Abstract

In Diabetes mellitus type 1, autoimmune destruction of the pancreatic β-cells results in loss of insulin production and potentially lethal hyperglycemia. As an alternative treatment option to exogenous insulin injection, transplantation of functional pancreatic tissue has been explored. Previous studies in our lab have shown that MIN6 cells encapsulated as aggregates maintain their viability throughout 4 weeks of culture in 20% oxygen as many 150 μL aliquots in 1.5 mL centrifuge tubes at a concentration of 3.4 x 10⁶ cells/mL. While we methacrylate our PEG with 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (Irgacure 2959), produces free radicals which attack the vinyl carbon-carbon double bonds of dimethacrylated PEG (PEGDM) inducing crosslinking at the chain ends. Crosslinking can be achieved within 10 minutes. PEG hydrogels constructed in such a manner have been shown to favorably support cells dependent on oxygen.

An inherent consequence of encapsulation is isolation of the cells from a vascular network. Supply of nutrients, notably oxygen, is therefore reduced and limited by diffusion. This reduced oxygen availability may especially impact β-cells whose insulin secretory function is highly dependent on oxygen. Capsule composition and geometry will also impact diffusion rates and lengths for oxygen. Therefore, we also describe a technique for identifying hypoxic cells within our PEG capsules. Infection of the cells with a recombinant adenovirus allows for a fluorescent signal to be produced when intracellular hypoxia-inducible factor (HIF) pathways are activated. As HIFs are the primary regulators of the transcriptional response to hypoxia, they represent an ideal target marker for detection of hypoxic signaling. This approach allows for easy and rapid detection of hypoxic cells.

A video component of this article can be found athttp://www.jove.com/video/3521/.

Protocol

1. PEGDM synthesis and photoactive PEGDM macromer solution preparation

1. Weigh 2g of PEG (linear, 10,000Da) into a 40mL glass vial with a hard plastic cap.
2. Add approximately 308μL of methacrylic anhydride and loosely cap the vial.
3. Microwave the vial on high for 2 minutes in a standard domestic microwave. Heating heat resistant gloves, thoroughly vortex the vial, then microwave on high for an additional 5 minutes.
MIN6 cells are cultured in RPMI 1640 medium supplemented with 10% FBS, 7mM glucose, 100 units/mL penicillin/streptomycin, and 0.5 μg/mL amphotericin B in a humidified environment at 37°C and 5% CO₂.

To release the cells from the treated culture flask surface, aspirate the medium, rinse the cells with 37°C calcium/magnesium-free HBSS, aspirate the HBSS, then add 2 mL of 37°C trypsin-EDTA solution and return the cells to the incubator for 3 minutes.

Firmly jar the side of the flask to knock the cells free, then add 8 mL of 37°C medium and vigorously pipette the cell suspension up and down taking care not to introduce bubbles.

Pipette the cells into a sterile 15 mL centrifuge tube and perform a cell count using a hemocytometer or other cell counting procedure.

In preparing to infect the cells with the maker virus, first determine the total number of cells to be infected and calculate the volume of virus suspension required to achieve the desired multiplicity of infection (MOI) (50 to 100 infectious particles per cell).

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex thoroughly to ensure dissolution of the PEGDM. We typically prepare 1mL of this solution at a time. Dialyze the solution against deionized water for 5 days in dialysis tubing with a molecular weight cut-off of 1,000 Da. Replace the water once below (3.2-3.9).

Collect the PEGDM by vacuum filtration and allow it to dry. Precipitate PEGDM in 200 mL of cold, stirred diethyl ether by adding the solution dropwise. Dissolve the PEGDM in an adequate amount of deionized water (typically 100 -150 mL). Dialyze the solution against deionized water for 5 days in dialysis tubing with a molecular weight cut-off of 1,000 Da. Replace the water once daily.

Aliquot the solution into appropriate containers and freeze at -80°C overnight. Lyophilize the solution for 4 days to yield a purified white PEGDM powder. Store the powder at 4°C when not in use.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.

To prepare a photoactive PEGDM solution, first prepare a 0.5 weight percent photoinitiator stock solution by dissolving Irgacure 2959 in deionized water. Vortex the solution and store it in the dark.
A representative example of MIN6 aggregates encapsulated in a PEG hydrogel is shown in Figure 4. The crosslinked gel will be solid throughout, taking the shape of the vessel in which the reaction was performed. A gel with smooth outer surfaces is preferable for implantation to aid in prevention of a foreign body response. Within the gel, cell aggregates should be fully enclosed in the matrix and homogenously distributed to allow for better nutrient transport.

Representative images of hypoxic signaling in MIN6 aggregates are pictured in Figure 5. Identical MIN6 aggregates infected with the marker virus were cultured in either 20% O₂ (5a) or 1% O₂ (5b) for 44 hours before image capture.

**Figure 1.** Schematic of the activity of our hypoxia marker system. Adenoviral insertion of the Ds Red DR gene and upstream HRE promoter allows for hypoxia-induced production of the fluorescent protein under control of HIF-1.

**Figure 2.** Procedural flow chart for the single-step encapsulation of dispersed MIN6 cells in a crosslinked PEGDM hydrogel. For each gel, the dispersed cells are suspended in 40μL of the photoactive PEGDM macromer solution. This is placed in a decapitated 1mL syringe and the hydrogel is formed under 365nm UV light after 10-12 minutes. Upon completion, the hydrogel disk is removed from the syringe, washed and placed in a medium-filled well plate for incubation.

**Figure 3.** Procedural flow chart for the dual-step encapsulation of aggregated MIN6 cells in a crosslinked PEGDM hydrogel. For each gel, a half-gel is first formed by UV crosslinking of 20μL of the photoactive PEGDM macromer solution for 8 minutes. MIN6 aggregates are carefully suspended in an additional 20μL of photoactive macromer solution which is added on top of the pre-formed half-gel. The full gel is formed by an additional 8 minutes of UV exposure with aggregates fully encapsulated in the medial plane of the gel.
Figure 4. Image of a 40μL hydrogel under 20X magnification. MIN6 aggregates (~400,000 total cells) are clearly seen within the gel. Hydrogel diameter is approximately 6mm (bar = 1mm).

Figure 5. Fluorescent hypoxia signaling in aggregated MIN6 cells in a PEGDM hydrogel. Cells that were encapsulated and then placed in incubation at 20% O₂ for 44 hours do not display hypoxia signaling (a) while cells that were encapsulated then incubated in 2% O₂ for 44 hours display clear, ubiquitous signal. (bar = 100μm) (b).

Discussion

The method presented here offers a quick and simple technique for cell encapsulation in a PEG hydrogel with minimal use of non-physiological conditions. PEG represents a very useful encapsulation material for its biocompatibility and ease of modification. Simple variation of PEG percentage in the photoactive solution, for instance, may be used to adjust mechanical properties, such as compressive modulus, and transport properties through pore size. Also, PEG is easily modified by the addition of side chains. PEG hydrogels, therefore, represent both a promising clinical device and a flexible platform for in vitro research.

A method for tracking hypoxia in PEG-encapsulated cells has also been presented. This method is useful for the simplicity of hypoxia detection and for avoiding the need to sacrifice the cells of interest. The technique may be applied to a variety of types of cells in a variety of conditions making its usefulness broad. For instance, hypoxia as a cue for stem cell differentiation may be tracked in stem cell micromass cultures. However, this method can only be applied to disperse cell systems or system in which dispersed cells are later aggregated. Also, detection of the fluorescent signal may be difficult in larger or denser tissues.

Disclosures

No conflicts of interest declared.

Acknowledgements

Thanks to the Kristi Anseth lab of the University of Colorado, Boulder for generously supplying MIN6 cells. Funding for this project has been provided by the NSF.

References


