A Collagen-Chitosan Scaffold as a Possible Highly Vascularized Ectopic Site for Pancreatic Islet Transplantation

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Introduction:
One of the alternatives to the daily insulin injection for the treatment of type I diabetes is a transplant of insulin producing tissue. However, islet graft survival requires chronic immunosuppression and in trials done so far, only 15% of recipients remain insulin independent at 5 years post-transplant (1). A major problem is poor revascularization of islets that leads to metabolic exhaustion and cell death. This study was designed to engineer a highly vascularized scaffold that would be able to support islet graft survival. A gel was developed previously for cell delivery that promoted vascularization by host and transplanted cells (2); and may therefore be an ideal site for islet transplantation. Chitosan, a natural polymer of glucosamine and N-acetyl glucosamine, is biocompatible, biodegradable and has antimicrobial properties (3). Chitosan has been reported to promote rapid angiogenesis in corneal and skin tissue (4, 5). We developed and tested collagen-chitosan scaffolds as candidate materials for creating vascularized sites for islet transplantation for the treatment of diabetes.

Materials and Methods:
1% porcine collagen and 1.5% chitosan HCl solutions buffered with a 0.5M morpholinoethanesulfonic solution were mixed with 1-ethyl-3-(3-dimethyl aminopropyl) carbomide and N-hydroxysuccinimide on ice. Hydrogels with different ratios of collagen and chitosan (collagen:chitosan ratios of 1:1, 5:1, 10:1, 20:1 and 50:1) were made. The mixture (pH 7.4) was dispensed into cell culture plates and incubated at 37°C to form hydrogels. Circulating progenitor cells (CPCs), which can promote tissue vascularization, were isolated from the blood of healthy human donors and cultured on fibronectin. After 4 days, the adherent population (CPCs) was collected and added to freshly prepared gel before incubation at 37°C. To assess cell survival on the gels, CPCs were seeded onto the different hydrogels and subjected to 48 hours of serum deprivation to induce cell death. CPCs were then incubated with 4nM Syto16 and 30μM verapamil, followed by flow cytometry analysis for viable cells. In vivo experiments were performed using mice (CD-1 and Balb/C). Hydrogels were implanted subcutaneously with (in Balb/C mice) or without (in CD1 mice) human CPCs. Gels were harvested 1 week later, paraffin embedded, sectioned and analysed for vascularization and the expression of von Willebrand factor (vWF; an endothelial cell marker) and CXCR4 (the receptor for SDF-1, an angiogenic cell homing factor).

Results and Discussion:
Total CPC viability on collagen-chitosan matrices was increased compared to collagen only matrix (Figure 1). This suggests that these matrices may improve the retention and survival of transplanted angiogenic cells. Immunostaining of implanted scaffolds without CPCs, demonstrated up to a 3.7±0.8 fold increase in CXCR4 expression in collagen:chitosan gels compared to collagen-only. Also, up to a 2.0±0.1 fold increase in vWF^+ cells in collagen:chitosan gels compared to collagen-only was observed. Therefore, the addition of chitosan appears to improve the recruitment of angiogenic and endothelial cells from the host. For gels implanted with CPCs, greater vascularization was observed in collagen-chitosan versus collagen-only scaffolds. In addition, an increased number of vWF^+ and $CXCR4^+$ cells were observed in collagen-chitosan implants compared to collagen-only gels (Figure 2).

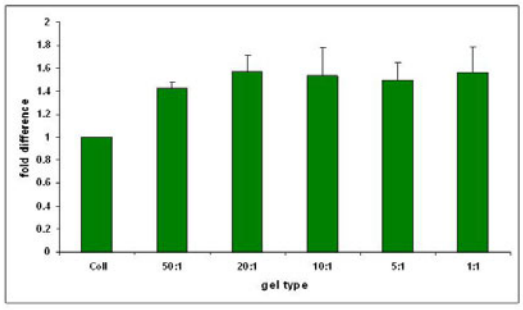

Fig. 1. The fold-difference in the number of viable CPCs after 48 hrs of serum deprivation assay.

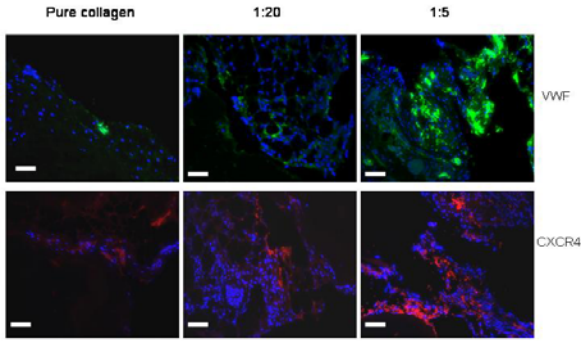

Fig. 2. Identification of cells expressing vWF (green), CXCR4 (red) in the scaffolds, 1wk after subcutaneous implantation. Cell nuclei are stained with DAPI (blue). Scale bar 50mm

Conclusions:
This study shows that the addition of chitosan to a collagen-based gel promotes the viability of angiogenic progenitor cells and greater in vivo vascularization. These results suggest that a collagen-chitosan matrix has the potential to be a highly vascularized site for the support of transplanted islets for the treatment of diabetes.

References:
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