Development of polyurethane - zwitterionic acrylate double network hydrogels as membranes for macro-device based cell encapsulation

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Statement of Purpose: Treatment of Type 1 diabetes (T1D) with islet transplantation has been shown to be a viable option.[1] Even though the results are encouraging, the treatment is fraught with drawbacks like thrombosis/bleeding, low islet availability, poor engraftment, etc. leading to need for repeat islet transplantation and chronic immunosuppression. Protecting islets from the immune system by encapsulating in various forms like microcapsules, fibers and macro devices have shown promising results in experimental models.[2-3] Compared to planar devices, encapsulation efficiency of bead and fiber devices is very low leading to large device volume and consequently high barrier for nutrient and oxygen diffusion. Most of the current devices in clinical development are planar devices made of PTFE, Polypropylene, etc. These devices are prone to fibrosis causing fouling. Hydrogel based macro devices have been explored in the early 1980 and 90's but no clinical development has been reported despite showing some excellent results.[4-5] Here, we report a novel polyurethane - zwitterionic acrylate double network hydrogel based membrane suitable for cell encapsulation macro device development.

Methods: All polyurethane polymers (HydroMed Series) were purchased from AdvanSource Biomaterials. 3-[[2-Methacryloyloxy)ethyl]dimethylammonio]propionate (CBMA), 2-methacryloyloxyethyl phosphorylcholine (MPC) and [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (SBMA) monomers were used to synthesize double network by UV initiated radical polymerization using 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone and N,N′-Methylenebis(acrylamide) as initiator and crosslinker respectively. HGX means hydrogel with average X % water content and BGX/Y means blend of HGX and HGY.

Results: Polyurethane hydrogel membranes were fabricated by dip coating process from 95:5 ethanol:water solution. The process was optimized to obtain membrane with thickness ranging from average 80 to 170 micron depending on the water content of the Polymer (result not shown (RNS)). Thickness of the membrane depends on fabrication condition like solution viscosity, solvent type, draw speed, etc. The above thickness is suitable as it provide diffusion barrier to radicals (ROS and NO).[6] The membranes were then evaluated for permeability using dye-tagged dextran 150 kDa and 10 kDa mol. Wt. Fig 1A & B, diffusion of dextran increased with increase in water content. This was expected as more free volume is available for the diffusion of dextran. The HG90 and HG80 membranes were highly permeable for 10 kDa dextran representing insulin and were highly effective at preventing the diffusion of 150 kDa dextran representing IgG confirming immune isolation potential of the membranes. To improve diffusion rate by decreasing thickness as well as to improve mechanical property, blending of the hydrogel was studied. Blending resulted in decrease of thickness as well as increase in tensile modulus of the membranes (RNS). To further understand the relationship between permeability and water content, Cryo-SEM of samples was performed. Fig 1C shows HG90 has an interconnected network structure with pore size of ~200 nm but HG80 structure was not distinguishable. Blending of HG80 and HG90 at 1:1 wt. ratio resulted in interconnected pore structure with size of ~100 nm: sufficient for high diffusion of insulin.

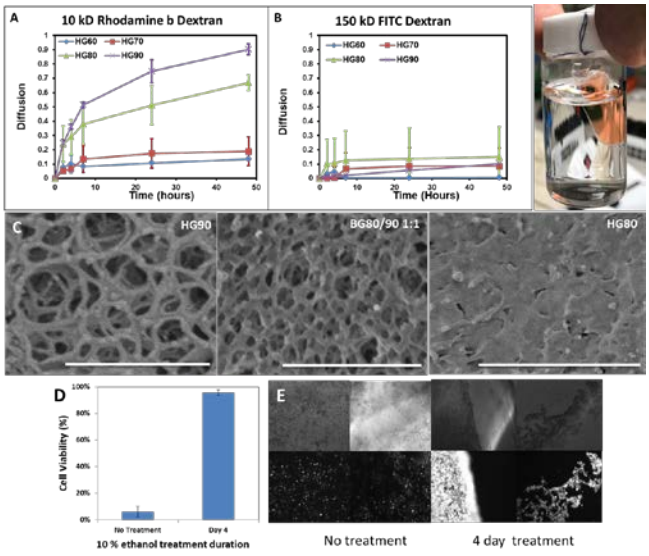

Figure 1. A&B) Diffusion of dye tagged dextran through membrane. C) Cryo-SEM image of hydrogel and blend (scale = 1 micron) D &E) viability and imaging of GFP-expressing Min6 cells

Membranes were filled with GFP-Min6 cells and evaluated to determine the ability to support cell viability on encapsulation. The membrane was toxic due to toxic leachables which were subsequently removed by treatment with 10% ethanol solution. Mechanical properties of membranes were also characterized. The tensile modulus of the HG80 and HG90 were 143.67 $\pm$ 77.17 and 28.59 $\pm$ 11 kPa whereas the blend tensile modulus was 93.32 $\pm$ 27.35 kPa.

Double network fabrication was carried out using three zwitterionic monomers. Initial process optimization was done using MPC monomer. Addition of zwitterionic monomers resulted in high swelling of membranes. For the MPC, 10% monomer concentration was optimum in terms of swelling behavior (90% water content) and mechanical properties. With 10% CBMA monomer, the swelling was very high (93%) and low mechanical properties. For 10% SBMA monomer, water content (89%) was low and high mechanical properties observed.

Conclusions: We have developed and characterized membranes suitable for addition to macro -devices for cell encapsulation. Membrane-macrophage interaction studies (in vitro and in vivo) are currently in progress.

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