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Subcutaneous Transplantation May Not Be an Appropriate Approach for the Islets Embedded in the Collagen Gel Scaffolds

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ABSTRACT

Background. Synthetic extracellular matrix (ECM) has been shown to be efficient to preserve the function of transplanted islets. In this study using a mouse model, we sought to determine whether subcutaneous transplantation was a convenient procedure for achieving normoglycemia.

Methods. We performed in vitro tests as well as morphologic observations and Western blotting to establish that embedded islets survived better than non-embedded islets. Streptozotocin-induced diabetic mice (BALB/c) were transplanted with ECM-embedded syngeneic islets via the subcutaneous (SC; n = 5) or subrenal capsule (SRC; n = 6) routes. We measured mean blood glucose levels at various points from pretransplantation to postoperative day 14, and examined immunohistochemistry staining for insulin in the transplant grafts on day 14.

Results. Islets transplanted with ECM gel retained better structure and developed a functional vasculature. Western blotting showed more caspase-3 expressed in the non-embedded islets, which indicated more islet cells undergoing apoptosis. On the first day after transplantation, glucose levels were significantly decreased in the SRC group compared with the SC group: 383.33 ± 44.50 mg/dL to 80.67 ± 16.85 mg/dL versus 414.00 ± 92.33 mg/dL to 278.28 ± 121.80 mg/dL ($P < .05$). Glucose levels were better maintained in the SRC group than the SC group over 14 days. Immunohistochemistry staining for insulin showed fewer islets in the SC group.

Conclusion. Embedded islets with ECM gel functioned better than non-embedded ones in vitro. However, the subcutaneous route may not be an ideal site for islet transplantation.

ISLET transplantation is a useful therapeutic option for type 1 diabetes mellitus. The subrenal capsule is used most frequently in animal studies. However, transplanted islets are unstable partly because of nonspecific inflammation and hypoxia, as well as the metabolic condition of the recipient. Approximately 60% of transplanted islets are lost in the first days.[1] In clinical practice, multiple transplantations are often required for one recipient to maintain normoglycemia.[2] Therefore, it is important to determine the efficacy of various transplant sites. Synthetic extracellular matrix (ECM) has been shown to be efficient to preserve transplanted islet function.[3] In this study, we sought to determine whether subcutaneous transplantation was a more convenient and easier procedure to achieve normoglycemia in a mouse model compared with the subrenal capsule (SRC), which is used most frequently in animal studies.

MATERIALS AND METHODS

Animals and Induction of Diabetes

Male BALB/c mice (Institute of Laboratory Animal Sciences, CAMS&PUMC, Beijing, China) were rendered diabetic through the tail vein by streptozotocin injection (200 mg/kg; Sigma Aldrich, St Louis, MO). Blood glucose levels were measured in whole blood (tail vein) using a One Touch Basic monitor (Lifescan, Milpitas, CA). Mice with blood glucose measurements >300 mg/dL on consecutive days were considered to be diabetic. Mice between 8

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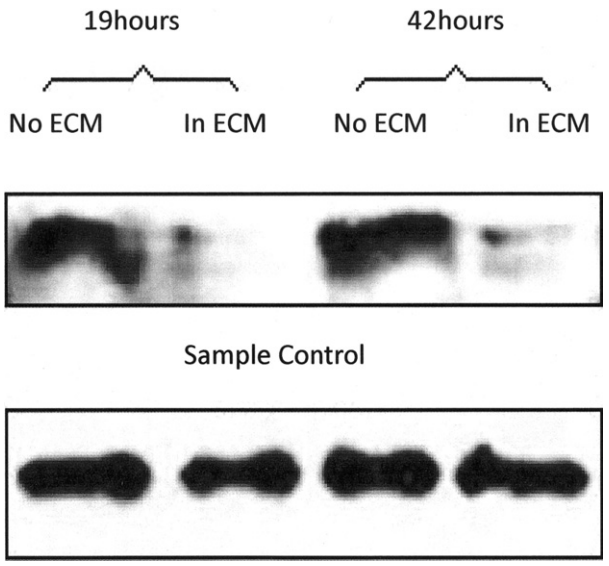

Fig 1. Caspase-3 Expression in Western blotting.

and 12 weeks of age were used as islet transplant recipients or islet donors. All studies were approved by our Animal Care and Use Committee.

Fabrication of Collagen Gel Scaffolds

Because the composition of basement membrane extract (BME) is similar to that of the normal peri-islet basement membrane in vivo, we used BME including laminin, collagen IV, and heparin sulfate proteoglycan (Trevigen, Gaithersburg, MD) to fabricate collagen gel scaffolds as previously reported.[3]

Islet Isolation and Scaffold Seeding

BALB/c male mice islets were isolated as previously described.[3,4] Preparations from three mice were mixed with 100 μg collagen gel at 4°C; BME is soluble at 4°C. The gel droplets were then seeded onto culture dishes for incubation at 37°C in 5% CO_2 and 95% air for 30 minutes to concrete. Thereafter, 3 mL of islet growth medium was added to each scaffold. Totally, 12 pieces of scaffolds were prepared from 36 mice: 1 for ex vitro assessment, and 11 for transplantation.

Assessment of Graft Function In Vitro

Before transplantation, we performed in vitro tests to assure that embedded functioned better than non-embedded islets using microscopic morphology and Western blotting.

Islet Transplantation

For islet transplantation, 11 mice were anesthetized with an intraperitoneal injection of 20 mg/kg 1% pentobarbital sodium. The two groups included: subcutaneous (SC; n = 5) versus subrenal capsule (SRC; n = 6). For the SC group, the shaved right subcostal region was sterilized with 75% alcohol, before performing a 0.5-cm incision. The gel scaffold was inserted subcutaneously and the wound closed by two metal clips. For the SRC group, the shaved abdominal midline was prepped in sterile fashion. Via a midline lower abdominal incision, we identified the kidney to wrap the scaffold into the capsule. The wound was closed in two layers with silk. Mice were allowed free access to food and water postoperatively. Blood glucose was measured as described above. All mice were sacrificed after a second surgery at 14 days post-transplant to remove the scaffolds.

Monitoring Blood Glucose Levels

Blood glucose levels were measured in the morning on postoperative days 0 (ie, preoperatively), as well as 1, 3, 5, 10, and 14. Normoglycemia was defined as a blood glucose value less than 150 mg/dL.

Histological Analysis

Histological analysis was performed to characterize the transplanted islet morphology. On postoperative day 14, explanted islet grafts were fixed in 4% paraformaldehyde. Immunohistochemistry was performed to confirm the presence of β-cells using guinea pig anti-insulin antibody (1:100; Zymed, San Francisco, CA). Digital images were acquired using a Spot camera attached to a Nikon Eclipse 50i microscope (Nikon, Tokyo, Japan).

Statistical Analysis

All results are reported as mean values ± standard error of mean. Differences between experimental groups were compared using the chi-square test. A P value less than .05 was considered statistically significant.

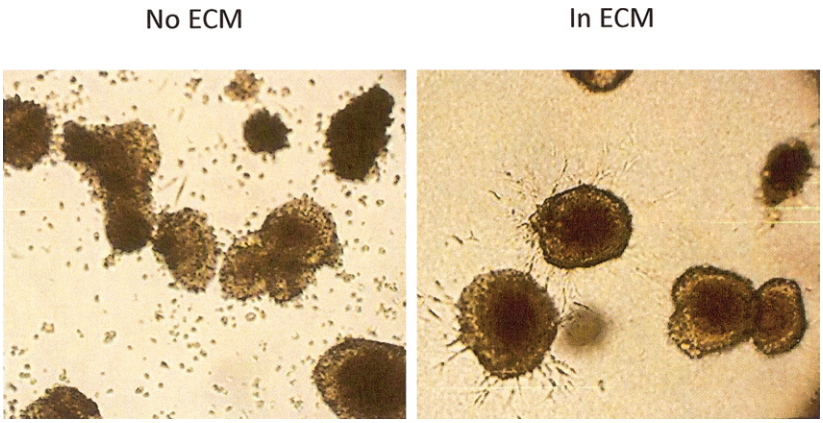

Fig 2. Microscopic view of the islets with or without extracellular matrix.