Extrahepatic transplantation of 3D cultured stem cell-derived islet organoids on microporous scaffolds

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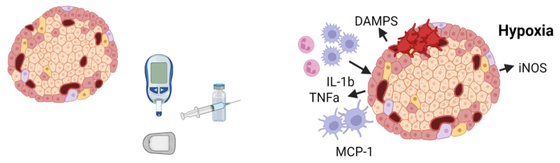
Background

- 1.6 million Americans are living with Type I Diabetes (T1D)
- Incidence and prevalence of T1D are rising with a current annual cost of $15 billion
- T1D is commonly managed by use of exogenous insulin, but risks of chronic complications persist
- Transplantation of native islets offers superior glucose control, but therapeutic benefits are hindered by lack of donors as well as poor islet survival[1, 2, 3]
- Stem cell derived islets offer an alternate method of generating β-cells[4]
- By providing scaffolding to the cells and creating aggregate clusters, we investigate the functionality of stem cell derived islet organoids
- Transplantation of beta cells immediately exposes cells to harsh inflammation that heavily involves the innate immune system



Methods

- Human pluripotent stem cells were used to derive β-cells through a 6-stage differentiation protocol [5]
- Single cell dispersals were seeded onto microporous PLG scaffolds at either S5D1 or S6D1, cells were reaggregated into clusters at S6D7 and seeded onto scaffolds at S6D10
- Transplantation occurred on day of seeding into the epididymal fat pads of diabetic NSG mice
- Analysis occurred through glucose stimulated insulin secretion (GSIS), in vivo glucose monitoring, serum C-peptide analysis, intraperitoneal glucose tolerance test, and flow cytometry for immune cell markers

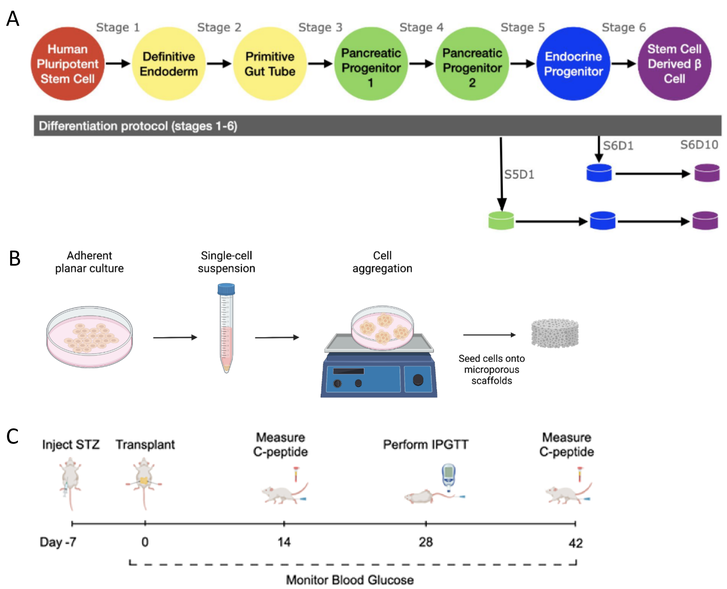


Figure 1: (A) β-cell differentiation and scaffold seeding protocol for S6D1 and S5D1 . (B) Reaggregation protocol and scaffold seeding for clusters. (C) Mouse transplantation and monitoring protocol from day -7 to day 42

Results

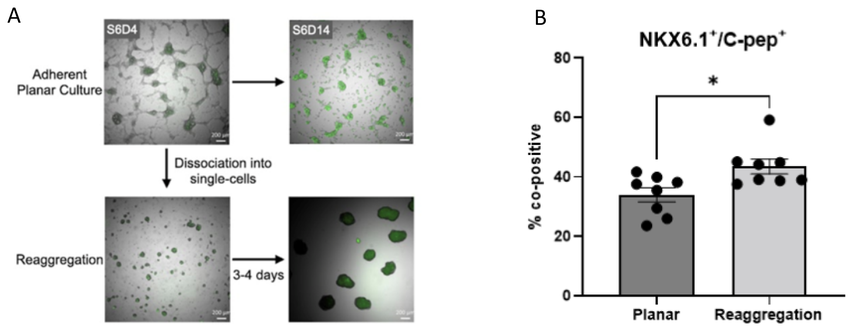


Figure 2: (A) Representative brightfield images of cells in planar culture and after reaggregation during the stem cell derived β-cell stage. 488 channel overlaid with brightfield images indicates insulin secretion of reaggregated and planar cells (B) Percent of co-positive cells for NKX6.1 and C-peptide for planar and reaggregated cells. α = 0.05, *p<0.05

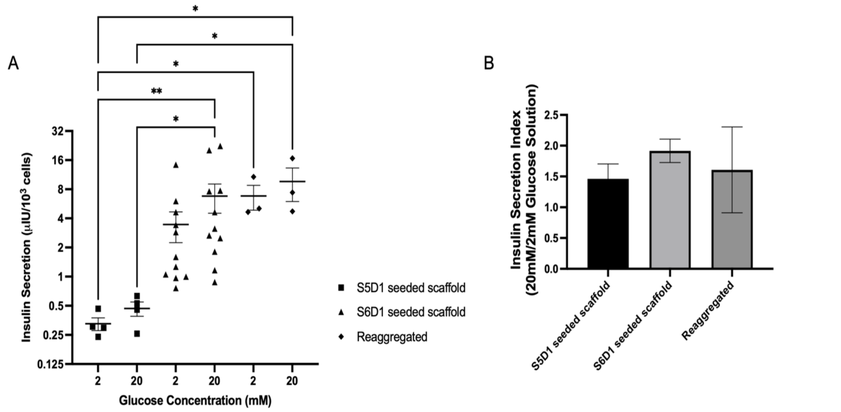


Figure 3: (A) Insulin secretion normalized to cell number during GSIS assays performed at S6D10. α = 0.05, n=4 S5D1, n=11 S6D1, n=3 Reaggregated, *p<0.05, **p<0.01. (B) Insulin secretion indexes for each condition

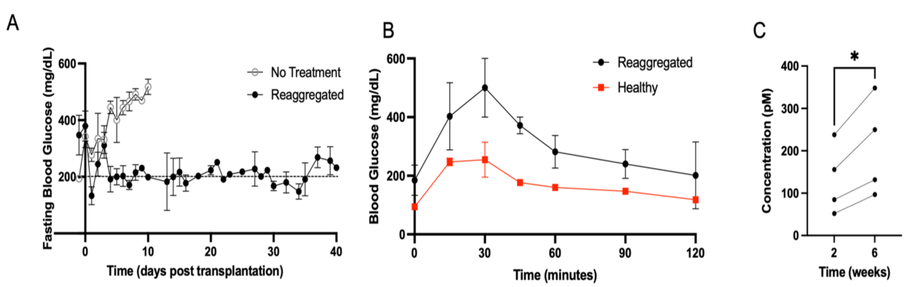


Figure 4: (A) Fasting blood glucose measurements from STZ-induced diabetic mice that received 10M reaggregated cells transplanted on two PLG scaffolds (5M cells/scaffold) (n=3) or no treatment (n=4). Blood glucose was measured daily for 10 days following transplantation, then 3 times per week for the duration of the study. (B) Blood glucose measurements taken during intraperitoneal glucose tolerance testing of mice that received reaggregated cells (n=2) and healthy mice (n=2). Mice were fasted for 4-6 hours, then received an intraperitoneal injection of glucose at 2g/kg body weight at t = 0. (C) Circulating C-peptide levels at 2 and 6 weeks post transplantation measured from serum collected through saphenous vein blood draw. Paired t-test performed (n=4 both time points, α =0.05).

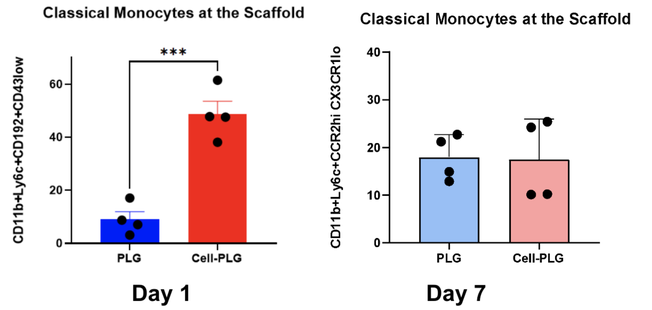


Figure 5: (A) Classical monocytes detected at 1 day post transplantation for scaffolds transplanted with or without stem cell derived beta cells. α = 0.05, ***p<0.001. (B) Classical monocytes detected at 7 days post transplantation for scaffolds transplanted with or without stem cell derived beta cells. α = 0.05.

Discussion

- Cells in later stages can reaggregate to form islet organoids and be seeded onto the scaffold for transplantation
- Reaggregated cells had greater insulin secretion in comparison to cultures from S5D1
- Reaggregated cells showed a significant difference in insulin secretion during GSIS assays at S6D10 in comparison to S5D1 seeded scaffolds
- Reaggregated clusters were able to reverse hyperglycemia though levels were not fully normalized
- Significantly higher count of monocytes at scaffold with cells, indicating dendritic cell response to foreign antigen
- Innate immune response levels off after 7 days

Conclusions

- 3D stem cell derived islet organoids supports cell maturation and enhances function
- Organoids produce insulin and reduce hyperglycemia
- S6D1 seeded scaffolds and reaggregated clusters secreted more insulin than S5D1 seeded scaffolds
- *Day 4 time point for immune cell study needs further investigation in order to understand how the innate immune system acts over time*
- Cell seeded scaffold transplantation causes an inflammatory immune response 24 hours after transplantation. Future therapeutics are needed to protect stem cell derived islet organoids from inflammation-related cell death

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