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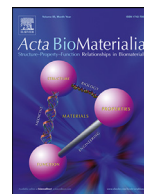
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Full length article

Glycaemic control in diabetic rats treated with islet transplantation using plasma combined with hydroxypropylmethyl cellulose hydrogel



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ABSTRACT

Islet transplantation is one of the most efficient cell therapies used in clinics and could treat a large proportion of patients with diabetes. However, it is limited by the high requirement of pancreas necessary to provide the sufficient surviving islet mass in the hepatic tissue and restore normoglycaemia. Reduction in organ procurement requirements could be achieved by extrahepatic transplantation using a biomaterial that enhances islet survival and function. We report a plasma-supplemented hydroxypropyl methylcellulose (HPMC) hydrogel, engineered specifically using a newly developed technique for intra-omental islet infusion, known as hOMING (h-Omental Matrix Islet filliNG). The HPMC hydrogel delivered islets with better performance than that of the classical intrahepatic infusion. After the validation of the HPMC suitability for islets *in vivo* and *in vitro*, plasma supplementation modified the rheological properties of HPMC without affecting its applicability with hOMING. The biomaterial association was proven to be more efficient both *in vitro* and *in vivo*, with better islet viability and function than that of the current clinical intrahepatic delivery technique. Indeed, when the islet mass was decreased by 25% or 35%, glycaemia control was observed in the group of plasma-supplemented hydrogels, whereas no regulation was observed in the hepatic group. Plasma gelation, observed immediately post infusion, decreased anoikis and promoted vascularisation. To conclude, the threshold mass for islet transplantation could be decreased using HPMC-Plasma combined with the hOMING technique. The simplicity of the hOMING technique and the already validated use of its components could facilitate its transfer to clinics.

Statement of significance

One of the major limitations for the broad deployment of current cell therapy for brittle type 1 diabetes is the islets' destruction during the transplantation process. Retrieved from their natural environment, the islets are grafted into a foreign tissue, which triggers massive cell loss.

It is mandatory to provide the islets with an 3D environment specifically designed for promoting islet transplantation to improve cell therapy outcomes. For this aim, we combined HPMC and plasma.

HPMC provides suitable rheological properties to the plasma to be injectable and be maintained in the omentum. Afterwards, the plasma polymerises around the graft *in vivo*, thereby allowing their optimal integration into their transplantation site.

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As a result, the islet mass required to obtain glycaemic control was reduced by 35%.

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1. Introduction

Cell therapy, through the replacement of functionally deficient cells, has been a breakthrough in the cure for chronic diseases such as diabetes. Type 1 diabetes is an autoimmune disease that develops largely in children, and its prevalence has been continuously increasing. Type 1 diabetes occurs due to the destruction of insulin-secreting cells in the pancreatic islets by the body's immune system. The inability of cells to store glucose as the energy source indicates lifelong insulin injection as the therapeutic option for the patients. However, despite intensive insulin therapy, the control of glycaemia can be difficult, with some patients suffering from damaging periods of out-of-range glucose concentrations. As the replacement of insulin-secreting cells becomes the only option for such severe cases [1], pancreatic islet allo-transplantation can be done for patients with brittle diabetes [2]. However, only few patients have access to this therapy, as it is not routinely used in clinics in most countries. The threshold islet mass to control glycaemia is often sourced from several pancreases, making it difficult to validate islet transplant as a treatment for type 1 diabetes [3]. The high requirement of pancreas, combined with the insufficient supply of human tissues, limits wider application of this therapy [4].

The liver, the actual transplantation site, was chosen because of the facilitated accessibility [5], important vascularisation leading to adequate partial pressure in oxygen for islet survival [6] and first hepatic pass of insulin (important for hormone optimal function [7,8]); the islet infusion is performed directly in the portal vein [9]. Therefore, the islets are in direct contact with the blood, triggering a reaction known as 'instant blood-mediated inflammatory reaction' (IBMIR). This reaction destroys approximately 40% of the freshly infused islet mass within the first 24 h of transplantation [10,11], further increasing the high demand of pancreases. To avoid this dramatic loss, alternative sites have been investigated to preserve the islet mass *in vivo*. among the alternative sites (the muscle, the bone marrow, and the anterior eye chamber) [12–14], the omentum has emerged as the site of choice for islet transplantation [15–18]. On the basis of promising preclinical results, recent clinical trials were conducted to test the possible application of this method in humans [19]. The omentum presents not only the same advantages as the liver (access, first hepatic pass of insulin, and vascularisation [20,21]) but also additional characteristics that are highly useful for islet transplantation, such as plasticity of the tissue and a large surface that enables the co-transplantation of islets with relevant scaffolds [15,22–24]. Owing to the absence of islet/blood contact and IBMIR in this scenario, at least 40% of the islet mass is expected to be preserved after transplantation. Consequently, the islet requirement, and that of pancreas, should decrease to almost half of the current levels for diabetes reversion. However, to the best of our knowledge, no study has assessed this impact thus far.

Therefore, with an aim to improve omentum transplantation outcomes and reduce the islet requirement, we implemented a surgical technique known as h-Omental Matrix Islet filling (hOMING) [17,25]. Functional grafts were obtained using an inert carrier (alginate) to implant islets close to the vessels inside the omental tissue [17,24]. Despite the absence of the IBMIR reaction and facilitated access to oxygen, hOMING needs to be refined by a

functionalised environment, for it to outperform hepatic transplantation. On the basis of previous studies from our lab and others, the use of plasma was chosen as a strategy to activate islet survival and insulin secretion. Fibrin, the main component of the plasma and a chameleon protein, creates a network surrounding the islets, recognised as a transient matrix [26], similar to the healing process. Integrin interaction with RGD motifs of fibrin [27–30] activates signalling pathways involving cytoskeleton modification and insulin vesicle transport [31,32] or anti-apoptotic and pro-survival proteins [33]. Thus, this environment decreases anoikis and potentiates insulin secretion [28]. Plasma also contains fibronectin and growth factors (EGF, FGF, etc.), the key factors that improve the performance of the islets *in vitro* [34,35]. Plasma had been already used as a carrier for islet transplantation. Thus, deposition of fibrin-embedded islets on the surface of the omentum presented encouraging results [29,36,37]. The hOMING technique delivers the islets inside the tissue, and if plasma is integrated into the injectable matrix, a fibrin nest should surround the grafted islets. For hOMING to be efficient, one requirement is to prevent islet leakage out of the tissue [17] and maintain the islet mass at the site of implantation. For this purpose, the islets have to be embedded into an injectable viscous matrix. As plasma is a liquid, it cannot be used as it is to deliver islets in the omentum. Thus, viscosity modifications would permit the use of plasma for hOMING. Hydrogels, especially hydroxypropyl methylcellulose (HPMC), can play this role as a transient water-soluble carrier mixed with plasma and cells [38,39]. This candidate is hydrophilic, viscoelastic, inert, biocompatible, and bioresorbable, with mechanical properties adapted to the injection [40]. It is also currently approved by the FDA for use in controlled release formulations [41] for eye surgery or bone repair [42–45].

The objective of the present study was to reduce the number of donors required to restore insulin independence in a single recipient, by providing a plasma-based environment for supporting islet implantation in an alternative recipient site, *i.e.* the omentum. Our study involved the *in vitro* characterisation of the carrying hydrogel, *i.e.* plasma supplemented with HPMC, followed by the *in vivo* validation in combination with hOMING, in a syngeneic rat model.

2. Materials and methods

2.1. Antibodies

Guinea pig anti-insulin antibodies were purchased from Abcam (ab7842, Cambridge, UK), goat anti-rabbit-HRP and goat anti-mouse-HRP antibodies were purchased from Sigma-Aldrich (G2654, F3648, A0545, and A9044, St. Louis, MO, USA), while the rabbit anti-CD31 and rabbit anti-TPB antibodies were obtained from Cell signaling (3528 and 8515, Danvers, MA, USA). The anti-guinea-pig-Texas red antibody was purchased from Vector Laboratories (TI-7000, Burlingame, CA, USA), while the goat anti-rabbit-Alexa 488 and goat anti-mouse-Alexa 488 antibodies were obtained from Life Technologies (10236882 and 10256302, Carlsbad, CA, USA). The mouse anti-HIF-1 α antibodies were purchased from Novus (NB100-105, Littleton, CO, USA). The rabbit anti- β 1 integrin and p-FAK antibodies were purchased from Santa Cruz (Santa Cruz, Dallas, Texas, USA), and the secondary anti-rabbit antibody was obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Animals

Male Wistar and Lewis rats were supplied by Janvier Laboratory (Le Genest-St Isle, France). The rats were housed under pathogen-free conditions in standard collective cages in a temperature-controlled room (23 ± 1 °C) with a 12-h light: 12-h dark cycle. They were fed with the SAFE-A04 diet (Villemoisson-sur-Orge, France). Food and water were available *ad libitum*.

2.3. HPMC preparation

Dried HPMC hydrogels were obtained from the Centre de Transfert de Technologie du Mans (CTTM, Le Mans, France). HPMC hydrogels (3% p/v) were obtained by adding 3 mL of solution (medium or plasma) to lyophilised HPMC. The HPMC was placed at 37 °C overnight for proper expansion and gently homogenised before use.

2.4. Rat islet isolation and collection of citrated plasma and serum

Pancreatic islets were isolated from adult male Wistar rats (200–250 g) for *in vitro* studies and from male Lewis rats for *in vivo* studies by standard collagenase (Sigma-Aldrich) digestion and Ficoll (Eurobio, Les Ulis, France) purification [46]. Blood from the rats was collected from the thoracic aorta into 4.5-mL Vacutainer citrate tubes or dry tubes during the isolation procedure and centrifuged (10 min, $3000 \times g$, 4 °C). Citrated-plasma or serum was then aliquoted and stored at -20 °C until use.

2.5. Cell culture

Rat islets were cultured under three different conditions. The islets cultured in complete M199 medium (Gibco, Fisher Bioblock, Illkirch Graffenstaden, France) supplemented with 10% heat-inactivated foetal bovine serum (FBS, Sigma-Aldrich) and 1% AB/AM (Antibiotic/Antimycotic, Gibco) were referred to as 'Control', and those cultured in hydrogel reconstituted with 3 mL complete medium or plasma were, respectively, referred to as 'HPMC' and 'HPMC-Plasma'. The islets were cultured for 24 h before the analyses.

2.6. Scanning electron microscopy of islets

The islets were cultured for 24 h at a density of 200 IEQ in culture medium (M199), in plasma gel (600 μ L) (obtained by coagulation of citrated plasma with 0.5 U of thrombin (T7009-1KU, Sigma)) or in 3% HPMC hydrogel reconstituted in complete M199 (600 μ L) in polystyrene 24-well plates for cell suspension (Greiner, Merck, Germany) with 1 mL of complete M199. Purquoi c'est ici ça ? C'est trop bizarre non? Plasma gels were shown to activate the integrin pathway and survival by decreasing anoikis [27]. Fibrin contained in plasma possesses RGD motifs recognised as a ligand for the integrin. Polymerised plasma gel, and more generally plasma, can therefore act as a substitutive extracellular matrix for the islets and provide them the right signals coming from the environment. The islets were fixed using a 2.5% glutaraldehyde–1% paraformaldehyde solution. Post-fixation treatments were realised with 1% osmium tetroxide. The samples were dehydrated using a gradient series of ethanol solutions and hexamethyldisilazane, followed by metallisation. Observations were performed using a scanning electron microscope (SEM; FEI Sirion FEG, Fei, Hillsboro, OR, USA).

2.7. Oxygen measurements

Oxygen diffusion was assessed in cell-free M199 (3 mL) in a 6-well plate containing an oxygen sensor, 600 μ L of plasma with

thrombin (0.5 U) to form a clot at the surface of the oxygen sensor covered with 2.4 mL of complete M199 and 3 mL of complete M199 supplemented with 3% of HPMC in a plate containing an oxygen sensor. The height of the medium above the islets was identical in all conditions. The islets were then subjected to hypoxia (pO_2 15 mmHg; 2% O_2 , 5% CO_2 , 93% N_2 , Linde Gas, Saint Priest, France) for 12 h in a hypoxic chamber. The chamber was then opened, and oxygen measurements were recorded until equilibration with the atmosphere. Oxygen measurements were carried out using a four-channel fibre-optic oxygen metre (Oxy-4) and non-invasive oxygen sensors (PreSens GmbH, Regensburg, Germany) glued to the bottom of the culture dish. The data are expressed as percentage O_2 saturation.

2.8. Rheology measurements

HPMC hydrogels were prepared as described in the cell culture subsection of the Methods section. HPMC was prepared in the cell culture medium 'HPMC Medium' or in the rat plasma 'HPMC-Plasma'. Rheological properties were measured using a Kinexus Ultra+ rheometer (Malvern Panalytical, UK). For the determination of shear viscosity and viscoelastic properties (G' and G''), we used a plate of 20 mm diameter, 0.5 mm gap and 200 μ L of HPMC hydrogel. Shear viscosity measurements were carried out with a shear rate ranging from 10^{-1} to 10^2 s^{-1} . Measurement of viscoelastic properties was performed with a frequency ranging from 0.1 to 1 Hz at a fixed strain of 0.5%.

2.9. Islet viability and apoptosis

The viability of islets after 24h of culture in the different conditions was analysed using FDA/PI staining (Sigma). Representative images were obtained using a Nikon Eclipse 50i microscope and Nis-Element-BR software (Nikon, Amstelveen, The Netherlands). Caspase-3 activation in islet cytosolic extracts (NE-PER, Fisher) was assessed using Quantikine® enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). Results were expressed as percentage of the control.

2.10. Islets functionality

Ten islets equivalent in each treatment were incubated in Krebs Ringer bicarbonate (KRB) solution supplemented with 10% FBS and 4.4 mmol/L glucose (Sigma). The islets were then stimulated with KRB solution containing 22.6 mmol/L glucose. Finally, the same batch of islets was incubated with KRB solution with glucose at 4.4 mmol/L. Each incubation step was performed for 90 min at 37 °C in humidified air with 5% CO_2 . The supernatants were collected after each incubation time and stored at -80 °C. Insulin measurements were performed using a rat insulin ELISA kit (Merckodia, Uppsala, Sweden). The results were expressed in pmol/L of insulin release per IEQ.

2.11. Western blotting

Proteins were extracted using a Nuclear and Cytoplasmic Extraction kit (NE-PER, Fisher) to obtain the cytosolic, nuclear, and membrane proteins. Equal protein concentrations (Ne-PER, Fisher) from each sample were mixed with sample buffer, loaded, separated by electrophoresis on a polyacrylamide gel (Criterion™ XT 4–12% Bis-Tris, Bio-Rad) and dried-transferred onto a nitrocellulose membrane (iBlot™ Life Technologies, Carlsbad, CA, USA). The membranes were probed with primary antibodies (4 °C overnight). Secondary antibodies were incubated for 1 h at room temperature. Blots were developed using a visualisation solution (Immuno-Star™ WesternC™ kit, Bio-Rad) and recorded with a ChemiDoc (Bio-Rad).

Integrin and p-FAK were assessed, respectively, in membrane and cytosolic protein extracts. Islet hypoxia was assessed by HIF-1 α translocation into the nucleus. Nuclear extracts were analysed by western blotting as described above. The results were normalised using the level of TBP (TATA-binding protein) and plotted as percentage of the control.

2.12. Diabetes induction and monitoring

Experimental type 1 diabetes was induced in Lewis rats by a single intraperitoneal injection of streptozotocin (STZ, 75 mg/kg, Sigma). Diabetic rats with non-fasting blood glucose values > 5 g/L, as measured using a blood glucose monitor (AccuCheck, Roche, Basel, Switzerland) and C-peptide < 200 pM (ELISA kit (MercoDIA, Uppsala, Sweden)) were included in the study as recipients for islet grafts.

Insulin pellets (Linshin, Toronto, ON, Canada) were used to maintain normoglycaemia of diabetic rats and to increase their body weight, and consequently, the omental surface. Insertion of the pellet was realised under gas anaesthesia (isoflurane, Baxter, Maurepas, France). Briefly, rat neck skin was pierced using a 16 G trocar, and the pellet was inserted using a guide and a stylet. The pellet diffused 3 IU of insulin per day; its efficiency was measured by the decrease in glycaemia after implantation and monitored weekly for one month.

2.13. Islet transplantation

Concomitant with islet transplantation, the insulin pellets were retrieved. The remnant insulin protects the graft from glucotoxicity on the first day post implantation. Rats (~300 g) were divided randomly into seven groups, with the first 4 receiving either 7660 IEQ/kg or no islets: (1) Islet infusion through the portal vein, referred to as 'Liver' ($n = 6$), (2) islet transplantation in the omentum with intra-tissular implantation of the hydrogel containing islets, referred to as 'HPMC' ($n = 8$), (3) hydrogel supplemented with plasma containing islets, referred to as 'HPMC-Plasma' ($n = 8$), (4) no implantation, referred to as 'Sham' ($n = 5$). For the second set of experiments, 3 groups were constituted with fewer islets. Rats were divided randomly into three groups: (5) rats receiving 5700 IEQ/kg islet infusion through the portal vein ($n = 8$), referred to as 'Liver 5700 IEQ/kg' ($n = 8$), rats receiving 5700 IEQ/kg (6) ($n = 10$) or 4900 IEQ/kg (7) ($n = 7$) islet transplantation into the omentum with an intratissular-implanted hydrogel supplemented with plasma, referred to as 'HPMC-Plasma 5700 IEQ/kg' and 'HPMC-Plasma 4900 IEQ/kg', respectively.

For the hydrogel groups, we used the h-Omental Matrix Islet filling (hOMING) technique, wherein the islets were transferred into 300 μ L of hydrogel and injected using an atraumatic needle into the omental tissue in linear movements through several entry points. The grafted omental tissue was then reintroduced carefully into the peritoneal cavity. The intra-portal graft was performed as previously described [27]. Rats were then stitched, placed on a heated carpet until they woke up and treated with an anti-inflammatory agent (Metacam[®], 1 mL/kg, Boehringer, Germany) and an antibiotic (Baytril[®], 5%, 10 mg/kg, Bayer, France), once a day for 4 days post-surgery. Insulin (Lantus[®] 6 IU/200 g of body weight, Sanofi) was administered subcutaneously at the same time every day if the rats remained diabetic (glycaemia > 2 g/L).

2.14. In vivo plasma polymerisation assessment

Dextran beads of average diameter 100 μ m (Cytodex 1, Sigma) were mixed with HPMC-Plasma and transplanted into the omental tissue. Omenta were explanted 1 h later, snap-frozen in OCT and

sectioned in 4 μ m thickness cryosections. MSB staining (Martius-Scarlet-Blue method) was used to highlight the red fibrin fibril [47,48].

2.15. Metabolic follow-up

Body weight, glycaemia and blood samples collected in heparin tubes from the tail vein were taken 1 day before the transplantation and at regular times during the study (D1, D0, D3, D7, D14, D21, D28, D42, D56, D63, D66 (or D + +3), D68 (or D + +5) and D70 (or D + +7)). D + +n indicates the days after graft retrieval for the omentum groups. The blood glucose level was measured using a blood glucose monitor (AccuCheck, Roche, Basel, Switzerland) and the results were expressed as g/L. The C-peptide level was measured using an ELISA kit (MercoDIA, Uppsala, Sweden), and the results were expressed in pmol/L.

2.16. Intraperitoneal glucose tolerance test (IPGTT)

IPGTT was performed on days 7, 14, 21, 28 and 56. Briefly, non-fasting rats were placed in clean cages with no food or faeces for 4 h prior to IPGTT and provided with *ad libitum* water access. Glycaemia was recorded using finger prick samples from the end of the tail with a blood glucose monitor. Baseline glycaemia was measured at t_0 , and then intraperitoneal injection of 2 g of glucose/kg body was performed. Blood glucose levels were measured at 15, 30, 60 and 120 min after glucose injection. Food was reintroduced at the end of the test. Area under the curve was calculated and represented for each time.

2.17. Omental graft explantation and sacrifice

Two months (63 days) after intra-omental grafting, omental tissues were explanted from all the hydrogel groups, with or without islets, under gas anaesthesia. The retrieved organs were then weighed and placed in 4% paraformaldehyde for histological analysis. Seven days later, all the rats were sacrificed and livers from intra-portal graft recipients were collected.

2.18. Histology and immunofluorescence analysis on grafts

Liver or omenta, retrieved at day 63, was sectioned and embedded in paraffin. The sections were stained with haematoxylin and eosin (H&E) or incubated with the primary antibodies guinea pig anti-insulin (1/20) and rabbit anti-CD31 (1/50). The secondary antibodies were conjugated to Alexa Fluor 488 (1:200; goat anti-guinea pig IgG (H + +L) or Texas-Red goat anti-rabbit IgG (H + +L). Slides were then mounted with mounting medium containing 1.5 μ g/mL 2-(4-amidinophenyl)-1H-indole-6-carboxamide (DAPI) (FluorSave Reagent, Millipore) and observed using a Nikon Eclipse 50i microscope and Nis-Element-BR software. All the digital images were acquired using a fluorescence microscope equipped with a camera. CD31 (vessels) in the transplanted islets was assessed by green immunofluorescence quantification using Nis-Element BR-software. The intensity of the green staining was recorded then scaled and rationalised by the size of the islet as indicated by the insulin staining. At least 15 different islets of various sizes were used for each treatment.

2.19. Statistical analysis

Statistical analyses were performed using Statistica software (StatSoft, Maisons-Alfort, France). The results of the *in vitro* tests were analysed using either one-way analysis of variance (ANOVA) with least-square difference (LSD) Fisher post-hoc test for parametric data or Mann-Whitney test or Kruskal-Wallis and

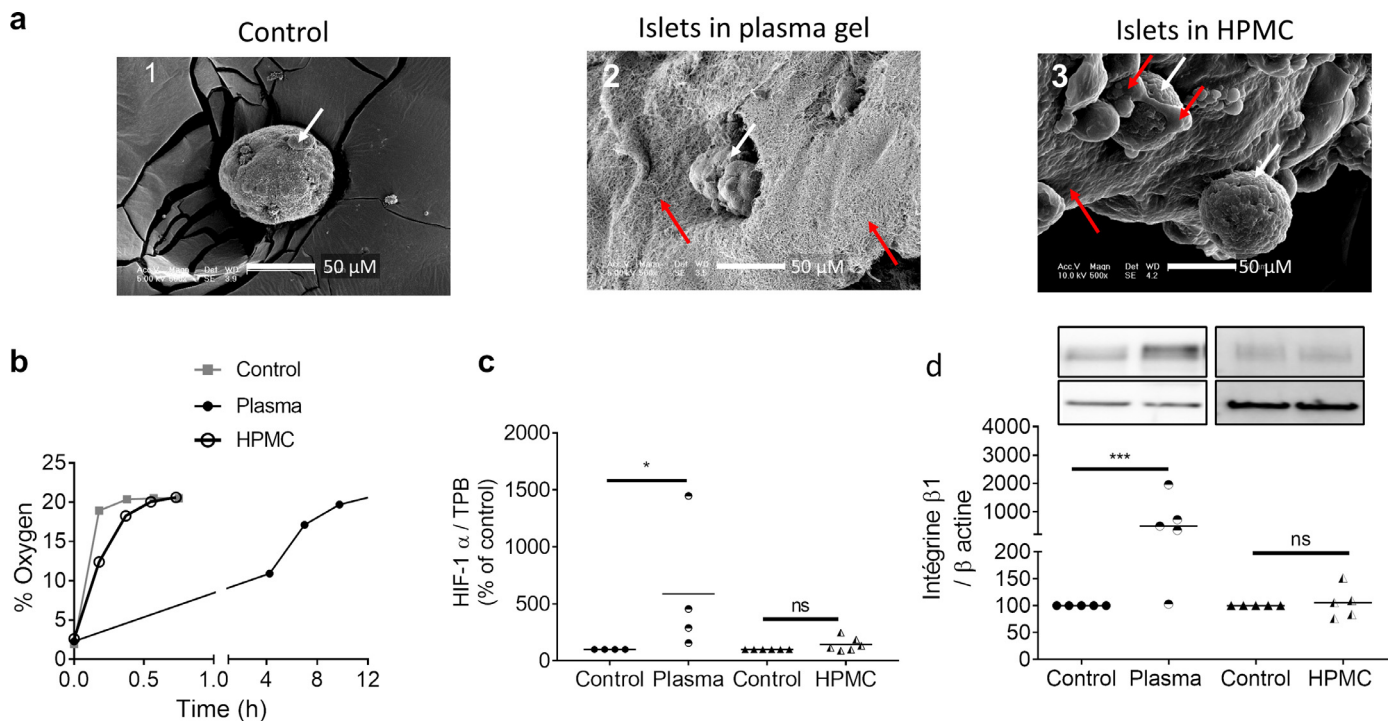


Fig. 1. Oxygen diffusion in HPMC. (a) Representative scanning electron microscopic (SEM) images of the islets cultured under free floating condition (1) in a plasma polymerised gel (2) and in HPMC (3). The islets have a spheroid structure with an irregular surface as expected, indicated by white arrows. Plasma and HPMC were designated by red arrows. The fibrillar constitution of the plasma, formed by the polymerisation of fibrinogen into fibrin, formed a nest around the islets. The HPMC hydrogel has a smooth surface and covers the islets. Original magnification: $\times 500$. (b) Oxygen equilibrium between the M199 medium, plasma gel and HPMC hydrogel after hypoxia (2%) and re-exposition to normal atmosphere. The equilibrium with air is longer for the polymerised plasma than for the control (complete M199) or HPMC. (Graph shows oxygen diffusion of one representative experiment; $n = 3$) (c) HIF-1 α translocation was measured in the nuclear fraction of rat islets (CTL vs. plasma $n = 4$; and CTL vs. HPMC $n = 6$) after culture for 24 h in complete M199, polymerised plasma or HPMC. * $p < 0.05$ vs control. Mann-Whitney test was performed between plasma or HPMC and their respective control. (d) Integrin expression at the surface of islets after 24 h culture in HPMC, polymerised plasma gel or complete M199 ($n = 5$), analysed by Western blot and quantified using ImageJ. Individual results were plotted on the graph, with mean represented by bar. *** represents significant difference between plasma gel and control; p value < 0.001 , ns represents no significance between HPMC and control (Mann-Whitney test).

Dunn's multiple comparison test for non-parametric data. The *in vivo* results were analysed using repeated-measures ANOVA or one-way ANOVA with an LSD Fisher post-hoc test. The results are presented as the mean \pm standard error of the mean (SEM). p values less than 0.05 were considered statistically significant and are represented as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

2.20. Study approval

All the animal experiments were performed according to the National Institutes of Health guidelines, with the authorisation number AL/60/67/02/13.

3. Results

3.1. Hypoxia reduction in HPMC

Islets cultured in a polymerised gel comprising thrombin-polymerised fibrin were embedded in a dense fibrillar network (Figs. 1(a) 2). This fibrillar structure created a delay in oxygen diffusion, as observed in Fig. 1(b), and triggered hypoxia-inducible factor 1 α (HIF-1 α) translocation into the nuclei of rat islets (Fig. 1(c)). In contrast, islets cultured in the HPMC hydrogel were embedded in a smoother gel (Figs. 1(a) 3), allowing proper oxygen diffusion (Fig. 1(b)), which did not trigger hypoxia in the rat islets (Fig. 1(c)). The HPMC is used as drug additive because of its viscoelastic properties, and the addition of HPMC increases the viscosity of the solution (culture medium, or in our case, the plasma). Thus, the thickness of the solution increases, which increases the time of oxygen diffusion. However, the polymerised

plasma is a structure formed of fibril entanglement as shown in the SEM observation. The rigid structure slowed down the oxygen diffusion through the matrix.

3.2. HPMC compatibility with hOMING technique

Islets injected in the omentum using the hOMING technique preserved islet morphology (Supplemental Fig. 4)(Fig. 2(a) and (b)) when compared with that of intra-portal infusion. Islets transplanted in the omentum with HPMC significantly decreased glycaemia (AUC = 178.5 ± 23.7 vs. sham AUC = 307.8 ± 11.2 , $p = 0.0018$) and C-peptide levels during the two months of follow-up (AUC = 7213 ± 767 vs. sham AUC = 4466 ± 884 ; $p = 0.0417$) (Fig. 2(a) and (b); Suppl 1). However, the glycaemia and C-peptide levels were not better than those of the animals transplanted with free islets in their liver (Glycaemia AUC liver = 112.3 ± 7.1 ; $p = 0.0564$ vs. HPMC; AUC liver = $13,006 \pm 634$, $p = 0.0003$ vs. HPMC). No modification in integrin expression was observed in the presence of HPMC (Fig. 1(d)). Furthermore, no impact in islet vascularisation (no difference in CD31 staining (Fig. 2(c) iii; iv and (d))) was detected.

3.3. Impact of plasma supplementation on HPMC rheological properties

To ensure conservation of injectability, the effect of plasma addition to HPMC hydrogels in terms of shear viscosity was first tested. As shown in Fig. 3(a), the preparation of HPMC hydrogels with plasma resulted in a significant decrease in viscosity over the range of shear rates tested, compared with the HPMC prepared in

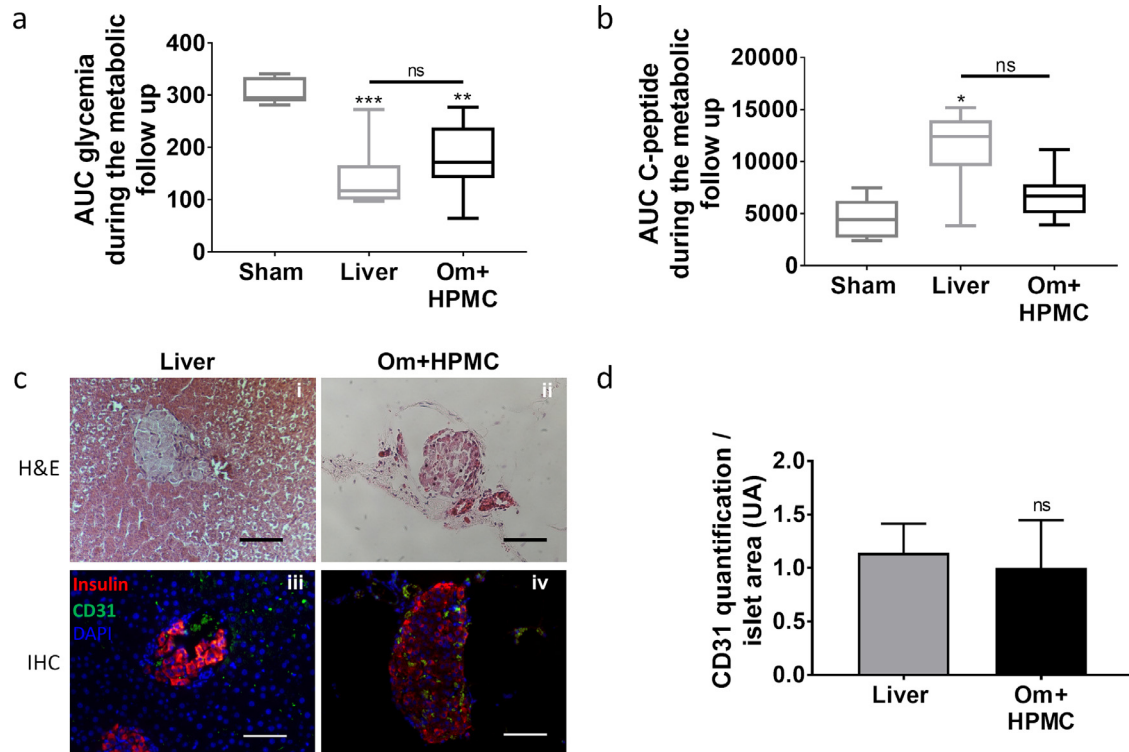


Fig. 2. Validation of HPMC for hOMING application. To validate HPMC for islet transplantation using hOMING, glycaemia and C-peptide levels of rats implanted in the omentum using HPMC (Om+HPMC; $n = 8$) were compared with those of rats implanted in the liver without the hydrogel (Liver; $n = 5$) or Sham ($n = 5$) for 2 months post-transplantation. (a) AUC of glycaemia measured for two months post-transplantation in diabetic rats (Sham: no islet ($n = 5$); Liver: hepatic islet transplantation ($n = 6$); omental islet transplantation with HPMC ($n = 8$)). AUC of glycaemia was measured for each rat. The results are expressed as Box (25th to 75th percentiles) and Whisker plots (5th and 95th percentiles). Symbols represent significant differences between Liver, HPMC for hOMING and Sham. * represents a p value < 0.05 , ** represents a p value < 0.01 and *** represents a p value < 0.001 . Kruskal–Wallis analysis of variance (ANOVA) was used with Dunn’s multiple comparison test (b) AUC of C-peptide levels measured during two months post-transplantation in diabetic rats (Sham: no islet ($n = 5$); Liver: hepatic islet transplantation ($n = 6$); omental islet transplantation with HPMC ($n = 8$)). AUC of C-peptide levels was measured for each rat. The results are expressed as Box (25th to 75th percentiles) and Whisker plots (5th and 95th percentiles). Symbols represent significant differences between Liver, HPMC for hOMING and Sham. * represents a p value < 0.05 . Kruskal–Wallis analysis of variance (ANOVA) was used with Dunn’s multiple comparison test. Glycaemia and C-peptide levels can be found in Fig. 4. (c) The structure (i) and (ii) and vascularisation (iii) and (iv) of islets 2 months post-implantation. Haematoxylin-Eosin (H&E) staining of an islet in the liver (i) and in the omentum (ii) two months post-transplantation. Vessel staining of transplanted intrahepatic (iii) or intra-omental islet (iv). Red: Insulin, Green: C31, Blue: 2-(4-amidinophenyl)-1H-indole-6-carboxamide (DAPI). Original magnification: $\times 200$. Scale bar: 50 μm . (d) CD31 (vessels) in the transplanted islets was assessed by immunofluorescence quantification of at least 15 different islets in each condition using Nis-Element BR-software. The intensity of the green staining was recorded and scaled by the size of the islets. Mann-Whitney test, no significant difference was observed.

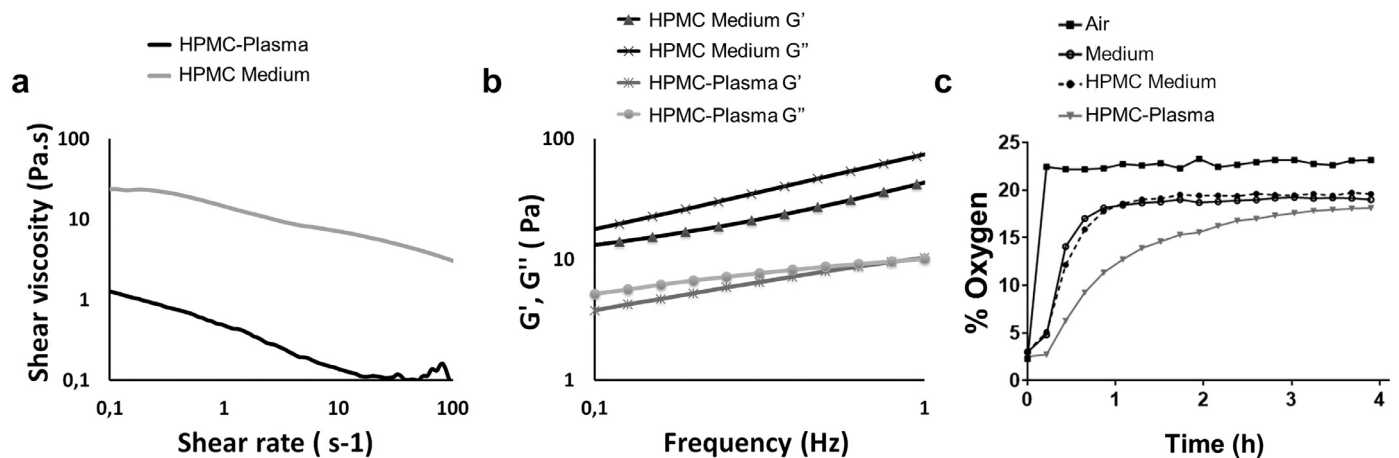


Fig. 3. Rheological properties and oxygen diffusion of HPMC after Plasma supplementation. (a) Shear viscosity as a function of shear rate for the HPMC matrix prepared either in cell culture medium ‘HPMC Medium’, in rat plasma ‘HPMC-Plasma’ or in plasma +thrombin ‘HPMC-Plasma + Thrombin’. The results are plotted as the shear viscosity in $\text{Pa s} = f(\text{Shear rate}(\text{s}^{-1}))$. (b) Storage modulus (G') and loss modulus (G'') as a function of frequency for the HPMC hydrogels prepared under the same conditions. Viscosity properties G', G'' (Pa) = $f(F(\text{Hz}))$. (c) Oxygen diffusion through the HPMC hydrogels.

the cell culture medium. The viscosity decreased in the presence of plasma by a factor of 10 and 30, depending on the applied shear rate. Next, the viscoelastic properties of the HPMC hydrogel prepared in the cell culture medium or with plasma were investigated. The viscoelastic properties of the HPMC hydrogel, storage (G'), and loss (G'') moduli, were determined as a function of frequency between 0.1 and 1 Hz. G' represents the elastic or recoverable component, and G'' is the viscous component. In Fig. 3(b), G'' is higher than G' over the frequency range for all conditions tested, indicating that the viscous component was predominant. Therefore, the HPMC hydrogel behaved more like a viscous fluid than like a solid across all the tested conditions. Moreover, HPMC-Plasma presented a significant decrease in both G' and G'' by a factor ranging between 2 and 3, depending on the frequency. This means that the addition of plasma within the HPMC hydrogel decreased the overall rheological properties. In parallel, as plasma was shown to decrease oxygen diffusion, measurements of oxygen levels were performed (Fig. 3(c)), which showed that oxygen diffused in a similar manner in the HPMC hydrogel (HPMC medium) as in a liquid (culture medium). Plasma supplementation modified the oxygen diffusion; the oxygen diffused less rapidly in this hydrogel than in the HPMC medium.

3.4. In vitro and in vivo assessment of HPMC-supplemented plasma efficiency for hOMING

Fluorescein diacetate/propidium iodide (FDA/PI) staining (Fig. 4(a)) showed that islet viability was similar for embedded and free islets, with few cells stained for necrosis. Levels of activated caspase 3 were lower in islets in plasma-supplemented HPMC (Fig. 4(b)) (Control vs. HPMC-Plasma, $p < 0.05$, $n = 6$), revealing a lower level of apoptosis in the presence of plasma. Glucose stimulation did not differ significantly between control and hydrogel culture conditions (Fig. 4(c)). However, an increase in insulin secretion was observed for stimulated islets in HPMC supplemented with plasma (Control vs. HPMC-Plasma $p < 0.05$). The relative increase in the basal level was compensated by the increase in stimulated conditions. Therefore, the ability of glucose sensing was maintained as shown by the index of stimulation (Fig. 4(d)). HIF-1 α translocation was similar under the different conditions (Fig. 4(e)). Islet transplantation at 7660 islet equivalents (IEQ) per kg restored glycaemic control in the three groups receiving islets (i.e. Liver, HPMC, and HPMC-Plasma), with a significant reduction in glycaemia ($p < 0.001$ D0 vs. D7) (Fig. 5(a)) along with a significant increase in C-peptide levels ($p < 0.001$ D0 vs. D7) (Fig. 5(b)), compared with the pre-transplant conditions. No statistical difference was observed between the groups regarding body weight (Fig. 5(c)). Intratissular grafting with HPMC-Plasma showed the same efficiency as intraportal grafting, in contrast to the HPMC alone group, which presented lower graft efficiencies (D42 $p < 0.05$ HPMC-Plasma vs. HPMC). Low glycaemia levels observed at day 0 were due to the remnant insulin from the pellets. The levels of glycaemia measured in the liver group and the HPMC-Plasma group were similar during the study. Differences in this cohort were observed between the HPMC-Plasma group and the HPMC alone group. The C-peptide levels revealed lower production in the HPMC group than in the HPMC-Plasma throughout the follow-up duration. Intra-peritoneal glucose tolerance test (IPGTT) profiles indicated normal graft function in all groups receiving islets (Fig. 5(d)–(i)). We observed a rapid increase in glycaemia at 15 min, followed by a progressive decrease to reach the normal level (<2 g/L) after 2 h. At day 21 after implantation, the IPGTT area under curve (AUC) value of the HPMC-Plasma group was significantly lower than that of all the other groups (Fig. 5(j)). After 56 days, glycaemia of the liver group had increased up to 2 g/L after 120 min (Liver, 2.15 ± 0.47 ; HPMC-Plasma, 1.86 ± 0.31).

3.5. Advantages of HPMC-Plasma over HPMC alone

To detect whether the fibrin contained in plasma can be polymerised *in vivo*, creating a nest for islets, injection of plasma-containing beads (mimicking islets) was carried out, and after 1 h, the omentum was retrieved and fixed. Sections from the omentum, obtained at 1 h after HPMC and HPMC-Plasma injections, revealed the presence of fibrin fibres (red) surrounding the dextran beads (blue) (Fig. 6(a) iii and iv) in the HPMC-Plasma treatment. In contrast, no fibre was observed in treatment with HPMC alone (Fig. 6(a) i and ii). The presence of plasma is correlated with the maintenance of integrin signalling in islets *in vitro*, as shown by the western blot analysis of integrin and FAK phosphorylation (Fig. 6(b)). Furthermore, the presence of plasma significantly increased the post-implantation vessel density (CD31 staining) (Fig. 6(c) and (d)), compared with the islets transplanted with HPMC alone. Eighty per cent, 75% and 50% of the rats reached insulin independence at day 21 in the Liver, OM+HPMC-Plasma and OM+HPMC groups, respectively.

3.6. Decrease in islet requirements with the combination of HPMC and plasma

On the basis of the encouraging results obtained for the first cohort, a 25% lower islets mass (5700 IEQ/kg) than that in the first cohort was transplanted. Only the intra-omental islet transplantation succeeded in reversing diabetes through a significant decrease in glycaemia (D4–D63; $p < 0.001$) and a significant increase in C-peptide levels (D3–D63 $p < 0.01$), compared with intra-portal grafting (Fig. 7(a) and (b)). When stimulated by an IPGTT (Fig. 6(c)), rats that received an intra-omental implantation of islets with HPMC-Plasma showed a significant decrease in the AUC values across all time points compared with the liver recipients. As shown previously, when the omental graft was retrieved, glycaemia increased and C-peptide levels decreased to the pre-transplant levels (Fig. 7(a) and (b)). With a reduction of 35% in the islet mass (4900 IEQ/kg), glycaemic regulation was also observed, with an 11-day delay in the full C-peptide production (C-peptide level at 7 days: 396.8 ± 205.5 pmol/L for 4900 IEQ/kg vs. 750.0 ± 341.1 pmol/L for 5700 IEQ/kg). Glycaemia below 2.5 g/L was observed in 5 out of the 8 transplanted rats in both groups against none in the Liver group.

When fasting glycaemia or post IPGTT glycaemia was compared, the liver group appeared to be different from the other two treatments, with higher glycaemia levels, indicating no function of the graft (Fig. 7(d)).

4. Discussion

In the present study, we show that a plasma-enriched HPMC hydrogel injected using the hOMING technique improved graft performance thanks to properties such as appropriate oxygen diffusion, adequate matrix network surrounding the graft, enhanced vascularisation and preserved islet morphology and structure. As a result, control of glycaemia was obtained along with a reduction of up to 35% in the rat islet transplanted mass. This result demonstrates the efficiency brought about by the combination of positive effects of the recipient site, the matrix and the implantation technique (hOMING) (Fig. 8).

4.1. Biomaterial properties

Polymerised plasma with thrombin showed incompatibility with hOMING (Supplemental Fig. 6). Polymerisation of plasma forms a dense network of entangled fibres (as shown in the SEM observation). This increase in density triggers hypoxic events due

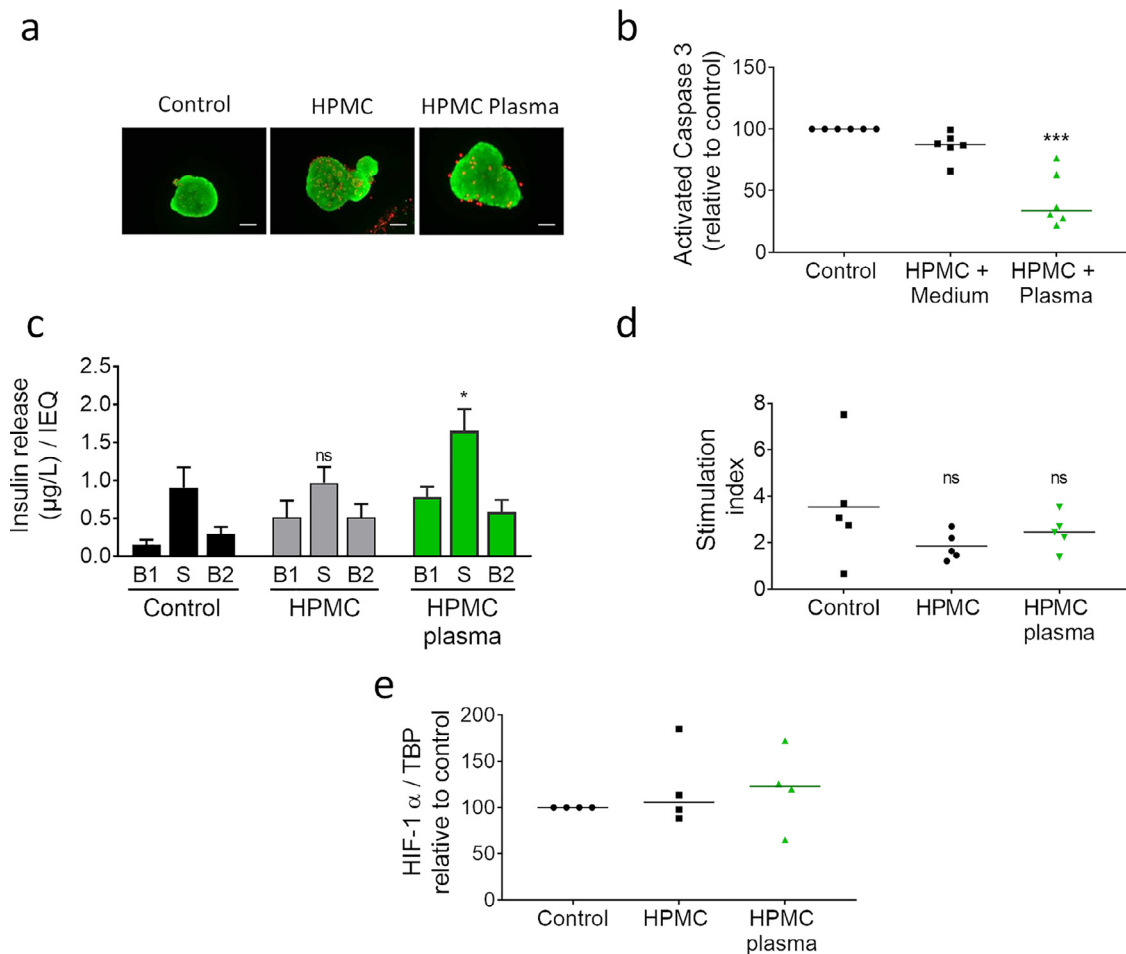


Fig. 4. Validation of the combination of HPMC and plasma *in vitro*. (a) Representative fluorescein diacetate/propidium iodide (FDA/PI) viability staining of rat islets cultured under different control or hydrogel conditions after 24 h. ($n = 5$). Original magnification: $\times 200$, scale bar = 50 μm . (b) Active caspase-3 level reflecting the apoptosis of rat islets cultured under different conditions (control or hydrogels) for 24 h. Kruskal–Wallis analysis of variance (ANOVA); $*p < 0.05$ vs. control ($n = 6$). (c) Glucose-stimulated insulin secretion test of rat islets after 24 h of exposure to the hydrogels. 'B1' and 'B2' represent the responses of islets after incubation with a low glucose concentration of 4.4 mM, and 'S' represents the stimulating conditions in 22.6 mM glucose. $*p \leq 0.05$ vs. control. Kruskal–Wallis analysis of variance (ANOVA) was used with Dunn's multiple comparison test ($n = 5$). (d) Stimulation index of the rat islets cultured under different conditions (control or hydrogels) for 24 h ($n = 5$, ns). (e) HIF-1 α translocation was measured in the nuclear fraction of rat islets ($n = 4$) after a culture for 24 h in complete M199, HPMC or HPMC-Plasma (ns).

to the high material content. Such a structure creates a barrier for diffusion and results in tortuosity; hence, it slows down the oxygen diffusion through the matrix. Furthermore, the plasma clot cannot be injectable, as it is a solid. Thus, the increase in plasma viscosity was induced by the addition of HPMC polymer chains. It was necessary to check whether the supplementation of plasma with HPMC is brought about by any drastic modification in the rheological properties of HPMC, preventing the use of the combination for hOMING application. The addition of plasma significantly decreased the viscosity and the viscoelastic properties of the HPMC hydrogel. However, after plasma addition, HPMC still behaved like a viscous fluid, with its G'' value always higher than its G' value. Therefore, HPMC-Plasma combination remains injectable (Supplemental Fig. 3), which is a key property for the hOMING application. Moreover, with a decreased viscosity, the injection might even be facilitated. In addition, HPMC at this very low concentration (3% w/w) is a hydrogel composed mostly of water; thus, its properties in terms of oxygen diffusion are almost equivalent to a pure water solution. This is another advantage of our composite, as no hypoxia was further triggered in the islets, as compared to polymerised plasma [28]. Finally, two months post implantation, the cells were integrated in the tissue, between the adipocytes without any evidence of the presence of HPMC. Erosion and swelling properties of HPMC was described to be responsible

for the resorbability of the materials [49], which is an advantage for our application, enabling islet settlement in direct contact with the tissue [50–52]. The hydrogel acts as a sponge with body fluids until the viscous properties decrease and the molecules of HPMC enter into the circulation for subsequent elimination [38,53,54].

4.2. HPMC validation for islet implantation using hOMING

Pancreatic islets are primary 3D mini organs composed of different types of cells organised in a way that allows their cooperation for the control of blood glucose levels. The preservation of this specific structure depends on the conditions of the implantation. Our hOMING technique and the fact that the transplantation was performed without any contact with the blood preserved the structure and the composition of the islets (Supplemental Fig. 4). Yet, fragmented islet capacity to express insulin and to function *in vivo* was maintained. Graft function was even more efficient in the liver than in the omentum, in which islets presented a preserved morphology. The difference in extracellular matrix composition between the two tissues [55,56] could be responsible for the difference in graft performance. Thus, the presence of plasma at the moment of implantation appeared essential to outperform hepatic transplantation outcomes.

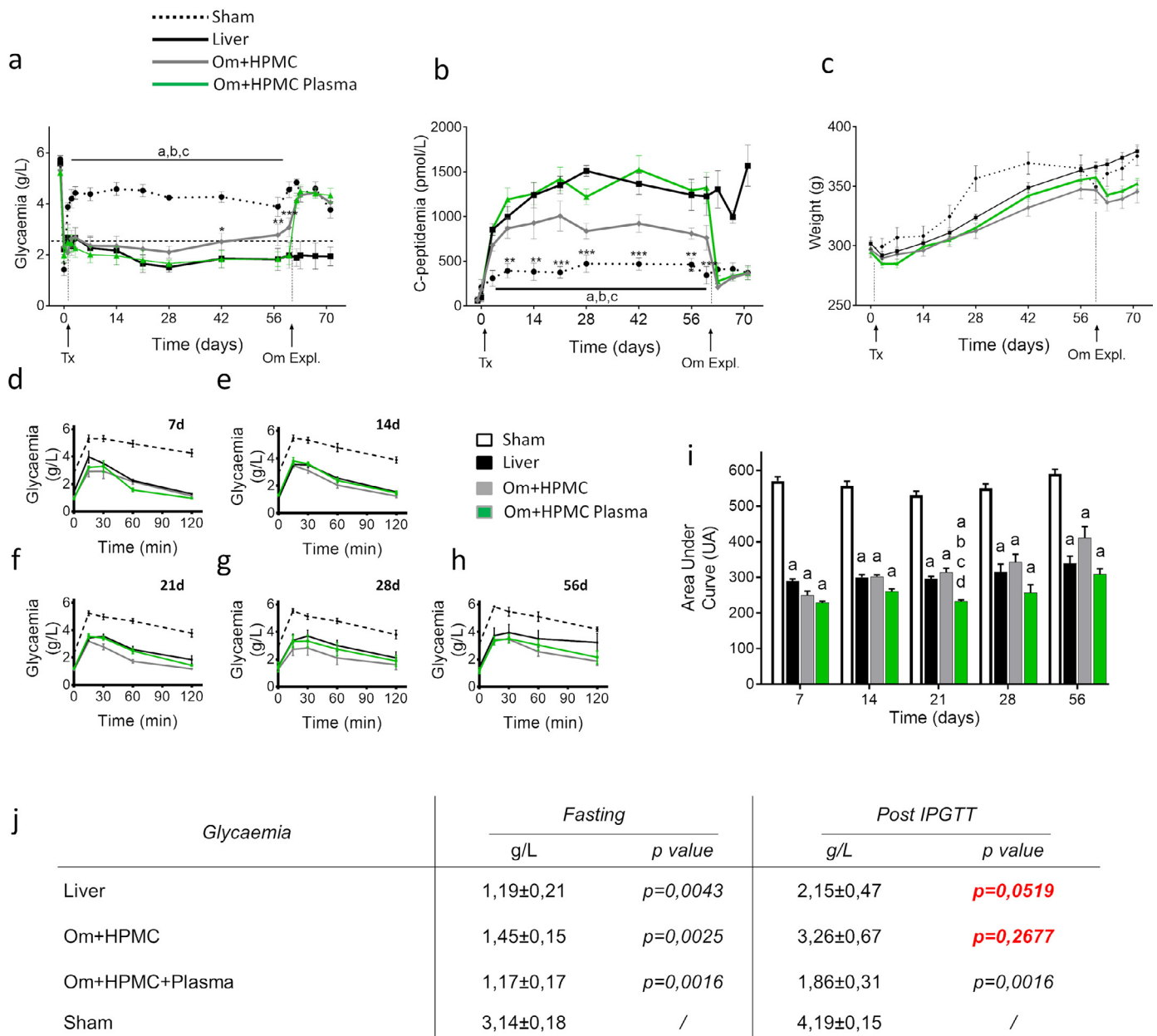


Fig. 5. Plasma supplementation to HPMC improved intra-omental graft function. (a) glycaemia (non-fasting) of rats transplanted with 7660 IEQ/Kg in the omentum with HPMC+plasma (Om+HPMC-Plasma; $n = 8$) or with HPMC (Om+HPMC; $n = 8$) in the liver ($n = 5$) with 7660 IEQ/Kg and of Sham ($n = 5$). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$: Om+HPMC vs. Om+HPMC-Plasma ($n = 8$), (a)–(c): $p < 0.001$ sham ($n = 5$) vs. liver ($n = 5$), vs. om+HPMC ($n = 8$), vs. om+HPMC-Plasma ($n = 8$), respectively. (b) C-peptide levels of transplanted rats with 7660 IEQ/Kg. (Om+HPMC-Plasma, $n = 8$; Om+HPMC, $n = 8$; Liver, $n = 5$) * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ Om+HPMC vs. Om+HPMC-Plasma; (a)–(c): $p < 0.001$ sham vs. liver, vs. Om+HPMC, vs. Om+HPMC-Plasma, respectively. Repeated-measures, one-way and Kruskal–Wallis analysis of variance (ANOVA) were used with Dunn’s multiple comparison test. Tx: transplantation and pump retrieval; Om Expl.: explantation of the omental graft. (c) Weight of the transplanted rats receiving 7660IEQ/kg for 2 months. A decrease in weight was observed after each surgical intervention, after islet transplant (day 0 to day 5) and after omental explantation (day 63 to day 70). (Om+HPMC Plasma, $n = 8$; Om+HPMC, $n = 8$; Liver, $n = 5$) Recipients of the intra-portal graft experienced only the first drop in weight, as they did not undergo graft explantation ($^5P < 0.05$ vs. liver). Sham received every day exogenous insulin (Lantus® 6 IU/200 g of body weight), which explained the increase in body weight. (d)–(h) Intra-peritoneal glucose tolerance test (IPGTT) performed on transplanted rats at days 7, 14, 21, 28 and 56, respectively (Om+HPMC-Plasma, $n = 8$; Om+HPMC, $n = 8$; Liver, $n = 5$). (i) Area under the curve (AUC) of IPGTT profiles. * $p < 0.001$ vs. sham; $^b p < 0.05$ vs. liver; $^c p < 0.05$ vs. omentum HPMC; and $^d p < 0.05$, based on one-way analysis of variance (ANOVA). Note that the AUC of the Om+HPMC-Plasma group at each time point is lower than the value in all the other groups. Free islet graft in the omentum using hOMING was previously shown to be non-functional [17]. (j) Fasting and post-IPGTT glycaemia at 56 days post-transplantation. The liver and omentum HPMC groups exhibited a glycaemia level higher than 2 g/L. Comparison of the fasting glycaemia and the Sham group with one-way analysis of variance (ANOVA) revealed that all the treatment groups were statistically different from the Sham group. Comparison of IPGTT glycaemia revealed the absence of difference between the liver or HPMC groups and the Sham, even though the HPMC-Plasma levels were different. Significant value using one-way analysis of variance (ANOVA) is highlighted in red.

4.3. Effect of the biomaterials on islets in vitro and in vivo

The addition of plasma reduced the islet apoptosis rate, confirming that plasma properties were conserved even when mixed with hydrogel plasma. Plasma contains fibrin, of which, RGD

sequences are essential for the activation of survival pathways through integrins [27,28]. β -cell integrin activation was shown to increase the insulin secretion [57] and decrease apoptosis [27,58].

Integrins need both biological/biochemical (as RGD ligands) and mechanical stimuli (as pressure forces) to decrease caspase-3

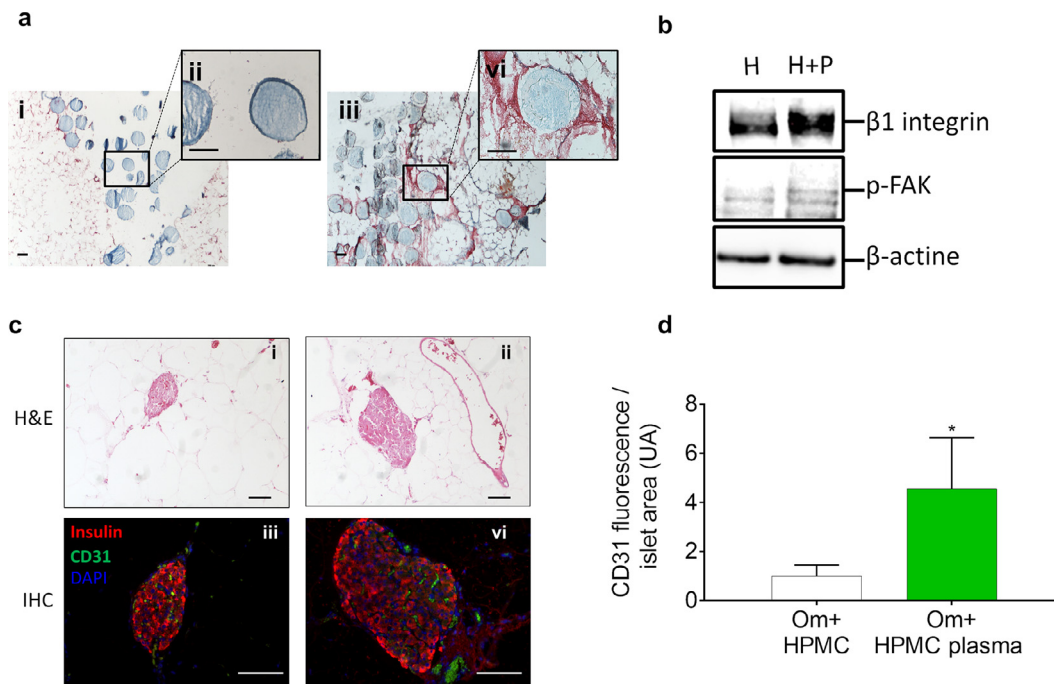


Fig. 6. Benefits of plasma in the HPMC. (a) Fibrin staining (MSB) of dextran beads co-transplanted either with HPMC alone (i) and (ii) or with HPMC-Plasma (iii) and (vi), 1 h post infusion. Fibrin fibres in formation are stained in red, and beads are stained in blue. Scale bar: 50 μ m. (b) Integrin expression at the surface of islets and FAK activation (by phosphorylation) as a target of $\beta 1$ integrin, when exposed to HPMC or HPMC-Plasma *in vitro* during 24 h culture, were studied by western blot. Fibrin present in the plasma activates the interaction between islets and HPMC+plasma, modulating the survival pathway (representative Western blot of three ($n = 3$) independent experiments). (c) Haematoxylin-Eosin (H&E) staining of an islet in the liver (i) and in the omentum (ii) two months post transplantation. (iii & vi) Vessel staining of transplanted intrahepatic (iii) or intra-omental islet (vi) (at graft retrieval, i.e., 2 months post-transplantation). Red: Insulin, Green: CD31, Blue: 2-(4-amidinophenyl)-1H-indole-6-carboxamide (DAPI). Original magnification: $\times 200$. Scale bar: 75 μ m. (d) Quantification CD31 (vessels) in the transplanted islets was assessed by immunofluorescence quantification of at least 15 different islets in each condition using Nis-Element BR-software. The intensity of the green staining was recorded and scaled by the size of the islets. Plasma in HPMC potentiated the islet re-vascularisation post-transplantation, $*p < 0.05$ Om+HPMC-Plasma vs. Om+HPMC (Mann-Whitney).

activation. With the properties of a hydrogel, the force/pressure applied by HPMC-Plasma on the cells was lower than that of a stiff gel such as polymerised plasma. As a result, the decrease in caspase-3 activation was lower in HPMC-Plasma (50%) than in polymerised plasma (75%) *in vitro* [27]. However, the *in vivo* HPMC permeability allows the circulating thrombin to rapidly polymerise the fibrinogen into fibrin, as shown by the fibril formation, observed within 1 h post implantation. The polymerised plasma might better reflect the *in vivo* condition, and the benefits of plasma on islets might be better potentiated *in vivo* than observed in our *in vitro* results. The maintenance of integrin at the islet cell surface, owing to the RGD motif of fibrin, makes them ready to anchor in the adipose tissue. Consequently, post-implantation decrease of anoikis should help maintain a large mass of functional islets in the long term, thus decreasing the islet requirement.

This hypothesis was confirmed by the first metabolic follow-up (glycaemia and C-peptide) post implantation, realised on STZ-diabetic rats grafted with 7660 IEQ/kg. An improved graft function was obtained using the plasma-supplemented HPMC during the study. On the contrary, it appears that even if the graft transplanted with HPMC alone or in combination with serum (i.e. plasma without fibrin) (Supplemental Fig. 5) successfully reversed diabetes, it did so for a shorter time and in a less potent manner (lower C-peptide levels and higher glycaemia).

Oxygen supply is another key aspect for islet survival and function. It has been established that hypoxia impairs insulin secretion ability [59], as revealed in our study by the C-peptide level in the HPMC condition, wherein the islet presented a lower vessel density. On the other hand, islet transplanted with HPMC-Plasma exhibited a high level of vascularisation, higher than that in the Liver, while also enhancing islet function. The correlation between the promotion of vascularisation by adapted matrices and graft

function has also been observed previously [60–62]. In our study, the presence of plasma, especially fibrin, most probably triggers the MAPK pathway and angiogenic sprouting [63] through integrin signalling. Furthermore, plasma, known to mediate vascular endothelial cell remodelling through the fibroblast growth factor receptor-1 (FGFR1) on β -cells [64], promotes the angiogenesis around and inside the islets.

In our study, we observed a loss in glycaemic control in HPMC and HPMC-serum (Supplemental Fig. 4) after 1.5 months, revealing the start of graft loss. While a period of 2 months was not long enough to detect a decrease in C-peptide levels, the IPGTT results confirmed this hypothesis. It is well known in clinics that a massive loss in islet mass within few days post-implantation impairs graft function in the long term. It is hypothesised that the glucotoxicity generated by the decrease in islet functional mass is deleterious for islet viability. This leads to a further decrease in islet mass, generating even more glucotoxicity. As this vicious cycle progresses with much fewer islets, the glycaemia is less and less controlled, increasing the glucotoxicity and the destruction of the graft. This phenomenon explains the need for 5000 IEQ/kg for the first islet infusion, a mass correlated with insulin independence [65–67]. Islets co-transplanted with HPMC-Plasma performed better than islets in HPMC alone or those in HPMC-serum because of a reduction in anoikis and cell death and a rapid and denser re-vascularisation, leading to insulin independence for the duration of the study.

4.4. Decrease in islet requirement

A reduction by at least a fourth of the original needs is dramatically important, as it translates to a decrease in organ procurement and the risk of recipient sensitisation. It appeared that

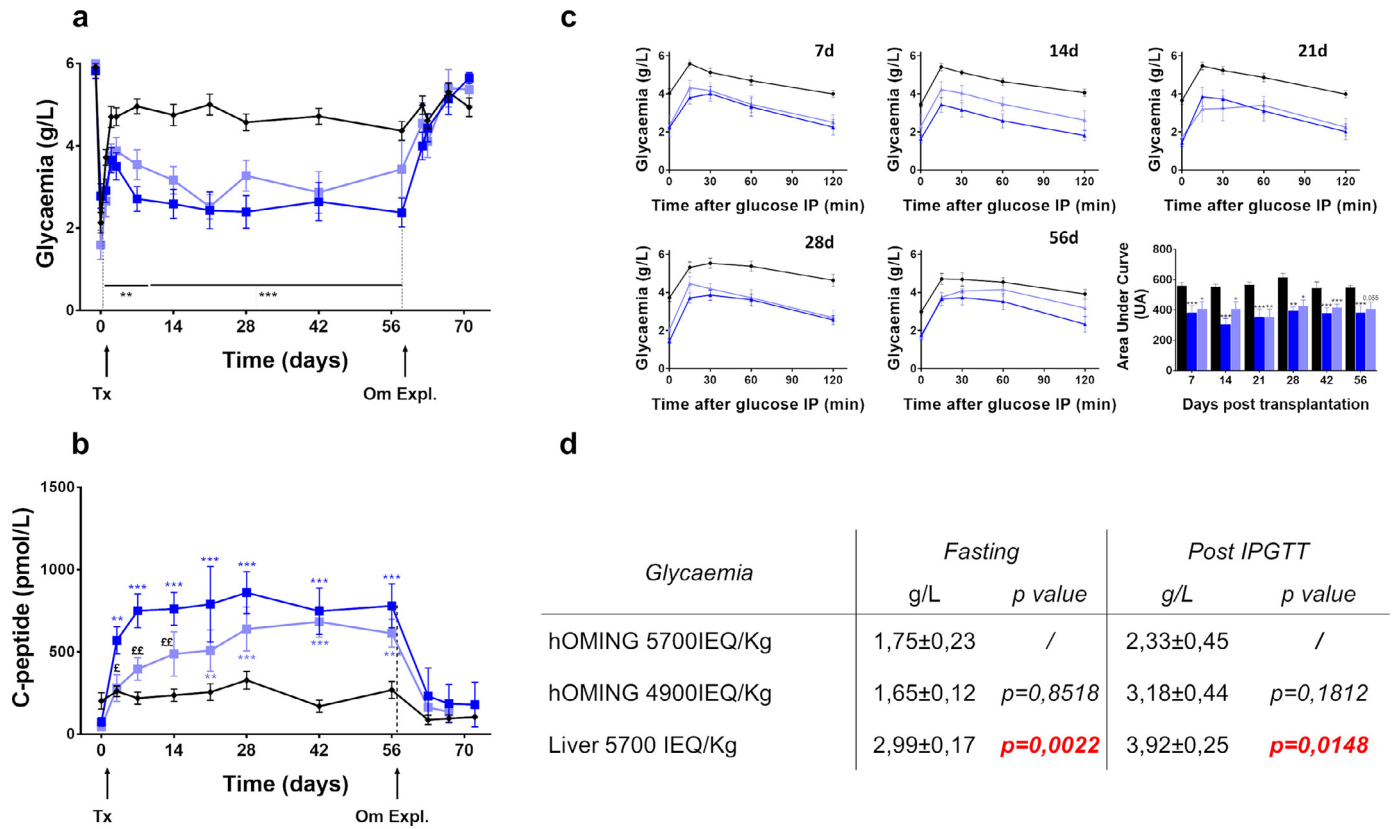


Fig. 7. Better performance of the graft with fewer islets, overperforming hepatic grafts. (a) Glycaemia (non-fasting) in rats transplanted either intraportally ($n = 8$) or intra-ommentally with 5700 IEQ/kg ($n = 10$) or with 4900 IEQ/kg ($n = 7$). $** p < 0.01$ Om+HPMC-Plasma 4900 vs. Liver and $*** p < 0.001$ vs. Liver. (b) C-peptide levels of rats transplanted either intraportally or intra-ommentally with 5700 IEQ/kg or 4900 IEQ/kg. $** p < 0.01$ vs. Liver and $*** p < 0.001$ vs. Liver. (c) Intra-peritoneal glucose tolerance test of transplanted rats at different time points, and the corresponding area under the curve (AUC). $p < 0.001$ (intra-ommentally with 5700 IEQ/kg ($n = 10$) or with 4900 IEQ/kg ($n = 7$)) as compared to the liver ($n = 8$). (d) Fasting and post-IPGTT glycaemia at 56 days post-transplantation, Liver and Om HPMC + plasma 4900IEQ/kg as compared to the Om+HPMC-Plasma 5700IEQ (fasting: baseline glycaemia before glucose injection, Post-IPGTT: glycaemia 120 min after glucose injection). The liver group exhibited a glycaemia level significantly different from the hOMING groups. Repeated-measures analysis of variance (ANOVA) was used with Fisher LSD post-hoc test. Tx: Transplantation and pump retrieval. Om. Expl.: Explantation of omentum. Black curve: Transplantation of 5700 IEQ/kg in the liver; dark blue curve: transplantation of 5700 IEQ/kg in the omentum; light blue curve: transplantation of 4900 IEQ/kg in the omentum.

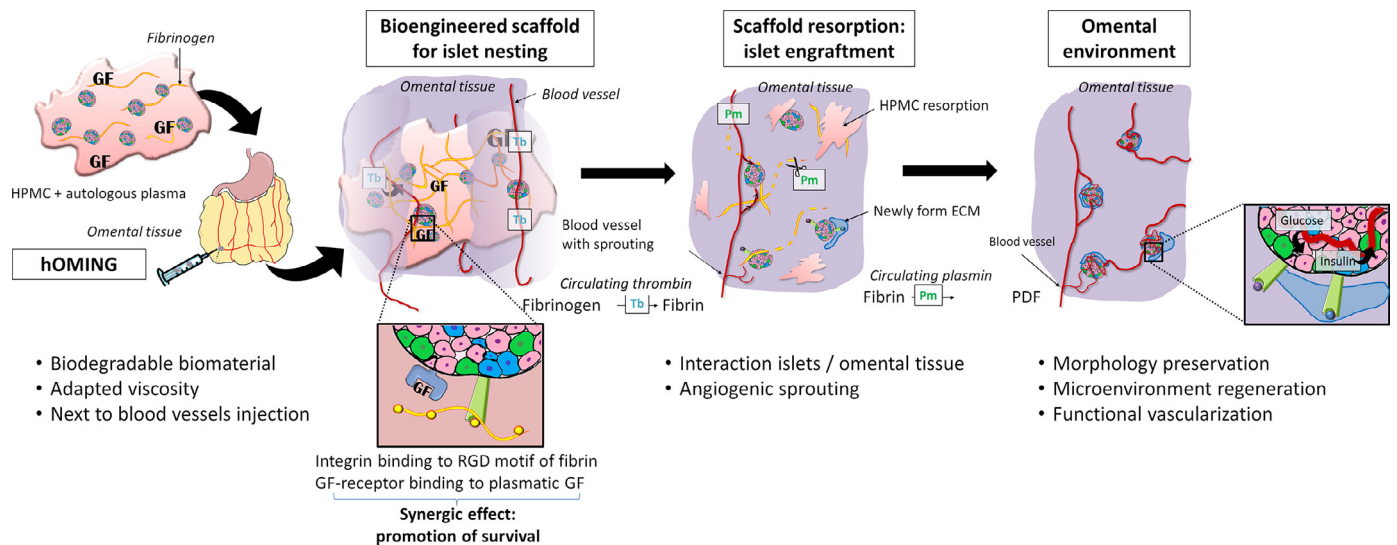


Fig. 8. HPMC-Plasma as a suitable carrier for hOMING. First, the islets are mixed with HPMC-Plasma. HPMC as a carrier fulfils the list of needs for hOMING (such as viscosity and biodegradability). The islets are then transplanted into the omental tissue using hOMING, allowing deposition of the islet-carrier mix next to the blood vessels. Next, quickly after implantation, the circulating thrombin (Tb) can mobilise the plasmatic fibrinogen into fibrin, creating a nest for the islet, which, thanks to plasma, is enriched in growth factor. HPMC is resorbed by the recipient's body. The fibrin nest around the islet is also progressively resorbed by circulating plasmin (Pm), which yields the products of fibrin degradation. At the same time, the islets create their anchorage by producing ECM to integrate into the omental tissue. Next, the vessels sprout thanks to plasmatic growth factor (GF) and islet secretion. At the end, the islets become well integrated to the omental tissue, without any damage, due to the smooth process of implantation. Microenvironment and vascularisation are reconstructed, providing the islets with all their needs for proper functioning. GF: Growth factor, RGD: Arg-Gly-Asp motif, Tb: Thrombin, Pm: Plasmin, ECM: Extracellular matrix.

the production of C-peptide was similar across all treatments, with a glycaemic regulation of the transplanted rats. The progressive function of the graft appeared to be slower with 4900 IEQ/kg than with 5700 IEQ/kg, indicating the efficiency limit of this islet mass.

4.5. Advantages of the omental recipient site

The reduction in islet number was also made possible by the host tissue, the omentum. Numerous studies have shown the advantages of omentum [15,18,21,68], especially the capacity of the omentum to promote vascularisation. However, it is likely that this is not the only advantage of using omentum as a host tissue. The capacity of the tissue to store glucose under insulin control, as shown by the elevation of omentum weight and adipocyte size in the presence of islets (Supplemental Fig. 2), promoted the efficiency of the graft. The proximity of the islets and adipocytes means that insulin released in the direct environment of the adipocytes activates receptors and enhances glucose storage. The capacity to store glucose in an ‘unlimited manner,’ in the fatty acid form of adipose tissue [69,70], is a major advantage of the omentum when compared with the liver. Indeed, the liver [71] is sensitive to hyperinsulin secretion, often leading to local steatosis [72,73] and local insulin resistance, which impair graft function [74]. Thus, the first hepatic pass could be improved using the omental approach. Considering the risk of insulin resistance development caused by accumulation of fat storage, we verified and confirmed that adipocyte dysfunction was not observed. Indeed, leptin levels were comparable after intra-portal and intra-omental islet transplantation (Supplemental Fig. 2). Moreover, the omental space is also suitable to accept a co-graft of islet with a carrier, which is impossible to realise in the liver.

4.6. Conclusion

We hypothesised that islet preservation improves graft function, owing to the advantages cited above as well as the specific kinetics of implantation, as shown in Fig. 7. The hydrogel disappears quickly post-implantation (within a few hours), representing the time necessary for the circulating thrombin to polymerise the plasma fibrinogen. After the disappearance of the hydrogel, only the plasma remains around the islets, forming a strong interaction that increases the number of integrins recruited at the membrane [27,28]. Plasma, as a biodegradable scaffold, is then slowly and naturally degraded by plasmin, providing time to build interactions with the omental tissue. Finally, conservation the islet mass during the first week post-implantation leads to well-integrated and functional islets, as observed on 2-month-old-grafts. In conclusion, a reduction in islet requirements was made possible by a combination of different factors: the decrease in islet death due to the anoikis reduction and the increased vascularisation, as well as the properties of the hOMING technique and the implantation site. The fact that the use of these biological products and omentum has already been validated in clinics should allow easy transfer of this technique to clinics.

Declaration of Competing Interest

There is no conflict of interest.

CRediT authorship contribution statement

Anais Schaschkow: Methodology, Writing - original draft, Data curation, Formal analysis. **S  verine Sigrist:** Writing - review & editing. **Carole Mura:** Data curation, Formal analysis. **Julien Barthes:** Writing - review & editing. **Nihal Engin Vrana:** Writing - review & editing. **Elodie Czuba:** Data curation, Formal analysis. **Florent**

Lemaire: Data curation, Formal analysis. **Romain Neidl:** Writing - review & editing. **Caroline Dissaux:** Writing - review & editing. **Anne Lejay:** Writing - review & editing. **Philippe Lavalle:** Writing - review & editing. **Catherine Bruant-Rodier:** Writing - review & editing. **Karim Bouzakri:** Writing - review & editing. **Michel Pinget:** Writing - review & editing. **Elisa Maillard:** Conceptualization, Supervision, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2019.11.047.

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