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Extra-Hepatic Islet Transplantation: Validation of the h-Omental Matrix Islet filliNG (hOMING) Technique on a Rodent Model Using an Alginate Carrier

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Abstract

Following the tremendous development of hydrogels for cell therapy, there is now a growing need for surgical techniques to validate *in vivo* scaffold benefits for islet transplantation. Therefore, we propose a newly designed surgical procedure involving the injection of hydrogel-embedded pancreatic islets in the omentum, which is considered a favorable environment for cell survival and function. Our technique, called h-Omental Matrix Islet filliNG (hOMING) was designed to test the benefits of hydrogel on islet survival and function *in vivo*. Islets were implanted in the omentum of diabetic rats using the hOMING technique and alginate as an islet carrier. Blood glucose and C-peptide levels were recorded to assess graft function. After 2 months, grafts were explanted and studied using insulin and vessel staining. All rats that underwent hOMING exhibited graft function characterized by a glycemia decrease and a C-peptidemia increase ($P < 0.001$ compared with preoperative levels). Furthermore, hOMING appeared to preserve islet morphology and insulin content and allowed the proper revascularization of grafted islets. The results suggest that hOMING is a viable and promising approach to test *in vivo* the benefits of hydrogel administration for islet transplantation into the omental tissue.

Keywords

type 1 diabetes, islet transplantation, omentum, h-omental matrix islet filling, biomaterials, alginate

Introduction

Hydrogel development is currently attracting the interest of the cell therapy research community. Indeed, numerous studies on hydrogels, from the most basic to the most functionalized, have demonstrated benefits to stem or primary cell environments in regenerative medicine and tissue engineering[1]. When injected *in vivo*, hydrogels support cell survival and aid the implantation process at transplantation sites[2]. For islet transplantation, the use of scaffolds was advanced recently by the demonstration of good cell viability/function results and improved graft survival *in vivo*[3]. Most scaffolds are stiff and can be easily implanted on the omentum surface and fixed by folding the tissue. However, this technique is incompatible with viscous hydrogels. Therefore, the aim of this study was to design a surgical technique allowing the implantation of islets into the omentum with a hydrogel. The idea was to use the omentum as a "bag" holding the graft inside the tissue based on the micro-fat transfer technique (lipofilling) used in reconstructive surgery[4]. The combination of the site and the hydrogel should house the grafted cells and support their viability and function by providing them with nutrients, oxygen, and mechanical support. Thus, we implemented a new surgical method named hOMING (h-Omental Matrix Islet filliNG). To test the feasibility and the

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outcomes of hOMING as a new islet implantation technique, we used alginate, a biomaterial widely used in islet transplantation[5]. Composed of polysaccharides from algae, it is inert and fully biocompatible[6], making it an ideal candidate to validate hOMING in a rodent model.

We hypothesize that our new method of implantation will allow proper graft function to extend its application to other type of scaffolds. We aimed to propose a novel tool able to evaluate the therapeutic and regenerative potential of the scaffolding in islet transplantation and/or in other cell therapies.

Materials and Methods

Animals, Hydrogel Preparation, and hOMING Realization

All animal experiments were performed according to National Institutes of Health guidelines under authorization number AL/60/67/02/13. Adult male Lewis rats (200–250 g) were supplied by Janvier Labs (Le Genest Saint Isle, France) and were housed in pathogen-free conditions in standard collective cages in a temperature-controlled room (23 $\pm$ 1°C) with a 12 h/12 h light/dark cycle. Food and water were available *ad libitum*. Healthy rats were used as islet donors and diabetic rats were used as recipients. Diabetes was induced by intraperitoneal injection of 75 mg/kg streptozotocin (Santa Cruz Biotechnology, Dallas, TX, USA) and insulin pellets (LinShin, Toronto, ON, Canada) were used to prevent diabetic complications.

Upstream validation of alginate safety was performed on alginate-embedded islets (1.5%, Novamatrix, Sandvika, Norway) using classical viability (live/dead) and function (glucose-stimulated insulin secretion) tests as described previously[7]. After 24 h of culture, no differences in viability or insulin secretion were observed compared with that in free islets (Fig. 1A).

The hOMING technique was performed as follows. First, islets were isolated from healthy Lewis rats as described previously[7]. Then, just before transplantation, $\sim$2300 islets equivalent (7660 IEQ/kg) were mixed with 150 µL of sterile alginate under a sterile hood and stored on ice until grafting. Diabetic recipients ($n = 8$) were anesthetized using isoflurane and, after insulin pellet retrieval, opened to expose the peritoneal cavity. The omentum was spread over sterile hydrated compresses. The islet–alginate mixture was loaded in a "no death volume" syringe (BBraun, Melsungen, Germany) preloaded with 150 µL of empty alginate through an atraumatic needle (BBraun). The needle was inserted as far as possible between the omental sheets and a small amount of the islet–alginate mixture was injected in a line ($\sim$30–50 µL; Fig. 1B). Control of proper injection was visualized by omental tissue extension. Injections were repeated until no mixture remained in the syringe (3–5 entry points) and the omentum was then reintroduced carefully into the peritoneal cavity and the rats were surgically closed. Short-term histological evaluation was performed 1 day after transplantation using dextran beads and hematoxylin and eosin staining following explantation (Fig. 1B). A control group received CMRL medium (Thermo Fisher Scientific, Waltham, MA, USA) as an islet carrier. Islets were resuspended in 300 µL of CMRL medium and injected in the omental tissue as described previously for the hydrogel.

Metabolic Follow-Up and Graft Assessment

Blood samples were collected in heparin tubes from the tail vein before transplantation and at regular times during the study. Blood glucose levels were measured using a blood glucose monitor (AccuCheck, Roche, Basel, Switzerland) and the results are expressed in grams per liter. C-peptide levels were measured using an enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden) and the results are expressed in picomoles per liter. Data are represented as the mean and minimum/maximum values. After 2 months, the omental grafts were explanted. Omenta were fixed in 4% paraformaldehyde and paraffin embedded. Antibodies against insulin (Ab7842, Abcam, Cambridge, UK) and CD31 (for vessel staining; 555025, BD Biosciences, Franklin Lakes, NJ, USA) were used in combination with Texas Red (TI-7000, Vector Laboratories, Burlingame, CA, USA) and Alexa Fluor 488 (A-11034, Life Technologies, Carlsbad, CA, USA) secondary antibodies on 4-µm-thick sections to assess the morphology and vascularization of transplanted islets.

Statistical significance was determined using Statistica software (Statsoft, Maisons-Alfort, France) and repeated-measures analysis of variance (ANOVA) with Tukey's honest significance difference test as a *post hoc* test and is represented as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Results

hOMING Using Alginate As a Carrier Provides Functional Grafts

After implantation using hOMING, the glycemia of diabetic recipient rats decreased significantly ($P < 0.001$) compared with pretransplantation levels and was maintained at approximately 3 g/L throughout the study. After 2 months, the omental grafts were explanted and glycemia increased back to pretransplantation levels (Fig. 2A). An inverse pattern was observed for the C-peptidemia level: plasma C-peptide levels increased significantly ($P < 0.05$) after transplantation using hOMING, were maintained at 700–800 pM, and then decreased to pretransplantation levels after explantation (Fig. 2B). However, no glycemia or C-peptidemia modifications were obtained in the control group.

Two months after implantation, islets had integrated into the omental tissue with preserved shape and insulin content (Fig. 2C). The implanted islets were supported by a dense vasculature, as shown in Figure 2C. Furthermore, once

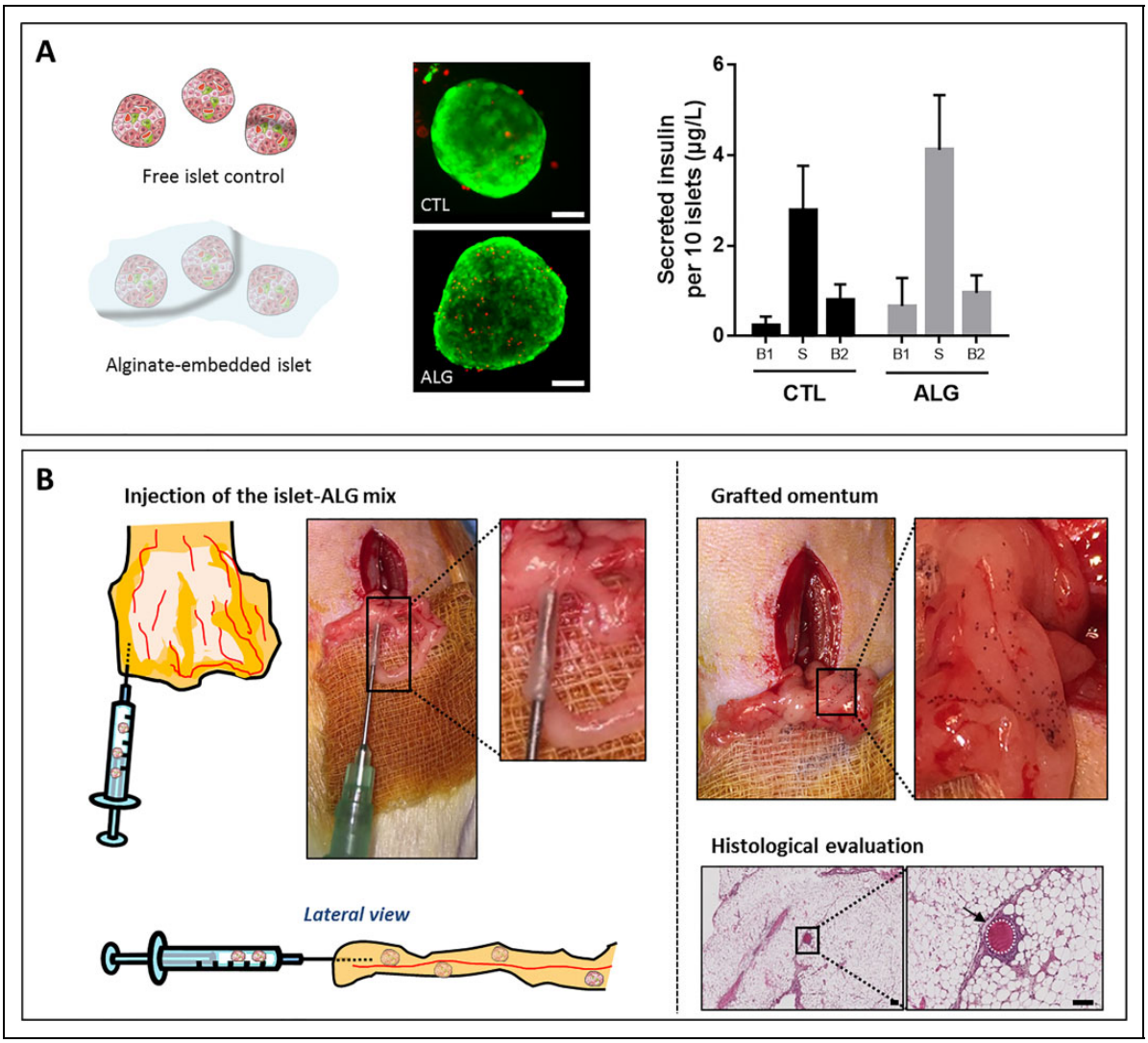

Fig 1. Validation of alginate safety and description of hOMING technique. (A) Representative viability images using fluorescein diacetate (FDA)/propidium iodide (PI) staining showing control (CTL) and alginate-embedded (ALG) islets and corresponding function profiles (GSIS). Scale bar, 50 µm. (B) An atraumatic needle was inserted in the omental tissue (left) and the islet–alginate mixture (replaced here by blue-colored dextran beads for better visualization) was injected between the omental sheets (upper right). Hematoxylin and eosin staining of omentum explanted 1 day after bead injection is shown on the lower right. Alginate (arrow) is still visible near the beads. Scale bar, 100 µm.

implanted, islet function was maintained at the same level during the entire follow-up, as shown by the maintenance of the AUC as shown by IPGTTs throughout the study (Supplemental Fig. 1).

Discussion

Hydrogel and islet co-transplantation required a novel surgical technique called hOMING, which is presented in this study. Islet implantation using this method is simple, fast, and tuneable. The use of hOMING permits the preservation of islet morphology and glycemic regulation, validating the surgical procedure.

The hOMING technique requires a viscous carrier that can maintain islets inside the tissue until they are integrated, as attested by the non-functionality of islets injected alone in the omentum. Non-polymerized alginate properly fulfilled this role and the islets survived, as shown by C-peptide production in the hOMING-transplanted rats. The islets were injected between adipocytes, as shown with the dextran beads. The tissue plasticity and hydrogel viscosity allow a rapid rearrangement of the tissue. After 24 hours, the tissue wrapped up the microbeads and they were already in close vicinity to the adipocytes.

Moreover, this technique allows islet grafting in close vicinity to vessels but without direct contact with the blood, thus avoiding mass islet loss due to the instant blood-mediated inflammatory reaction in the liver[8]. However, islet function was not optimal in all cases, as shown by the disparate results obtained in recipient rats. Because islets

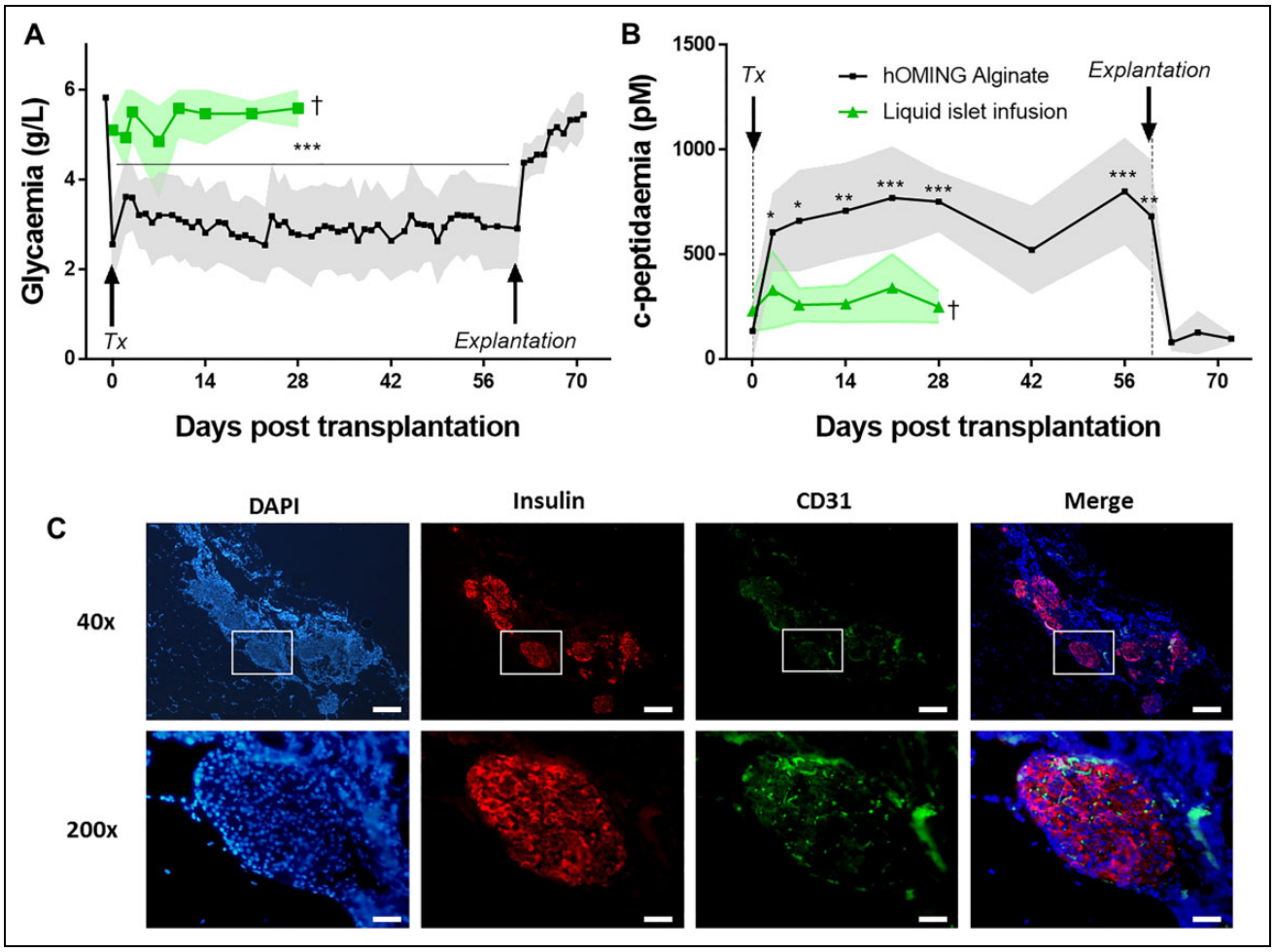

Fig. 2. Metabolic and morphologic graft assessment after intra-omental islet implantation using the hOMING technique. (A) Glycemic follow-up and (B) plasmatic C-peptidemia levels of diabetic rats that underwent hOMING using alginate or injection of free-floating islets in CMRL. Grey and green shadows represent the minimal and maximal recorded values at each time point, respectively. Arrows represent either transplantation (Tx) or omentum retrieval (explantation). †Time of sacrifice. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with pretransplantation levels by repeated-measures ANOVA with Tukey's honest significance difference test as a *post hoc* test. (C) Immunofluorescent staining of islets implanted in the omental tissue using hOMING. Top scale bar, 150 μm; bottom scale bar, 40 μm. Blue: DAPI, red: insulin, green: CD31 (vessels).

require integrin-mediated interactions with the hydrogel for optimal islet[9,10] and graft[11,12] function, alginate was not sufficient to improve the islet transplantation outcomes. However, once implanted, islets grafted using hOMING maintained their function for 2 months without any change, as confirmed by the IPGTT results during follow-up.

Now that the feasibility and the potential of the technique have been validated, hOMING using "smart" hydrogels can be tested easily *in vivo*. If the carrier used for hOMING is designed to be a biomimetic environment serving as a transient extracellular matrix from isolation to islet re-implantation, then hOMING outcomes and islet survival *in vivo* could be improved dramatically.

In conclusion, islets injected between adipocytes in the omentum using alginate to maintain the graft in the omentum are functional and able to decrease glycemia. This proof-of-concept study of the hOMING technique showed it to be a novel, simple, atraumatic, and tuneable islet implantation method. Our results suggest that graft outcomes could be maximized by designing specific hydrogels for the hOMING technique to bring it into clinical use.

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Ethical Statement

Ethical approval for this study was obtained from the French Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (MENESR) and the local ethical committee Comité d'éthique en matière d'expérimentation animale de Strasbourg (CREMEAS) with the approval number 02600.01.

Statement of Human and Animal Rights

All experiments were performed in compliance with National Institutes of Health guidelines with the authorization number AL/60/67/02/13.

Statement of Informed Consent

Statement of Informed Consent is not applicable for this article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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