BIOENGINEERED PANCREAS: HUMAN INSULIN-SECRETING ISLET CELLS IN VASCULARIZED PIG KIDNEY SCAFFOLDS

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ABSTRACT

INTRODUCTION AND AIMS: We have studied tissue regeneration by seeding decellularized kidneys with progenitor cells, and our previous results have shown endothelial differentiation of mouse embryonic stem cells in the vasculature of rat scaffolds. In that a barrier to pancreatic islet cell transplantation is lack of adequate blood supply, we hypothesize that regenerated renal scaffold vessels (glomerular and peritubular capillaries) can serve to vascularize insulin-secreting cells seeded into what was the collecting system (Bowman's space and tubules, respectively). For the ultimate goal of human xenotransplantation, this "trans-organ" would have a pig kidney scaffold seeded by endothelial and islet cell lineages grown from induced pluripotent stem cells (iPSCs) derived from the diabetic patient.

METHODS: Based on our murine findings, we tested new protocols to decellularize, sterilize, and seed kidneys harvested from 12 kg juvenile pigs, using Triton X-100, SDS, DNase, and peracetic acid. Integrity of the vascular and collecting systems was studied using light microscopy and after infusion of fluorescent 10 micron polystyrene microspheres. Cell seeding techniques were developed using positive and/or vacuum pressure. Fluorescent cells were injected anteriorly (immortalized mouse lung fibroblasts or HeLa) via the artery and retrograde (immortal islet cells: TC-Tet or BLox5) via the ureter. Histology was performed to assess cell delivery patterns, integrity of the vascular and collecting system compartments, and for insulin secretion.

RESULTS: Microscopy demonstrated preservation of the decellularized renal scaffold architecture. Microspheres were visualized antegrade as far as the glomerular tufts, and retrograde into Bowman's space without mixing between compartments. Vacuum-assisted seeding improved cell delivery into vasculature and collecting systems: For the former, by demonstrating appropriate cells in the glomerular and peritubular capillary vasculature (e.g. GFP+). For the latter, by showing pancreatic islet cells in Bowman's space and tubules (e.g. insulin+).

CONCLUSIONS: We have successfully developed protocols to transition from our rat model to pig kidneys for decellularization, sterilization and cell seeding. We selectively deployed cells antegrade through the vasculature into glomerular tufts, and retrograde up tubules into Bowman's space. Pancreatic islet cells successfully grew in the collecting system and demonstrated insulin production. This approach is very promising for tissue engineering a pig kidney trans-organ using a patient's iPSCs, and thereby crafting a vascularized pancreas xenotransplant.

AIMS

♦ Develop the methodology to transition from rat to pig kidneys for harvesting, decellularization and sterilization.

♦ Study the acellular scaffold and establish the integrity of the vascular and collecting system compartments.

♦ Use microspheres to develop the pressure and perfusion methodology to non-destructively retrograde seed up the ureter, into the tubule collecting system and as far as Bowman's space.

♦ Establish the perfusion, pressure and incubation conditions to retrograde seed and grow living cells in the collecting system as far as Bowman's space.

♦ Use pancreatic beta cells as proof of concept that non-renal cells can be vascularized using a kidney scaffold.

METHODS

♦ Kidneys were rapidly harvested from pigs ages 2 wk (5kg), 3 wk (13 kg) and adults (45 kg).

♦ Decellularization was tested over a range of concentrations and time of sequential non-ionic and ionic detergents (Triton X-100 and SDS), water and DNase. Various pressure and flow parameters were studied.

♦ Peracetic acid & hydrogen peroxide were used for sterilization.

♦ Ranges of antegrade and retrograde perfusion pressure and flow parameters were studied using green and red fluorescent 10 µm polystyrene microspheres. The patterns of particle distribution were assessed by fluorescence whole organ imaging and microscopy.

♦ The methodology for antegrade and retrograde sterile seeding and incubation was studied using a variety of cell types including immortalized murine lung fibroblasts and HeLa cells.

RESULTS- I

♦ Pigs at approximately 3 weeks age and 13 kg were ideal for the decellularization and seeding protocols. They were advantageous in still having in the cortex a cascade of glomeruli in progressive stages of embryologic development (Figures1, 2).

♦ The best results were obtained using a 3 day protocol with 1% Triton X100 and 0.75% SDS, then water rinses and DNase.

♦ Architecture was preserved (Figure 3) using fluid pressure and flow parameters of approximately 40 mm Hg and 800 ml/hr.

♦ Particulate (Figures 4-6) and cell seeding (Figures 7-9) was possible with positive pressure limited to 60 cm Hg, but was improved by applying 80 mm Hg vacuum.

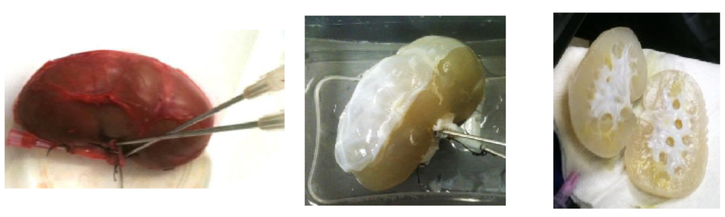

Figure 1. Pig kidneys, before and after decellularization

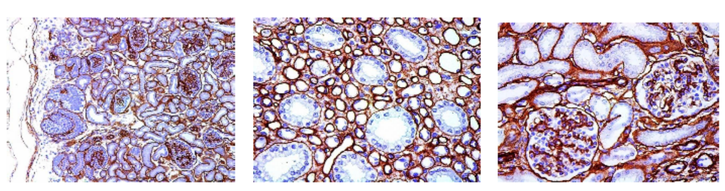

Figure 2: Pig kidney histology before decellularization (collagen IV IHC). Developing zone still present in outer cortex at 14 weeks (left). Inner medulla (middle). Glomeruli present in mid-cortex (right).

RESULTS- II

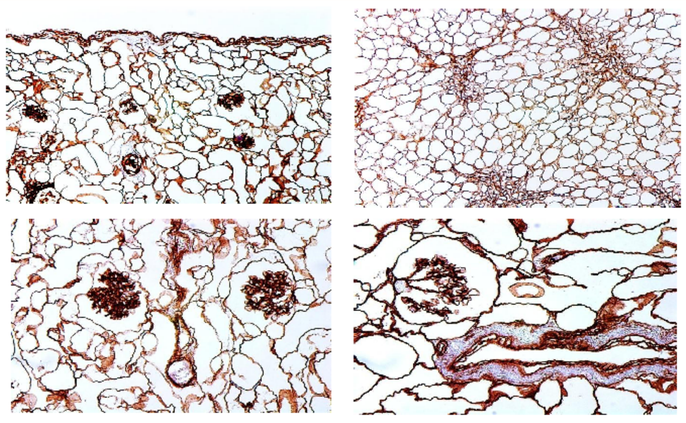

Figure 3: Pig kidney histology after decellularization (collagen IV immunohistochemistry). The scaffold has an intact architecture and retains collagen IV immunoreactivity.

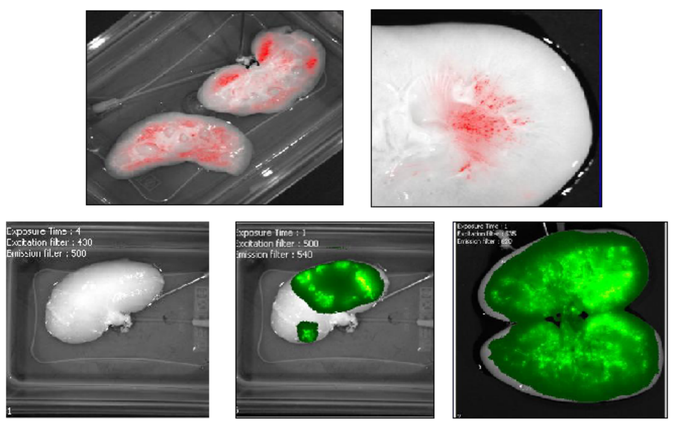

Figure 4: Fluorescence of kidney after perfusion of 10 µm polystyrene microspheres; red is arterial antegrade and green is ureteral retrograde perfusion.

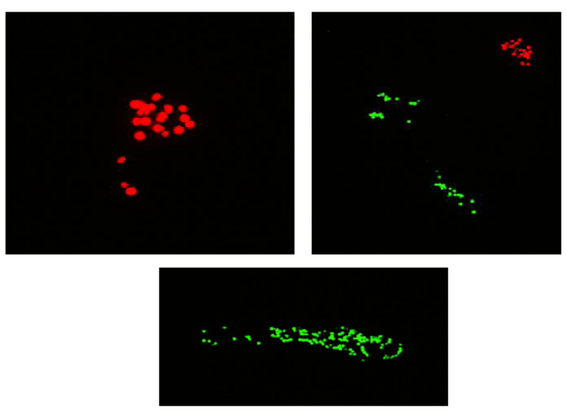

Figure 5: Fluorescent 10 µm polystyrene microspheres. Antegrade perfusion in renal artery (red) visualizing afferent arteriole and glomerulus (above) and retrograde perfusion in ureter (green) showing microspheres in tubules (below).

RESULTS- III

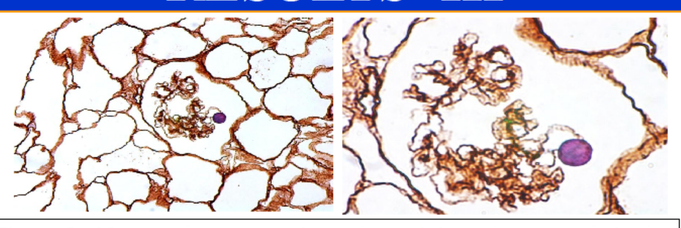

Figure 6. 10 µm polystyrene microspheres injected retrograde in the ureter and reaching Bowman's space. 40x and 100x

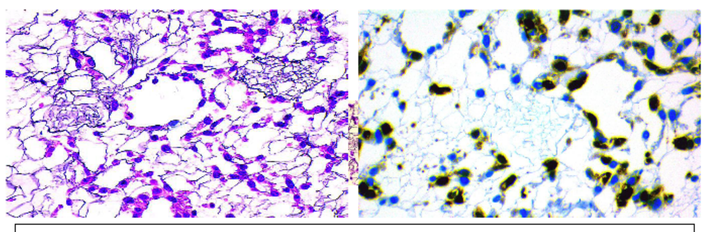

Figure 7: 3-day incubation of scaffold showing antegrade seeding of vasculature and peritubular capillaries (H&E and anti-GFP IHC)

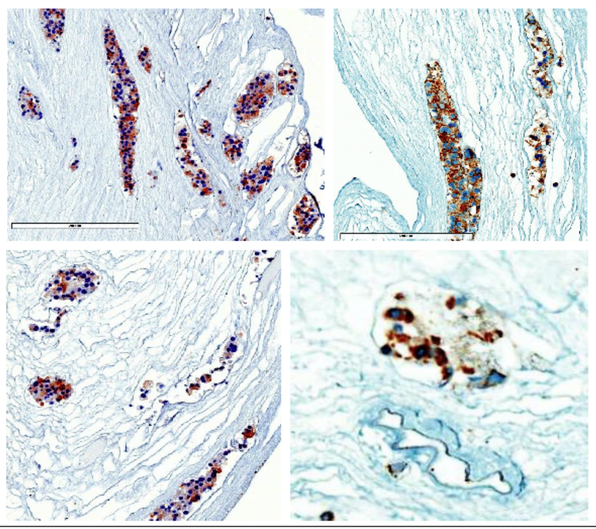

Figure 8. 3-day incubation of scaffold after retrograde seeding of pancreatic beta cells. Cells showing positive insulin immunoreactivity localize to the collecting tubules.

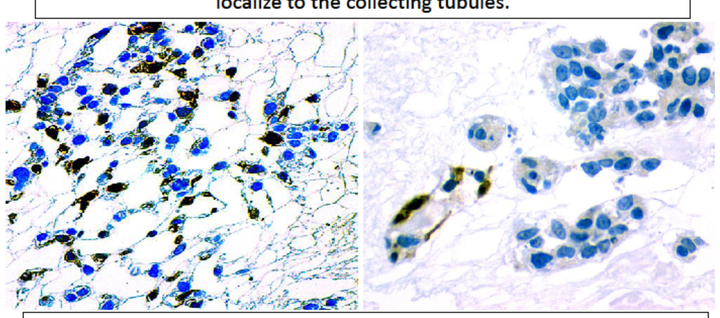

Figure 9: 3-day incubation of scaffold showing antegrade seeding of vasculature and retrograde seeding of collecting system. **Note the maintained integrity of the separate scaffold compartments. GFP+ cells outline a vessel while a different, non-GFP positive beta cell population occupy tubular structures.**

DISCUSSION & CONCLUSIONS

♦There has been much recent interest and progress in the use of bioscaffolds for tissue regeneration, including trachea, heart and bladder. Our protocols aim to develop methodology for the ultimate goal of xenotransplantation, wherein induced pluripotent stem cells from a patient would grow, differentiate and remodel an acellular pig kidney scaffold. We believe that this model can be used not just for renal regeneration but also as a platform for sustaining pancreatic beta islet cells, with the goal of treating diabetes. The kidney's extensive vascular supply would overcome a major barrier to islet-cell therapies in which there has been inadequate perfusion of the large numbers of beta cells that are needed for critical cell-to-cell interactions.

♦Our prior work focused on kidney vasculature regeneration, in which we demonstrated that arterial antegrade seeding of embryonic stem cells into the scaffold's renal artery succeeded in endothelial differentiation, appropriate new basement membrane formation and early lumen formation. The current experiments were directed to developing the technology to repopulate the collecting system: retrograde seeding up the ureter, whether for kidneys (e.g. with metanephric mesenchymal cells) or a pseudo-pancreas (e.g. with beta cell lineages).

♦In these experiments we have succeeded in transitioning from our prior rodent model to porcine kidneys, as we believe they would be architecturally appropriate for human xenografts. The delicate architecture of the kidney was preserved in our acellular pig kidneys. The integrity of the vascular and collecting system compartments was maintained and did not allow mixing; this conclusion was supported by the microsphere and cell perfusion protocols.

♦Our data show that a combination of positive and negative pressure profiles, as well as pressure-relief pumping systems, was successful for accomplishing our aim of "landing" first microspheres and then cells as far as the glomerular Bowman's space.

♦These results demonstrate that we were able to develop seeding and perfusion conditions that permitted cells to adhere, multiply and grow antegrade in the vasculature to glomerular tufts and (separately) retrograde to Bowman's space.

♦ These findings also demonstrate proof-of-concept that the kidney can host growth of other organ tissue: using the kidney's extensive system of blood vessels and the unique glomerular tuft to provide vasculature for pancreatic insulin-producing beta cells. Establishing a vascular supply to groups of islet cells that need to be large enough for appropriate cell-cell signaling and paracrine effects has been a barrier to pancreas bioengineering. We believe that a functional pseudo-pancreas trans-organ can be grown using the revascularized kidney scaffold and islet cells in the renal collecting system.

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I) Diabetes. I1) Basic research in diabetes.

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