Design considerations for alternate site nanofiber islet encapsulation devices

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INTRODUCTION AND OBJECTIVE

Nanofiber scaffolds could improve islet transplant success by physically mimicking the shape of extracellular matrix and by acting as a drug delivery vehicle. Much interest has arisen in improving the suitability of alternate islet transplant sites to replace the standard portal vein infusion technique. Scaffolds can be made into shapes suitable for the alternate transplant site chosen. If a subcutaneous site is selected, for instance, a flat pocket can be constructed of nanofibers. Any site must be pre-vascularized or very quickly vascularized following transplant in order to prevent hypoxia induced islet necrosis. The local release of the S1P pro-drug FTY720 induces diameter enlargement and increases in length density (Sefcik 2011). It was hypothesized that the local release of FTY720 would still induce increases in diameter and length density despite changes in cell physiology brought on by hyperglycemia. Preliminary data not only support this hypothesis, but suggest that local release of FTY720 may in fact work better in a diabetic environment.

The degradation rate and polymer characteristics are important interdependent considerations in the context of islet transplant device material. First, the release profile of incorporated factors must be timed to positively affect cellular function during the critical 7-10 days following islet transplant. Second, the polymer in nanofiber form or its soluable degradation products must not adversely affect islet function. Third, the construct must be durable enough to implant.

Table 1 : Polymer degradation characteristics

Polymer	Characteristic
PCL	Longer degradation time
PLAGA	Medium tunable degradation time
PHBV	Longer tunable degradation time

Equally important is the device construction. If a minimally invasive procedure is desired for implant of islets after a site preconditioning period, normal nanofiber contraction must be countered. Taking into consideration these requirements, the objective of this study is to determine a composite nanofiber scaffold that will support islet transplant when made into a device and to further investigate the microvascular remodeling that occurs under local release of FTY720 as a method to precondition the site.

MATERIALS AND METHODS

Nanofibers are electrospun from polymer solutions of PLAGA (poly(DL-lactide-co-glycolide), 50:50 LA:GA), PCL (polycaprolactone), PHB (polyhydroxybutyrate), PHBV (poly-3-hydroxy butyrateco-valerate) or the same PLAGA polymer mixed in equal parts with PCL, with and without FTY720. Electrospinning solvents of Methylene Chloride and HFP (1,1,1,3,3,3-Hexafluoro-2-propanol) were used. DMSO (dimethyl sulfoxide) was used to solvate FTY720 prior to addition to the HFP solvated polymer solutions. Enduragen® was used to modify the pocket design for preconditioning the subcutaneous site. Repeated measures of microvessel metrics were made from light microscopy images obtained over the 7 days following randomly oriented nanofiber mat implants in dorsal skinfold window chambers. RAVE, a MATLAB program (Seaman 2011), was used to quantify blood vessel metrics. Streptozotocin was used to induce chemical diabetes in C57bl6/j mice. Islets are assessed for viability (Propidium Iodide and Fluorescein Diacetate staining) and function (response to 28mM and 2.8mM glucose).

RESULTS AND DISCUSSION

Results from in vitro islet viability data have revealed interesting differences between polymers. Murine islets cultured with PLAGA nanofibers for 48 hours show a reduced viability (45% fibers verses 70% for media only controls), however the addition of FTY720 improves the viability to at least that of untreated islets (76%). No significant differences in Stimulation Index have been found (1.85 controls verses 2.0 PLAGA fibers). Human islets tested with PLAGA/PCL nanofibers, which are much more durable than fibers made of PLAGA alone, displayed a decrease in viability compared to the media only controls (68% PLAGA/PCL verses 78% media only) and an unexpected further decrease with the addition of FTY720 (60%). Both PHB and PHBV performed better in this assay (75% and 79% respectively, Figure 1). The Stimulation Index of islets tested in culture with fibers (no direct contact) verses in culture

between 2 layers of nanofibers (pocket, direct contact) for 24 hours demonstrated greater stimulation index when the islets were in direct contact verses no direct contact except for PHB (PLAGA/PCL, PLAGA/PCL + FTY720, PCL, PHB, were included).

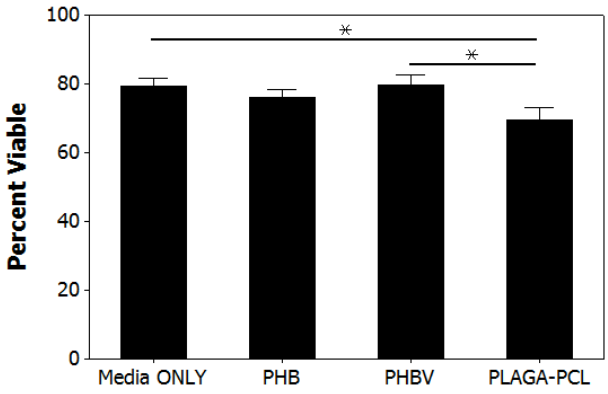

Figure 1 : Human islet viability in vitro based on PI and FDA staining. *p<0.05 T-test

Analysis of images from dorsal skinfold window chambers revealed the length density of vessels was significantly increased between recently induced moderately diabetic and non-diabetic animals ($p<0.05$) on Day 0 (the day of implant, Mod-Dia 3.37%, Non-Dia 4.55%) and Day 3 (Mod-Dia 3.53%, Non-Dia 4.64%). The difference between days was only significant at Day 7 compared to Day 0 in the moderately diabetic animals length density (Day 0: 3.37%, Day 7: 4.22%). Visual inspection of blood vessels in the peritoneal wall near a nanofiber pocket made of PLAGA/PCL displayed an increase in microvessel density when FTY720 was present 30 days post-implant.

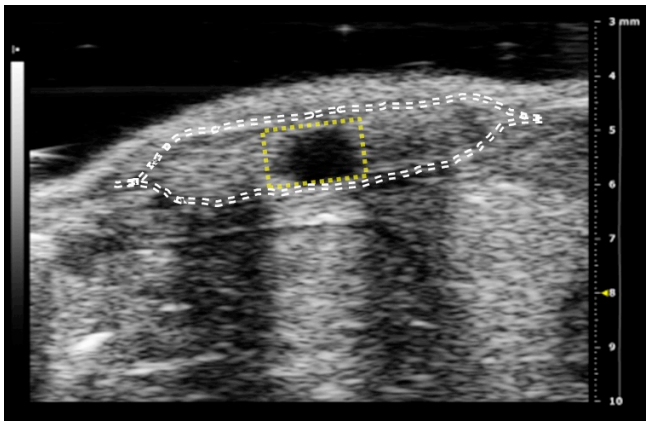

Figure 2 : A void exists one month following subcutaneous implant (outlined in yellow)

Preconditioned nanofiber pockets have been injected with islets. Confirmation of the void has been gathered using ultrasound (Figure 2, pocket outlined in with white dashed line). During the second procedure, only a 28G needle breech of the skin is required. Ultrasound can be used to guide the needle insertion.

CONCLUSIONS

Direct contact of islets with nanofibers promotes islet function in vitro, apparently over coming any possible adverse effect from soluble degradation products that may exist at higher concentration when in culture than would be experienced in vivo. PHB and PHBV support islet viability better than PLAGA/PCL does. FTY720 has been shown by our group (unpublished results) and others (Truong 2007) to have no harmful effect on islets in culture. The addition of FTY720 may increase the degradation rate, which would explain the unexpected decrease in islet viability when FTY720 was loaded in the fibers. Local release of FTY720 from nanofibers stimulates significant increases in blood vessel length density in non-diabetic animals and in moderately diabetic animals within a 7 day time frame relevant to islet transplant. An insert within the pocket allows a truly minimally invasive procedure for delivery of islets to the pocket following a site preconditioning period. Together these data support the continued investigation of local release of FTY720 from nanofibers made into a fiber pocket for improvement of alternate site islet transplant.

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