Developing Hydrogel Systems for the Formation of Islets of Langerhans from Single β-Cells

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Introduction: Type 1 diabetes mellitus (DM) is a disease characterized by destruction of the β-cells in the pancreatic islets of Langerhans[1]. The current primary treatment is daily multiple insulin injections. This treatment cannot provide normal physiological insulin on-demand feedback control, and therefore the insulin amount is not tuned to gylcemia. The goal of this project is to develop hydrogel based carrier systems that allows single β-cells to form functional islets in less than two weeks and in the meantime to allow complete vascular network formation in the hydrogel in less than 2 weeks for physiological function of islets (Fig. 1). We started from MIN-6 and primary β-cells for developing the hydrogel system, and then we will use human induced pluripotent stem cells (iPSCs) derived β-cells in the next set of in vitro and in vivo experiments. With optimized hydrogel system, we expect long-term survival and insulin secretion from islets by providing proper permissive support, relieving the immediate needs in vascular support due to high permeability to nutrient supply and waste removal, and promoting angiogenesis for proper insulin release and feedback. The working hypothesis is that β-cells may behave differently, in terms of viability, adhesion, morphology, proliferation, islet formation and function inside the hydrogels with different stiffness and adhesive components.

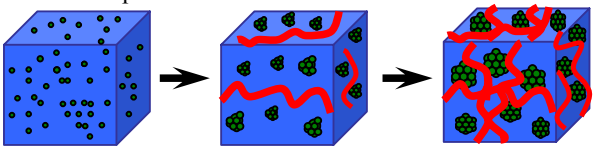
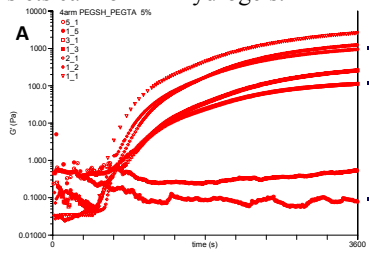


Fig.1: Model for the islets and vascular network formation in hydrogel system.

Materials/Methods: An in situ cross-linkable hydrogel based on multi-Arm PEG and modified laminin peptides, and other additives had been developed. Hydrogels formed at physiological conditions due to conjugate addition reactions. A rheometer (AR1000, TA Instruments Inc.) was used for the rheological characterization of all hydrogel samples. Murine pancreatic β-cells of the MIN-6 cell line were entrapped in hydrogel. LIVE/DEAD staining was used to evaluate cell viability of the MIN-6 β-cells inside the hydrogels. For β-cells glucose-stimulated insulin secretion, samples were incubated in a low glucose concentration solution for 45min, and then placed in a high glucose concentration solution for 1 h. The high glucose solutions were collected for insulin measurement with an Insulin ELISA kit.

Results/Discussion: Figure 2A shows the time sweep profiles of storage modulus (G') for the ratios of functional groups on multi-arm PEG and PEG acrylate at the same PEG concentration as 5%. The range of hydrogel storage modulus is increased from about 100Pa to 2600Pa (Fig.1B). As for cell viability, more than 90% cells survive in all samples up to 7 days. And β-cells entrapped in 5% 1:2 and 1:3 hydrogels started to aggregate after 4 days to form islets (Fig. 3). For insulin secretion, at 4 days, the β-cells encapsulated in the samples of 1:2 and 1:3 hydrogels secreted significantly higher amounts of insulin, and the cell-secreted insulin amount almost caught 2D plate control (Fig. 4). Peptides conjugated to hydrogels benefits islets formation (Fig. 5). With the increase of LN peptide concentrations, more islets can form in hydrogels.



Hydrogel	G' at 1hr (Pa)
1:1	2594
1:2	1248
2:1	953
1:3	259
3:1	113

Fig. 2: Rheological characterization of PEG based hydrogels with different ratios of functional groups on multi-arm PEG and PEG acrylate.

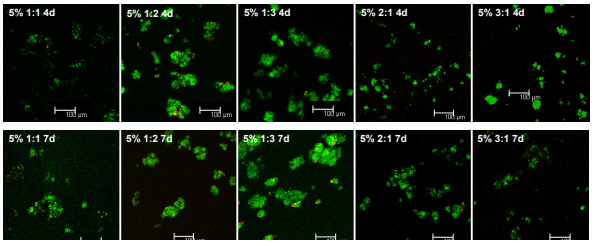


Fig. 3: LIVE/DEAD staining of β-cells encapsulated in PEG based hydrogels with different ratios of functional groups

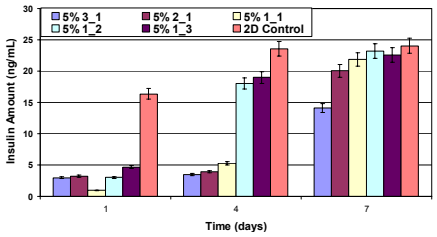


Fig. 4: Glucose-stimulated insulin secretion of β-cells encapsulated in PEG based hydrogels with different ratios of functional groups

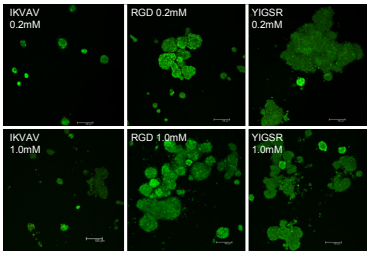


Fig.5: LIVE/DEAD staining of β-cells encapsulated in peptide functionalized PEG based hydrogels as 5% 1:2 at 12 days

Conclusions: Optimized injectable hydrogel system based multi-Arm PEG and modified laminin peptides, can provide permissive environment suitable for the formation of islets in vitro with insulin releasing function.

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References:

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