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Article

Design of Antibody-Functionalized Polymeric Membranes for the Immunoisolation of Pancreatic Islets

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1. Materials and Methods

Isolation of BALB/C mouse islets

All procedures were carried out in accordance with institutional guidelines and following experimental protocols approved by the Italian Ministry of Health. Five-week-old BALB/C male mice, supplied by Harlan Laboratories, were housed on a 12 h light/dark cycle and had access to food and water ad libitum except when fasted. All animals were sacrificed on the day of organ harvest and islet isolation. Islets were isolated from healthy 6–10 week old mice following standard islet isolation procedures [197]. All surgeries were carried out under isofluorane anaesthesia and surgical tools were cleaned and autoclaved for all procedures. Briefly, mice were anesthetized and euthanized by cervical dislocation, the abdomen exposed by laparotomy, and the liver was inverted to reveal the common bile duct. The ampulla of Vater was clamped to close the route from the common bile duct to the duodenum. 2 mL collagenase (Liberase TL, Roche Diagnostics) at 0.2 mg/mL in a modified Hank's Balanced Salt Solution (HBSS, Sigma-Aldrich) was injected into the common bile duct to distend the pancreatic tissue. Pancreata were excised and stored in groups of 2-3 per 50 mL falcon tube containing 5 mL of collagenase solution, on ice for <60 min before incubation in a water bath at 37°C for 15 min. Falcon tubes were then manually shaken for 10 seconds and the enzymatic reaction was stopped by adding an ice-cold supplemented Minimum Essential Medium (MEM) (Invitrogen) containing 10% Fetal Bovine Serum (FBS) (Invitrogen). Following the enzymatic digestion the tissue was washed and centrifuged for 2 minutes at 290xg and 4°C, the supernatant discarded, and the pellet resuspended in supplemented MEM and filtered through a 380 μm mesh to remove the large pieces of exocrine tissue and undigested tissue. The filtered tissue was then washed and purified by discontinuous gradient centrifugation, trough sequentially layered 1.11 g/mL, 1.096 g/mL and 1.066 g/mL gradient solutions (glucose/polysucrose, Mediatech). Centrifugation (1500xg, 4°C, 18 min) was carried out with no brake so not to disturb the density interface, and resulted in the accumulation of islets at the density interface between 1.096 g/mL and 1.066 g/mL and exocrine fragments in the pellet. Islets were then washed, handpicked and cultured with Roswell Park Memorial Institute Medium (RPMI-1640) (Invitrogen) supplemented with 10% FBS, 100 U/mL Penicillin and 100 μg/mL Streptomycin (all supplied from Invitrogen), in a humidified incubator at 37°C with 5% CO2.

2. Results

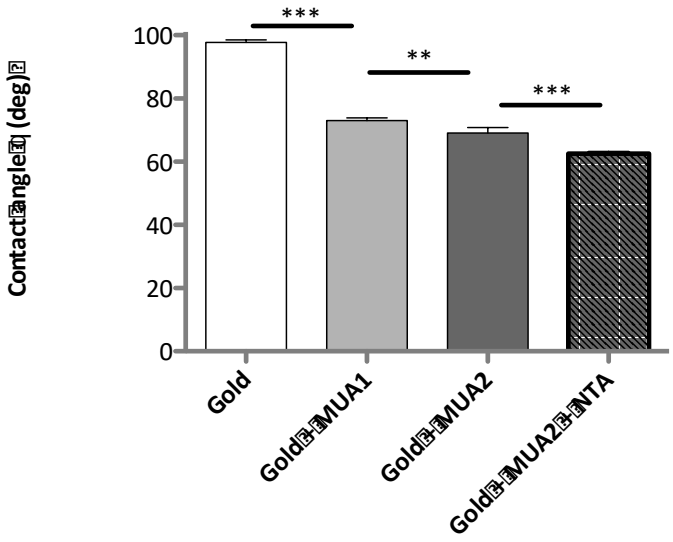

Figure S1. Values of contact angle of either gold surface, gold surface after 12 h and 60 h incubation with MUA, or gold surface after 60h incubation with MUA and NTA. Reported values have been averaged over 5 measurements. Error bars represent standard deviation (SD). (*** $p < 0.001$, ** $p < 0.01$)

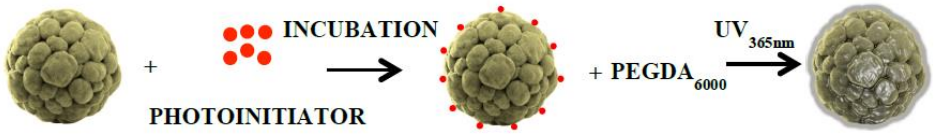

Figure S2. Schematic representation of the experimental design for the conformal encapsulation of islets through photopolymerization. Islets are incubated with the photoinitiator that adsorbs on the islet surface. The islet-photoinitiator complex is incubated with the PEGDA and exposed to UV to encapsulate the islet, inducing the crosslinking of the polymer.