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Survival and Function of Transplanted Islet Cells on an In Vivo, Vascularized Tissue Engineering Platform in the Rat: A Pilot Study[1]

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As in vivo tissue engineering of complex tissues and organs progresses, there is a need for an independently vascularized, alterable, and recoverable model. Current models of islet cell transplantation (release into the portal venous system, placement under the renal capsule, and microencapsulation) lack these qualities. We have developed a model of angiogenesis and spontaneous tissue generation in the rat that lends itself as a potential platform for tissue engineering. In this experiment, we examined the effectiveness of such a model in addressing some of the shortcomings of endocrine pancreatic transplantation. An arteriovenous loop was created in the groins of five adult inbred Sprague-Dawley rats, and placed within polycarbonate chambers. Isolated pancreatic islet cell clusters were placed within the chambers, suspended in a matrix of Matrigel®. The chambers were recovered at 3 weeks, and the newly generated tissue was processed for histologic and immunohistochemical analysis. By 3 weeks, spontaneous generation of angiogenesis and collagen matrix and deposition of a collagen matrix was observed. Surviving islet cells were identified by histology and their viability was confirmed via immunohistochemistry for insulin and glucagon. This study demonstrates the ability to maintain viability and functionality of transplanted islet cells on a tissue-engineered platform with an independent vascular supply. The model provides the ability to alter the graft environment via matrix substitution, cellular coculture, and administration of growth factors. The transplanted tissues are recoverable without animal sacrifice and are microsurgically transferable. This model may provide an in vivo culture platform for the study of islet transplantation.

Key words: Islet transplantation; Tissue engineering; In vivo vascularization

INTRODUCTION

A major obstacle to the successful treatment of diabetes is the lack of suitable insulin-producing tissue available for transplantation. Each year the number of new cases of diabetes far outstrips the number of donors available as sources of pancreatic tissue (11). In addition, whole gland and islet transplantation methods are fraught with difficulties, including poor tissue survival rates, the need for multiple donors to treat each individual, and the inability to examine and alter tissues after transplantation. Current successes in transdifferentiation of other cell types into insulin-producing tissues holds some promise for increasing donor tissue numbers (1,2,8,9,18,20).

Current models of islet transplantation, including release into the portal venous system, placement under the renal capsule, and microencapsulation, are encumbered by several significant restrictions. These include poor culture recovery rates, an unalterable microenvironment of the cells, irretrievability, and lack of an intrinsic blood supply (4,6,7,12,14,18). Fabrication of a relatively isolated, alterable, and readily accessible construct with an independent blood supply would allow in vivo culture and manipulation of transplanted cells, and could advance the efforts toward understanding the signals required for expansion or creation of insulin-producing tissue.

Our laboratory has been working with several in vivo three-dimensional tissue culture models that could potentially be used as a foundation on which to construct a new pancreatic "organoid." This current model is a

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modification of our original report by Tanaka et al. (19) in which an arteriovenous (AV) loop was sandwiched between layers of dermis. In that experiment, we showed that progressive outgrowth of new tissue occurred from the AV loop. Subsequently, by isolating the AV loop in a polycarbonate chamber, we demonstrated the spontaneous development of a three-dimensional, highly vascularized "flap" of tissue with its own matrix (15). Over 4–12 weeks, there was progressive expansion of the new tissue, with increasing collagen deposits (15). The addition of various matrices around the AV loop has been shown to affect tissue growth within the chamber (3). The model supports the survival of transplanted cells, as confirmed by the demonstration of viability of labeled fibroblasts and myoblasts within the chamber after several weeks (16). We went on to develop an equivalent model of vascularized tissue growth in the mouse using a closed silicone tube wrapped around the undisturbed inferior epigastric vessel pedicle in the groin. This too resulted in spontaneous angiogenesis and, when Matrigel® was included, adipogenesis was observed (5).

Following these successes in creating a stable in vivo vascularized flap of tissue that appeared to support the survival of various cell types, we hypothesized that this model would provide a suitable bioartificial platform on which to develop tissue-engineered constructs. The goal of this study was to prove the principle that an isolated arteriovenous loop would provide sufficient vascularization and matrix deposition to support the survival and function of transplanted islets.

MATERIALS AND METHODS

Inbred male Sprague-Dawley rats were used in the study. Donors were young, weighing 145–215 g, while recipients were adults, weighing 275–325 g. All experimental procedures were carried out under the supervision of a veterinarian, and were prospectively approved by the Animal Experimental Ethics Committee of St. Vincent's Hospital, Melbourne, Australia.

Islet Isolation and Chamber Preparation

Pancreatic islet clusters were isolated utilizing a previously published method (13). Following induction of anesthesia via intraperitoneal pentobarbitone sodium (35 mg/kg), the bile duct was isolated, ligated proximally, and cannulated with a 27-gauge needle. Seven milliliters of cold collagenase solution (1.3 U/ml collagenase P; Boehringer Mannheim Corp., Indianapolis, IN) with 100 U/ml penicillin G (CSL Limited, Melbourne, Australia) and 100 μg/ml streptomycin (CSL Limited) in DMEM medium (Gibco BRL Life Technologies, Grand Island, NY) was injected into the pancreas via the cannulated bile duct. The pancreas was then explanted and placed on ice.

Four pancreata were digested at a time. A total of 20 donors were used, with islets from four donors being transplanted into each recipient. They were placed in 10 ml of warm DMEM and incubated at 37°C for 20 min with occasional shaking. Digestion was monitored via intermittent sample inspection under an inverted microscope. Total digestion time did not exceed 45 min. Digestion was stopped with ice-cold DMEM. The tissues were filtered through a 425-μm filter to remove larger tissue chunks. Serial washing and sedimentation, to remove most of the acinar tissue, was performed three times with 50 ml ice-cold DMEM. The remaining tissue was then pelleted at $200 \times g$ for 1 min at 4°C. The pellet was resuspended in Histopaque-1077 (Sigma, St. Louis, MO). Fresh DMEM was overlaid without disturbing the solution interface. This was then centrifuged at 4°C with gradual speed increases from 25 to $800 \times g$ over 4 min, followed by 10 min at $800 \times g$. Isolated islets were recovered from the media/Histopaque interface via pipette extraction. They were washed three times with DMEM at 4°C. The final islet cell pellet (containing islets from four donors) was resuspended in Matrigel® matrix (BD Biosciences, Bedford, MA). A custom-molded polycarbonate chamber with an internal volume 0.5 cc (manufactured by the Department of Chemical and Biomolecular Engineering, University of Melbourne, Melbourne, Australia) was sterilized with 100% ethanol and sterile PBS, and then filled with the Matrigel®/islet suspension at 4°C. Incubation at 37°C prompted the Matrigel® to gel. A subset of the purified islets was kept in parallel in vitro culture and examined regularly for viability.

Arteriovenous Loop and Chamber Model (Fig. 1)

The AV loop/polycarbonate chamber model has been previously described (3,15,16,19). After induction of general anesthesia, an arteriovenous loop was microsurgically created in the right groin of five inbred Sprague-Dawley rats. A 2-cm vein graft, harvested from the left femoral vein, was used to create a shunt between the ends of the right femoral artery and vein, which were transected just distal to the branch point of the epigastric vessels. Meticulous microsurgical techniques utilizing 8-0 nylon sutures were employed. Patency of the grafts and the loops was confirmed. Polycarbonate chambers filled with a suspension of Matrigel® and pancreatic islet cells (see above) were then placed around each formed AV loop, such that the vascular pedicle entered and exited through a hole at the top of the chamber, with the vessel taking a circular course within the chamber. The chambers were secured proximally to the inguinal ligament and distally to the fascia of the thigh musculature using 6-0 nylon sutures. The loop's position and its circular course within the chamber were ensured by draping the epigastric branches of the vessels over the sides of the chamber prior to attaching the lid to the

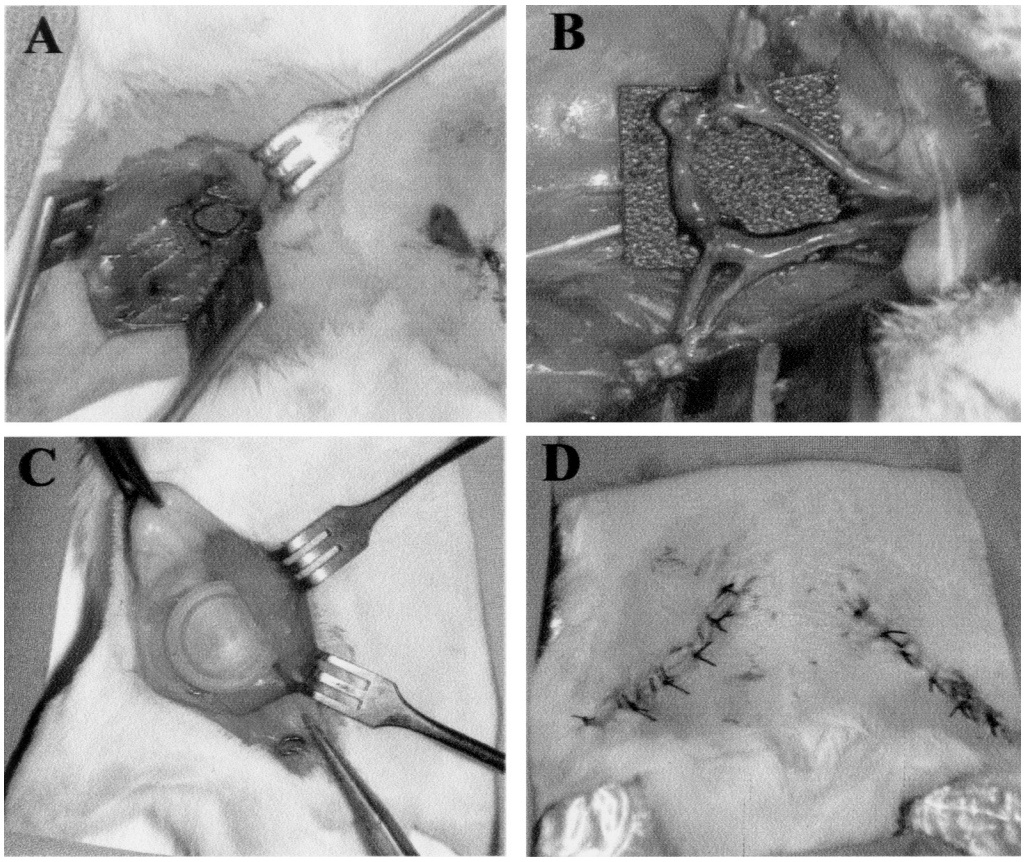

Figure 1. The AV loop/polycarbonate chamber model. (A) An arteriovenous loop is created between the divided ends of the right femoral artery and vein of a rat, using a 2-cm interpositional graft harvested from the left femoral vein. (B) Close-up view of the AV loop. The epigastric vessels protrude from the loop and are used as anchor points in the chamber. (C) Polycarbonate chambers, filled with a suspension of Matrigel® and pancreatic islet cells are then placed around the AV loop, such that the vascular pedicle enters and exits through a hole at the top of the chamber. (D) The inguinal skin is closed over the chamber.

chamber. The inguinal skin was closed over the chamber using 4-0 nylon sutures.

Incubation and Harvest

At 3 weeks, with the rats under general anesthesia, the operative sites were explored and patency of the vascular loops was assessed. Both the afferent and efferent vessels were inspected for high flow perfusion. The vessels were transected at the chamber opening, and the cut ends were inspected for patency. The chambers were then removed, and the newly formed "flaps" of tissue therein (Fig. 2) were preserved with 10% buffered formal saline for 4 h. Tissues were then processed routinely for histologic and immunohistochemical analysis. The animals were sacrificed after chamber harvest.

Histological Analysis

Serial sections, 5 μm thick, were made transversely across the "flaps" and placed on slides coated with AES (3-aminopropyltriethoxysilane, Sigma Chemical Co.). The sections were dried overnight at 37°C and deparaffinized in the standard fashion. Hematoxylin and eosin (H&E) staining and immunohistochemical studies were performed.

Immunohistochemical localization of functioning pancreatic alpha and beta cells was performed via staining for their respective endocrine products, glucagon and insulin. A commercial immunohistochemistry autostainer (DAKO, Carpinteria, CA) was used. Slides were washed with buffer, followed by a 5-min hydrogen peroxide incubation. The primary antibodies used were: guinea pig anti-insulin polyclonal antibody, diluted 1:10, and rabbit anti-glucagon polyclonal antibody, diluted 1:200 (DAKO). Following a 30-min primary antibody incubation, the components of the rabbit EnVision plus detection system (DAKO) were applied for 30 min, followed by DAB (DAKO) for 5 min. The slides were counterstained with hematoxylin. Rabbit nonimmune serum was substituted for negative controls, and normal pancreas sections were tested as positive controls.

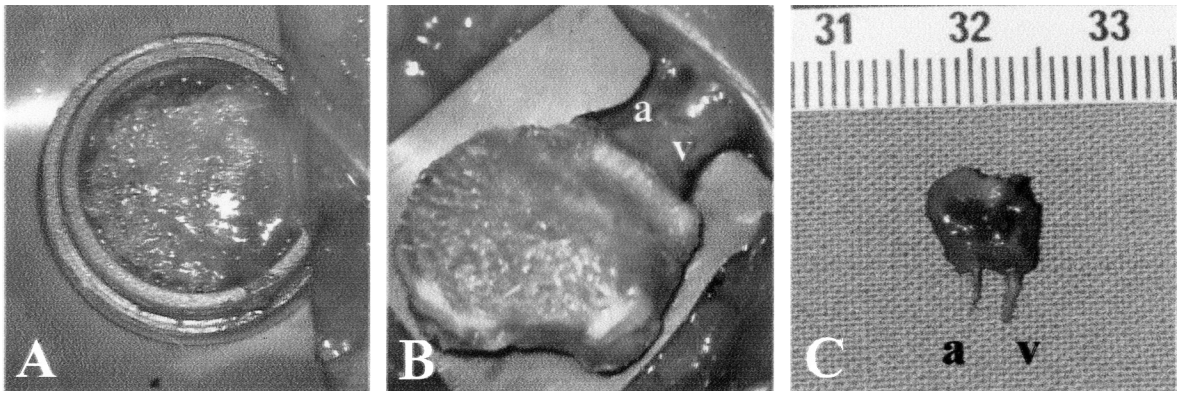

Figure 2. Appearance of vascularized flaps of tissue harvested from chambers after 3 weeks. (A) After removal of the lid, the newly formed tissue can be seen to fill the chamber. (B) The artery (a) and vein (v) of the AV loop pedicle can be dissected free, prior to flap harvest. (C) The flap can be removed for analysis, and is potentially microsurgically transferable based on the artery (a) and vein (v).

RESULTS

Four of the five animals survived for the 3-week duration of the study period. Patency was confirmed in all the AV loops upon exploration and harvest of the chambers and tissue flaps. The chambers were noted to be entirely filled with newly generated "flaps" of tissue, which had a relatively firm consistency and grossly resembled granulation tissue, with an arterial and venous vascular pedicle protruding from one end (Fig. 2).

Hematoxylin and eosin staining of tissue sections revealed intense spontaneous angiogenesis and collagen matrix deposition throughout the tissue flaps, similar to that observed previously in this model (Fig. 3) (3,15,16, 19). Higher power histological assessment allowed identification of islet structures, whose typical, round morphologic appearance had been somewhat distorted (Fig. 4). Immunostaining of serial sections demonstrated that the surviving islets maintained their functionality, via identification of insulin and glucagon production. Characteristic differential staining patterns were observed between locations of beta and alpha cells, as distinguished by variations in insulin and glucagon staining of adjacent sections from the same specimen.

Isolated, purified islets were also maintained in parallel in vitro culture. They were examined regularly, and were observed to maintain normal morphology and to sprout neurofilamentous growths characteristic of healthy islets in vitro.

DISCUSSION

In the current study we hypothesized that our in vivo model of angiogenesis and matrix deposition arising de novo from an arteriovenous loop isolated in a polycarbonate chamber would support survival and functionality of transplanted pancreatic cells. We chose a 3-week in vivo incubation period as a time by which we knew from previous experiments that the spontaneous tissue generation would be well under way (3,15,19). Furthermore, we supposed that any islet tissue that survived for 3 weeks would have surpassed initial hypoxic insults associated with the transplantation. The identification of surviving, functional transplanted islet cells after 3 weeks confirms our hypothesis and acts as a proof of principle that this vascularized tissue engineering platform can adequately support complex transplanted tissues. Distortion of the normal islet morphologic architecture may be the result of a transient hypoxic insult during the early stages of vascularization within the chambers. Similarly, processed islets cultured simultaneously in vivo maintained their morphology, suggesting that it was not the processing that interfered with the architecture of the islets seen in the retrieved constructs.

This system may one day prove to be a useful application for reversing insulin dependence in human diabetics. Our group has shown that the volume of vascularized tissue that can be engineered using this model can be increased in a small animal model by at least 400% (10). Theoretically, the ultimate size could be even larger in humans. A significant-sized construct would be necessary to house the requisite number of islets to achieve normoglycemia in this type of transplant. However, and perhaps more importantly, this model has several significant advantages that allow it to be a good method for studying in vivo transplantation of a wide variety of cell and tissue types. The incorporation of an intrinsic blood supply and the development of intense spontaneous angiogenesis are perhaps the most significant, allowing for rapid and complete vascularization of transplanted tis-

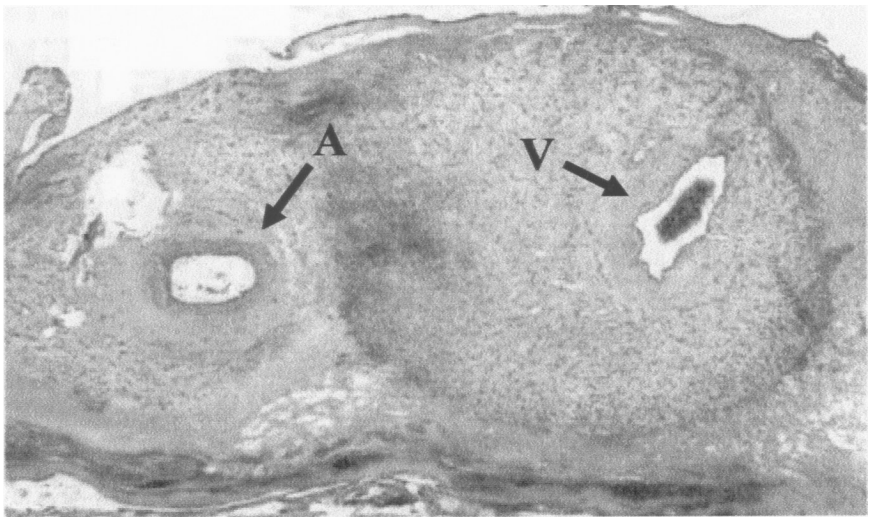

Figure 3. Cross section of a flap of tissue arising from the AV loop after 3 weeks (H&E). The artery (A) and the vein (V) of the A/V loop are seen in cross section. Note the intense angiogenesis throughout the tissue, completely vascularizing the newly formed collagen-rich matrix. Magnification: ×25.

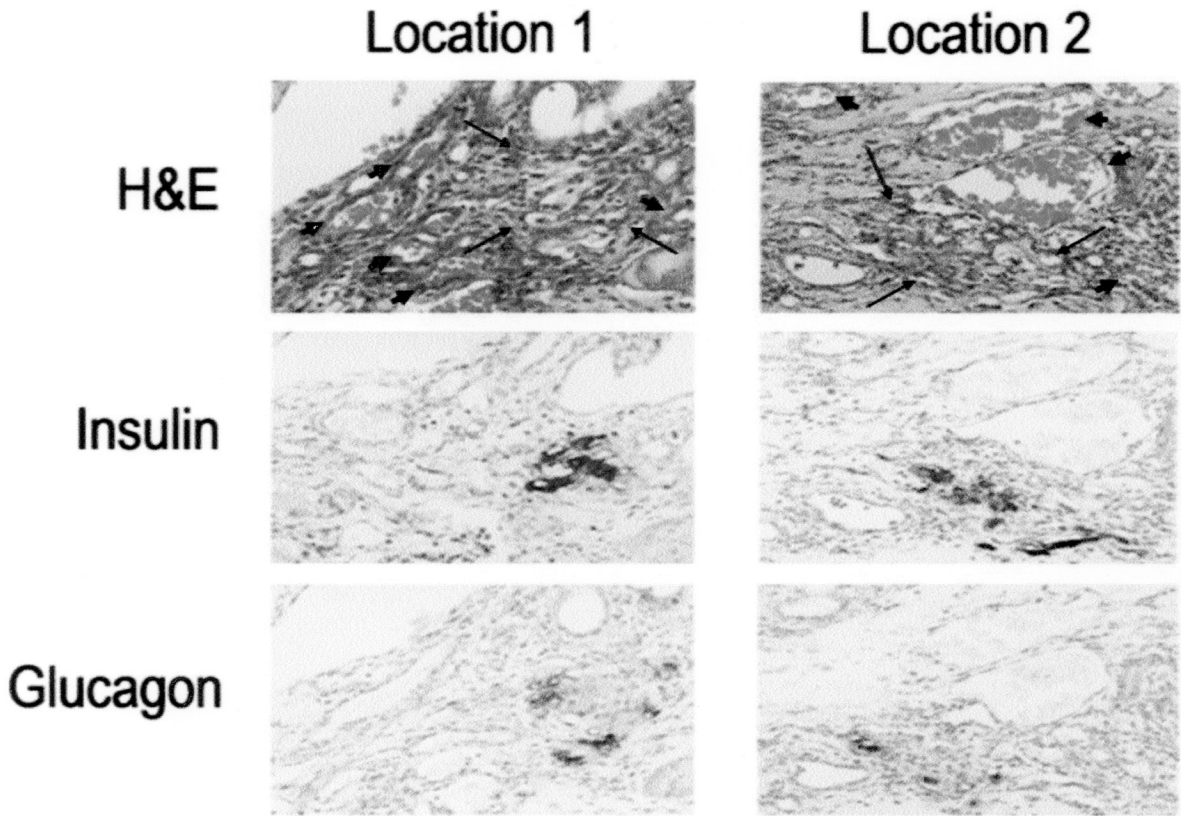

Figure 4. Serial sections of two different locations in harvested flaps at 3 weeks demonstrating survival and function of islet tissue. Slight distortion of the normal islet architecture is observed on the H&E-stained sections (between long arrows). Multiple new vascular channels are evidenced throughout the substance of the constructs, and are intimately associated with the islets (short arrows). Differential immunostaining of regions of beta and alpha cells can be seen on serial sections with insulin and glucagon antibodies, respectively. Magnification: ×100.

sues. The model also provides an easily accessible, alterable microenvironment for the transplant. Alteration of the local environment at the time of transplantation can be performed via the addition of various matrices, growth factors and cellular coculture. Ongoing access and/or alteration would be theoretically possible due to the isolated location of the transplanted tissue. Local administration of substances to affect the grafted tissues could reduce systemic side effects. Transplantation within and between recipients is possible due to the vascular pedicle supplying the flap. By demonstrating the survival and function of transplanted pancreatic islet tissue on this in vivo bioartificial, vascularized tissue engineering platform, we identify it as a potentially useful model for studying transplantation of pancreatic and other tissue types in vivo.

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