydrogels to induce apoptosis within Fas-sensitive (Jurkat) and Fas-insensitive (I9.2) T cell lines. 75/25 PEGDA/NPA hydrogels were conjugated with either no DX2 or 0.25 mg/mL DX2 in the same manner as stated above. 10000 cells/well of either Jurkat cells or I9.2 cells (ATCC, Manassas, VA), a caspase-8 mutant Jurkat cell line that is Fas-insensitive, were then added to these hydrogels. Annexin V-FITC binding studies were then performed following 2 days incubation. Quantitation of the fraction of apoptotic cells with all samples was calculated using the same techniques as described above.

## RESULTS

PEGDA-co-NPA hydrogels were photopolymerized prior to conjugation to MAH IgG (Figure 1A). NHS moieties were utilized to react to amine groups in the antibodies (Figure 1B). Preliminary results showed that a high level of MAH IgG was conjugated to the surface of prepolymerized PEGDA-co-NPA hydrogels. Indirect ELISA assays showed higher levels of MAH IgG conjugated to these hydrogels upon both increasing MAH IgG solution concentration (0–1 mg/mL) and fraction of NPA (5–50 mol %) comprising the hydrogels (Figure 2A). The highest degree of conjugation was observed with 50/50 PEGDA-co-NPA gels; however, these gels were less mechanically robust due to the lower quantity of cross-linking PEGDA monomer present within these gels. In addition, minimal color formation was evident when 100/0 PEGDA/NPA control gels were reacted with MAH IgG antibodies, indicating that the presence of antibody was due to conjugation to NPA groups and not due to physical adsorption of the antibodies to the hydrogel matrix (data not shown). Sandwich ELISA assays performed on 75/25 and 50/50 PEGDA-co-NPA hydrogels showed that conjugated MAH IgG retained the ability to recognize human IgG antigen, with greater detection sensitivity with gels that contain higher quantities of conjugated MAH IgG (Figure 2B, 75/25 gels). High nonspecific background absorbance was observed with 50/50 gels in the absence of human IgG (Figure 2C, 50/50 gels). Detection levels for human IgG become saturated at 25 ng/mL with both gel compositions. These ELISA results indicate that monoclonal antibodies can be conjugated to a high degree to preformed PEGDA-co-NPA hydrogels without significantly compromising the abilities of the antibodies to recognize their antigens.

Cryosectioning and immunostaining of MAH-conjugated hydrogels showed that MAH IgG conjugation was limited to the hydrogel surface, as indicated by the lack of fluorescence observed within the interior of the gels (Figure 3). No fluorescence was observed with hydrogels not exposed to MAH (Figure 3A). In addition, a thicker band of fluorescence on the hydrogel surfaces was evident with higher concentrations of MAH used for conjugation, which may be indicative of greater degrees of surface conjugation (Figure 3B,C).

Conjugation of DX2 anti-Fas MAbs to PEGDA-co-NPA hydrogels was performed using the methodology and conditions developed for MAH IgG discussed in the above section. Indirect and sandwich ELISAs were performed with PEGDA-co-NPA hydrogels prepared at varying ratios (100:0 to 50:50 PEGDA:NPA). These assays show similar trends for detection of protein conjugation and antigen recognition by DX2, respectively, as seen with ELISAs with model MAH IgG antibodies (Figure 4). A higher degree of DX2 conjugation was observed with higher concentrations of antibody added for conjugation (Figure 4A). However, minimal differences in conjugation efficiency were observed with gels polymerized with different concentrations of NPA, which is in contrast to MAH IgG results. Greater



**Figure 3.** Immunological staining of MAH IgG-conjugated PEG-co-NPA hydrogel cryosections. Hydrogels were conjugated with (A) 0 mg/mL, (B) 0.1 mg/mL, and (C) 0.5 mg/mL MAH IgG. Conjugated hydrogels were then frozen and cryosectioned at 50  $\mu\text{m}$  thickness. Sections were then stained with goat anti-mouse (H+L) F(ab')<sub>2</sub> Alexa 488 conjugate. Images were taken at 4 $\times$  (objective) magnification. Fluorescently stained sections were then observed by fluorescence microscopy.

sensitivity of rFas detection was evident with higher concentrations of DX2 conjugated to both 75/25 and 50/50 PEGDA/NPA hydrogels; however, significant background was evident with 50/50 hydrogels, most likely due to rFas entrapment within these gels (due to greater pore sizes) (Figure 4B,C).

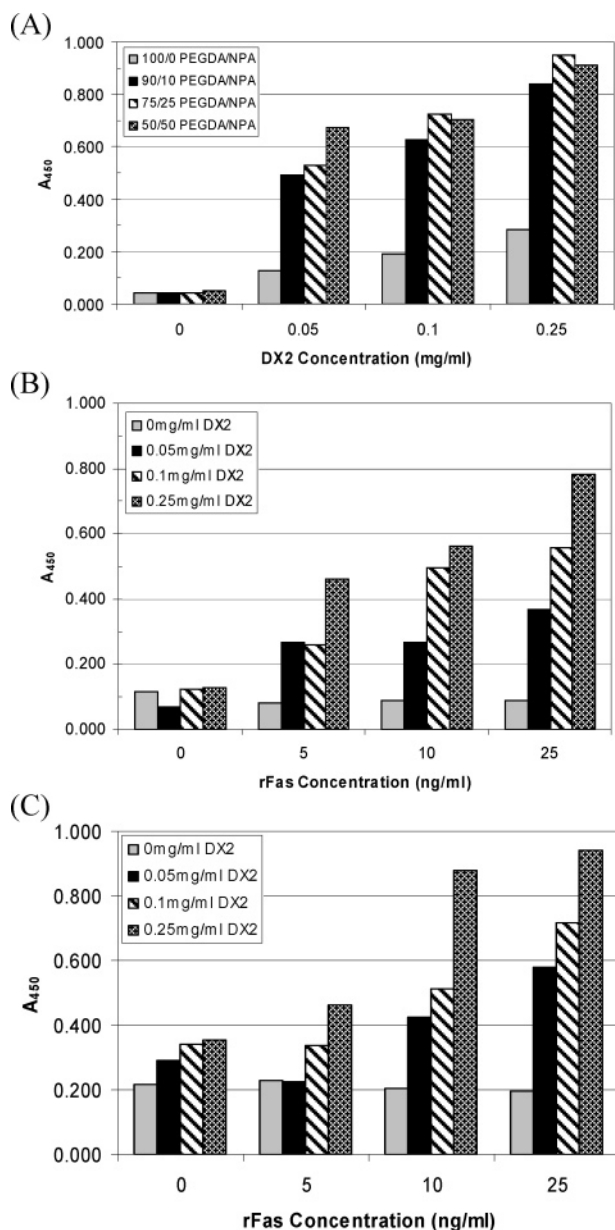
The ability of DX2-conjugated hydrogels to induce apoptosis within Jurkat cells, a model T cell line, was assessed by monitoring Annexin V binding. Annexin V binds to phosphatidylserine, which is usually located on the inner leaflet of the phospholipid bilayer of the cell membrane, but becomes translocated to the outer leaflet in dying cells. Binding of Annexin-FITC conjugates to apoptotic Jurkat cells can be visualized by fluorescence microscopy. Counterstaining of cells with propidium iodide can differentiate between apoptotic and necrotic cells, as PI can diffuse into and bind with the DNA only within necrotic cells. In our experiments, relatively few cells (less than 1%) were observed to be necrotic throughout all conditions tested, and therefore only apoptosis results are shown and discussed.

Experiments were performed to compare the ability of DX2-conjugated hydrogels to induce apoptosis within Jurkat cells compared to control hydrogels. Control hydrogels were either unconjugated or were conjugated with MAH IgG control antibodies that are not apoptosis-inducing. Fluorescence images screening for Annexin V fluorescence showed that while similar fractions of cells appear to induce apoptosis in the presence of both control hydrogel formulations, a significant increase in apoptotic cells was observed with hydrogels conjugated with DX2 (Compare fluorescence images from Figure 5A and 5B with 5C). These cell images were also used for quantitation of the fraction of apoptotic cells within each condition tested. Quantitative results confirm that an enhancement in apoptosis induction was observed with Jurkat cells exposed to DX2-conjugated gels. Only 5–7.5% of Jurkat cells were apoptotic in the presence of control gels, whereas approximately 20% of Jurkats appeared apoptotic with DX2-conjugated gels (Figure 6). Statistical analysis by student's *t*-test showed that all *p*-values were lower than 0.05, indicating statistical significance of the data. Other tests were performed to test the specificity of apoptosis induction by DX2 antibodies. I9.2 Fas-insensitive T cells were exposed to DX2-conjugated hydrogels to determine whether apoptosis induction occurred due to the specific action of the Fas/anti-Fas interaction or whether apoptosis induction was a nonspecific occurrence. Results from this study show that no enhancement in apoptosis was observed with I9.2 cells exposed to 0.25 mg/mL DX2 over levels induced with control gels lacking conjugated antibodies, whereas higher levels of apoptosis were observed within Jurkat cells as expected (Figure 7).

## DISCUSSION

Passive encapsulation technology has made great strides in providing protection for islets against cell–cell-mediated immunity by presenting a physical barrier between islet cells and activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells. However, inflammatory cytokines produced by these activated cells, such as TNF $\alpha$ , IL-1, and IFN $\gamma$ , can elicit a toxic response within islets, leading to  $\beta$ -cell destruction (13). PEG represents a functionally versatile inert polymer material that can be modified to attach biological moieties to actively promote and assist the long-term survival of islet grafts by down-regulating the immune response around encapsulated tissue. In addition, PEGylation has improved the serum half-life and stability of proteins. The studies discussed in this work describe the application of PEGylated antibodies into hydrogel network for the treatment of autoimmune diabetes, assessing the potency of antibodies conjugated to hydrogel surfaces to act in a therapeutic manner. Investigators have previously incorporated polymerizable antibodies and antibody fragments into hydrogels by free radical polymerization, but with the sole purpose for the diagnostic detection of antigens and biosensor applications (14, 15).

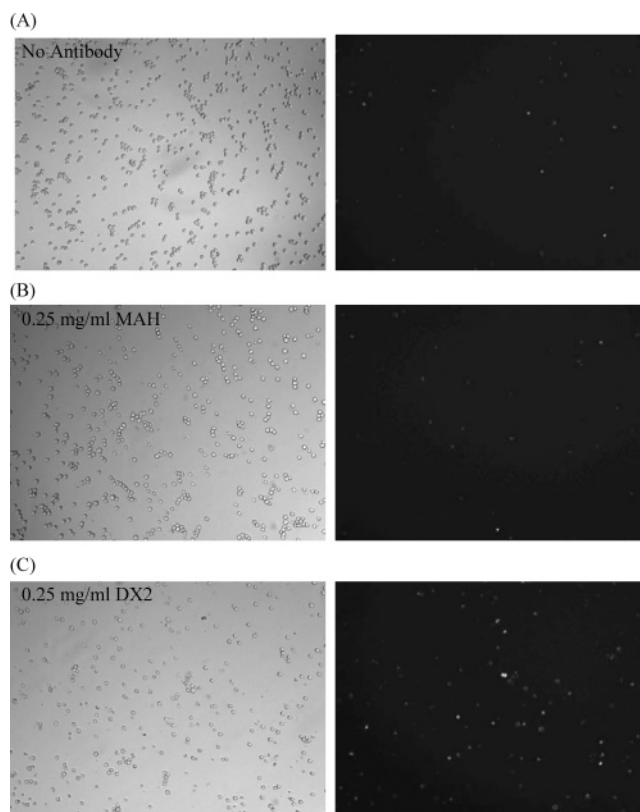
Studies have shown that conjugation of apoptosis-inducing anti-Fas IgG MAbs to PEGDA hydrogels provides a matrix that can actively work to suppress T cell mediated immunity. Fas is a 45kD cell surface protein belonging to the TNF receptor family of proteins that can induce apoptosis when engaged with Fas Ligand (FasL). Fas-mediated apoptosis is normally involved in clonal deletion of autoreactive T cells by negative selection (16). The Fas pathway is also responsible for the elimination of activated T cells following the completion of the immune response to infection. Many tissues and cell lines weakly express Fas, but Fas is highly expressed in activated, mature lymphocytes, making lymphocytes highly susceptible to Fas-mediated apoptosis (17, 18). FasL is the natural homotrimeric protein binding ligand for Fas, binding to three Fas receptor molecules to induce apoptosis (18). FasL expression is induced upon activation of cytotoxic T lymphocytes, which can then destroy Fas-expressing activated lymphocytes (17). Fas activation requires FasL multimerization or cross-linking of Fas receptors to initiate the signal transduction cascade for apoptosis (19). Binding of MAbs to Fas receptors can also induce apoptosis. The multimeric nature of Anti-Fas IgM antibodies effectively induce apoptosis by allowing for multiple cooperative binding events to cross-link several Fas receptors simultaneously on cell surfaces (20, 21). Monomeric IgG subclass anti-Fas antibodies do not induce apoptosis as effectively as the IgM subclass due to their inability to simultaneously cross-link multiple Fas receptors in the absence of exogenously added cross-linking molecules (22). DX2 is an anti-Fas monoclonal antibody (IgG1



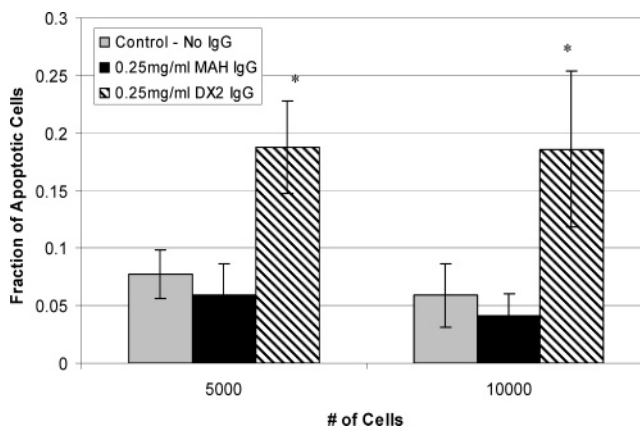
**Figure 4.** (A) Indirect ELISA for detection of DX2 anti-Fas MAB conjugated to PEGDA-*co*-NPA hydrogels. PEGDA-*co*-NPA hydrogels were photopolymerized at 100/0, 90/10, 75/25, and 50/50 (wt) ratios of PEGDA to NPA. Hydrogels were conjugated to DX2 at concentrations ranging from 0 to 0.25 mg/mL antibody. DX2 conjugated to the surface of hydrogels was detected using GAM HRP. Sandwich ELISAs were formed for (B) 75/25 and (C) 50/50 PEGDA-*co*-NPA hydrogels conjugated with DX2. Hydrogels were conjugated with 0–0.25 mg/mL DX2 and then exposed to rFas antigen. Sandwiches were completed using goat anti-Fas PAb, followed by detection with donkey anti-goat HRP secondary antibodies.

subclone) that can induce apoptosis in human T cells lines in solution upon antibody cross-linking (21, 23).

Antibody immobilization can induce cellular activation more effectively than soluble antibodies due to the ability to provide high local concentrations of antibodies for binding and receptor clustering that leads to enhanced transcriptional signal strength of activation signals or secondary messengers (24). Immobilization of anti-CD3 antibodies on solid surfaces has been shown to both enhance stimulation and activation of T cells and induce energy within T cells in the presence and absence of co-stimulatory factors, respectively (21, 24–26). Surface immobilization techniques were modified in our studies to account for the future presence of live islets to be encapsulated within

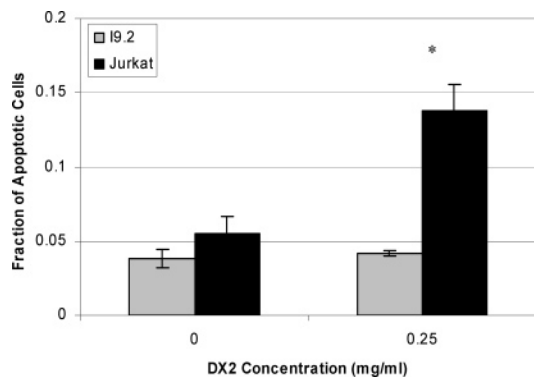


**Figure 5.** Brightfield and fluorescent images of Jurkat T cells exposed to hydrogels conjugated with various antibodies. Jurkat T cells were cultured for 2 days in 96-well tissue culture plates in the presence of PEG-*co*-NPA hydrogels conjugated with (A) no antibody, (B) 0.25 mg/mL MAH IgG control MAb, or (C) 0.25 mg/mL DX2 anti-Fas MAb. Following the culture period, Jurkat cells were removed from wells containing hydrogels and then assayed for the presence of phosphatidylserine using annexin V-FITC conjugates. Images were taken at 10 $\times$  (objective) magnification. Brightfield images show Jurkat cell density for each condition tested, whereas green fluorescence observed in fluorescent images (shown in grayscale) are indicative of annexin V-FITC conjugation to phosphatidylserine, which is indicative of apoptotic cells.



**Figure 6.** Quantitative evaluation of annexin V binding to Jurkat cells. The fraction of apoptotic cells was calculated by counting both apoptotic and total cell numbers from four random fields of view. Data were generated from both 5000 and 10000 Jurkat cells exposed to hydrogels prepared at the various experimental conditions described in Figure 4. \* denotes  $p \leq 0.04$  when results are compared to controls lacking conjugated antibody.

our hydrogel devices, which precluded the use of organic solvents for surface conjugation. Therefore, aqueous conjugation conditions at physiological salt concentrations were required. Copolymerization of PEGDA with NPA allowed for incorpora-



**Figure 7.** Quantitative evaluation of annexin V binding to both Fas-insensitive I9.2 and Fas-sensitive Jurkat T cells. 10000 I9.2 or Jurkat cells were exposed to hydrogels conjugated with either no antibody or 0.25 mg/mL DX2 anti-Fas MAb. \* denotes  $p \leq 0.001$  when results are compared to controls for the same cell line exposed to hydrogels lacking conjugated antibody.

tion of aqueous-reactive NHS groups prevalent both within the hydrogel as well as on the surface of hydrogels. Initial studies with MAH IgG model antibodies confirmed the ability to conjugate antibodies to the surfaces of NHS-reactive hydrogels, as well as demonstrated the ability of these antibodies to retain antigen recognition after conjugation. Surface conjugation was verified by cryosectioning and immunostaining of MAH IgG-conjugated hydrogels. These studies with MAH IgG also provided framework conditions to conjugate DX2 anti-Fas MAb to hydrogel surfaces at varying surface densities. Preliminary data indicated that DX2 antibodies adsorbed onto TCPS surfaces induced higher levels of Jurkat cell apoptosis compared to DX2 antibodies added in solution, suggesting that higher localized concentrations of DX2 promoted apoptosis induction (data not shown). In accordance with these results, immobilization of DX2 antibodies onto the surface of PEGDA-co-NPA hydrogels also enhanced apoptosis of Jurkat cells over passive, unconjugated hydrogel controls. These studies also showed that apoptosis induction was a unique quality observed solely with DX2 antibodies and demonstrated the specificity of apoptosis induction with DX2, as only Fas-sensitive cells underwent apoptosis in the presence of DX2.

The results presented in this study demonstrate the utility of surface conjugation of immunosuppressive moieties into encapsulation matrixes to down-regulate the immune response to transplanted tissues. Although the degree of T cell apoptosis with current systems may not be high enough to fully down-regulate immune responses to grafted tissue, this work demonstrates the potential that activation of passive barrier holds, whereby biological processes may be altered by the addition of biological moieties, favoring enhanced survival and function of encapsulated grafted tissue. Modifications to improve current systems, such as developing systems with higher degrees of grafting/clustering of immunosuppressive proteins on hydrogel surfaces, may lead to adequate and sustainable immunosuppression and allow for long-term graft survival. Research into active barrier systems will hopefully change traditional views and begin addressing the different shortcomings associated with current encapsulation systems pursued for treating diabetes mellitus.

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