Vegf-A mRNA transfection as a novel approach to improve mouse and human islet graft revascularisation

Willem Staels^{1,2} · Yannick Verdonck¹ · Yves Heremans¹ · Gunter Leuckx¹ · Sofie De Groef¹ · Carlo Heirman³ · Eelco de Koning⁴ · Conny Gysemans⁵ · Kris Thielemans³ · Luc Baeyens¹ · Harry Heimberg¹ · Nico De Leu^{1,6,7}

Received: 12 December 2017 / Accepted: 23 April 2018 / Published online: 22 May 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Aims/hypothesis The initial avascular period following islet transplantation seriously compromises graft function and survival. Enhancing graft revascularisation to improve engraftment has been attempted through virus-based delivery of angiogenic triggers, but risks associated with viral vectors have hampered clinical translation. In vitro transcribed mRNA transfection circumvents these risks and may be used for improving islet engraftment.

Methods Mouse and human pancreatic islet cells were transfected with mRNA encoding the angiogenic growth factor vascular endothelial growth factor A (VEGF-A) before transplantation under the kidney capsule in mice.

Results At day 7 post transplantation, revascularisation of grafts transfected with *Vegf-A* (also known as *Vegfa*) mRNA was significantly higher compared with non-transfected or *Gfp* mRNA-transfected controls in mouse islet grafts (2.11- and 1.87-fold, respectively) (vessel area/graft area, mean $\pm$ SEM: 0.118 ± 0.01 [$n = 3$] in *Vegf-A* mRNA transfected group (VEGF) vs 0.056 ± 0.01 [$n = 3$] in no RNA [$p < 0.05$] vs 0.063 ± 0.02 [$n = 4$] in *Gfp* mRNA transfected group (GFP) [$p < 0.05$]); EndoC-bH3 grafts (2.85- and 2.48-fold, respectively) (0.085 ± 0.02 [$n = 4$] in VEGF vs 0.030 ± 0.004 [$n = 4$] in no RNA [$p < 0.05$] vs 0.034 ± 0.01 [$n = 5$] in GFP [$p < 0.05$]); and human islet grafts (3.17- and 3.80-fold, respectively) (0.048 ± 0.013 [$n = 3$] in VEGF vs 0.015 ± 0.0051 [$n = 4$] in no RNA [$p < 0.01$] vs 0.013 ± 0.0046 [$n = 4$] in GFP [$p < 0.01$]). At day 30 post transplantation, human islet grafts maintained a vascularisation benefit (1.70- and 1.82-fold, respectively) (0.049 ± 0.0042 [$n = 8$] in VEGF vs 0.029 ± 0.0052 [$n = 5$] in no RNA [$p < 0.05$] vs 0.027 ± 0.0056 [$n = 4$] in GFP [$p < 0.05$]) and a higher beta cell volume (1.64- and 2.26-fold, respectively) (0.0292 ± 0.0032 μl [$n = 7$] in VEGF vs 0.0178 ± 0.0021 μl [$n = 5$] in no RNA [$p < 0.01$] vs 0.0129 ± 0.0012 μl [$n = 4$] in GFP [$p < 0.001$]).

Conclusions/interpretation *Vegf-A* mRNA transfection before transplantation provides a promising and safe strategy to improve engraftment of islets and other cell-based implants.

Keywords Cell therapy · Diabetes · Gene delivery · Graft revascularisation · Islet transplantation · Messenger RNA · Pancreatic beta cell · RNA delivery · VEGFA

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00125-018-4646-7>) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

✉ Harry Heimberg
Harry.Heimberg@vub.be

✉ Nico De Leu
Nico.De.Leu@vub.be

¹ Beta Cell Neogenesis (BENE), Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium

² Department of Paediatrics, Division of Paediatric Endocrinology, Ghent University, Ghent, Belgium

³ Laboratory of Molecular and Cellular Therapy, Vrije Universiteit Brussel, Brussels, Belgium

⁴ Department of Medicine, Section of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands

⁵ Laboratory of Clinical and Experimental Endocrinology, Katholieke Universiteit Leuven, Leuven, Belgium

⁶ Department of Endocrinology, UZ Brussel, Brussels, Belgium

⁷ Department of Endocrinology, ASZ Aalst, Aalst, Belgium

Research in context

What is already known about this subject?

- Clinical islet transplantation suffers from a major loss of donor cells in the initial period after transplantation
- Impaired graft revascularisation is an important reason for islet graft attrition
- Virus-based delivery of angiogenic triggers has been successfully exploited in animal studies to improve graft revascularisation, but risks associated with viral vectors have hampered clinical translation of this approach

What is the key question?

- Can *Vegf-A* mRNA transfection of donor islets before transplantation effectively improve graft revascularisation?

What are the new findings?

- *Vegf-A* mRNA transfection of islets yields a transient boost in secreted vascular endothelial growth factor A (VEGF-A) protein
- *Vegf-A* mRNA-transfected mouse and human islet cell grafts show enhanced revascularisation following transplantation under the kidney capsule in the mouse

How might this impact on clinical practice in the foreseeable future?

- Modified *Vegf-A* mRNA transfection before transplantation provides a promising and safe strategy to improve engraftment of islets and other cell-based implants

Abbreviations

GFP	<i>Gfp</i> mRNA transfected group
IVT	In vitro transcription
modVEGF	modified <i>Vegf-A</i> mRNA transfected group
SCID	Severe combined immunodeficiency
VEGF	<i>Vegf-A</i> mRNA transfected group
VEGF-A	Vascular endothelial growth factor A

Introduction

In clinical islet transplantation, it is estimated that up to half of the donor cells are lost in the initial period after transplantation [1–4]. Excessively high numbers of islet cells, often from multiple donors, are required to restore euglycaemia in type 1 diabetes via intrahepatic islet transplantation [4, 5]. Achieving insulin independence with lower islet masses or by using abundant alternative cell sources are key to making clinical islet transplantation a suitable and widely applicable treatment [4]. The intrahepatic transplantation site currently used in humans has multiple disadvantages that contribute to islet cell loss (reviewed in Staels et al. [6] and Cantarelli and Piemonti [6, 7]). Alternative transplantation sites are extensively explored for immunological and metabolic benefits or graft-retrieval purposes [8–10], but their overall benefit is often reduced by delayed graft revascularisation [7].

The vital role of a functional vascular network makes impaired graft revascularisation one of the main reasons for graft attrition [11]. While pancreatic islets in situ are highly

vascularised, revascularisation of transplanted islets takes several weeks and remains suboptimal compared with endogenous islets [12]. Triggering the host's angiogenic response by providing angiogenic growth factors has been successfully exploited in animal studies to improve graft revascularisation [13, 14]. However, the traditional viral-vector-based methods used in these studies have inherent risks of insertional mutagenesis or vigorous immune reaction [15]. In addition, prolonged overexpression of angiogenic growth factors may negatively influence vessel microarchitecture and islet graft function [16, 17].

In vitro transcribed mRNA represents an emerging class of gene therapy drugs that harbours the potential to circumvent many, if not all, drawbacks of viral-vector-based gene delivery. Its temporary expression provides unique opportunities [18], as a transient boost in the production and secretion of angiogenic growth factors may suffice to accelerate graft revascularisation. We explored the effect of mRNA-based delivery of the potent angiogenic factor vascular endothelial growth factor A (VEGF-A) in mouse and human islet cells before transplantation.

Methods

Cell culture and transfection Islets were isolated from C57BL/6JRj mice (Janvier, Saint Berthevin, France), cultured overnight, and the peri-islet capsule was digested immediately before mRNA transfection. EndoC-bH3 cells (SARL

Endocells, Paris, France) were cultured and immortalising transgenes were excised before transfection as described in Benazra et al. [19]. Human islets were obtained from the Leiden University Medical Center. Human islets isolated at the Leiden University Medical Center that could not be used for clinical transplantation were used in the studies according to national laws and if research consent was available. See electronic supplementary material (ESM) Table 1 for donor characteristics, and ESM Methods.

mRNA production *Mus musculus Vegf-A* (also known as *Vegfa*) was cloned in the vector pEtheRNA. In vitro transcription (IVT) was performed as previously reported [20]. Modified *Vegf-A* mRNA was produced by incorporating pseudouridine and 5-methyl-cytidine during IVT [21, 22]. The production of mRNA encoding green fluorescent protein (*Gfp*) was as previously reported [20]. See ESM Methods.

Measurement of insulin secretion Isolated mouse islets were cultured overnight and transfected the following day as described above. Islets were incubated for 2 h in Ham's F-10 (Gibco, Thermo Fischer Scientific, Waltham, MA, USA) with either 2 mmol/l or 20 mmol/l glucose. Insulin concentration was measured by ELISA (Mercodia, Uppsala, Sweden). See ESM Methods.

Cell transplantation The Ethical Committee for Animal Use of Vrije Universiteit Brussel approved the experiments. 8–12-week-old male C57BL/6JRj mice (Janvier) were used for syngeneic transplantation and 8–12-week-old male severe combined immunodeficiency (SCID)-beige mice (*CB17.Cg-Prkdc^{scid}Lys1^{bg-J}/Cr1*) (Charles River, Laboratories, L'Arbresle, France) for xenotransplantation of human islets or EndoC-bH3 cells. Mice were housed under standardised conditions (12 h dark/12 h light cycle) and fed a standard diet ad libitum. To evaluate graft vascularisation and size, mice were randomised to a non-transfected, or a *Gfp* or *Vegf-A* mRNA transfected group (henceforth referred to as 'no RNA', 'GFP', and 'VEGF') and received either 100 mouse islets, or 1×10^5 human islet cells or EndoC-bH3 cells under the kidney capsule. To evaluate the metabolic effect of mRNA transfection of the grafts, alloxan-induced diabetic C57BL/6JRj mice were randomised to no RNA, VEGF, or a modified *Vegf-A* mRNA-transfected group (henceforth referred to as modVEGF), received a marginal mass of 300 syngeneic mouse islets under the kidney capsule [13], and underwent metabolic measurements. Experimenters were blind to group assignment and outcome assessment. See ESM Methods.

Immunostaining Cells were fixed, embedded and stained for GFP for analysis of transfection efficiency. Islet grafts were fixed and processed as previously reported [23]. Beta cells were stained for insulin and NKX6.1 and alpha cells for

glucagon. Biotinylated tomato lectin was used for analysis of in vivo vascularization. See ESM Methods.

qPCR The relative mRNA expression levels of *Ppia*, *Rig1*, *Ifna1* and *Mx1* were analysed via qPCR as previously reported [24]. Data are presented as average dCq compared to the reference gene *Ppia*. See ESM Methods.

Statistics One-way ANOVA, two-way ANOVA, Kruskal–Wallis or logrank tests were used as indicated. Data are presented as mean $\pm$ SEM. A value of $p < 0.05$ was considered statistically significant. See ESM Methods.

Results

We evaluated the potential of liposomal mRNA delivery to islet cells. As intact islets tended to resist the introduction of mRNA, the peri-islet capsule was disintegrated to allow efficient transfection of peripheral islet cells. A representative transfection of mouse islets with *Gfp* mRNA is shown in ESM Fig. 1a–c. mRNA transfection did not affect the survival of mouse islets in culture (data not shown) or their glucose-stimulated insulin secretion (see ESM Fig. 1d, e). To assess the effects of transfecting *Vegf-A* mRNA on mouse islet grafts, the experimental design shown in Fig. 1a was followed. VEGF-A protein levels were significantly elevated in the culture medium of VEGF compared with no RNA and GFP controls at day 1 post transfection (mean $\pm$ SEM, $1.1 \times 10^3 \pm 56$ pg/ml [$n = 4$] in VEGF vs $2.05 \times 10^2 \pm 84.7$ pg/ml [$n = 5$] in no RNA [$p < 0.0001$] vs $2.46 \times 10^2 \pm 1.26 \times 10^2$ pg/ml [$n = 5$] in GFP [$p < 0.0001$]) (see Fig. 1b) but normalised rapidly afterwards (see ESM Fig. 1f). Transplantation of VEGF grafts under the kidney capsule resulted in a 2.11-fold (vs no RNA) and 1.87-fold (vs GFP) increase in graft vessel area at day 7 post transplantation (vessel area/graft area: 0.118 ± 0.01 [$n = 3$] in VEGF vs 0.056 ± 0.01 [$n = 3$] in no RNA [$p < 0.05$] and 0.063 ± 0.02 [$n = 4$] in GFP [$p < 0.05$]) (Fig. 1c–f).

The potential of *Vegf-A* mRNA transfection was further evaluated in the human pancreatic beta cell line EndoC-bH3 and in primary human islet grafts (the experimental designs are shown in Fig. 2a and Fig. 3a, respectively). Representative transfections of EndoC-bH3 cells and of primary human islet grafts with *Gfp* mRNA are shown in ESM Fig. 2a–d. The mRNA transfection did not affect cell survival in vitro (data not shown). EndoC-bH3 cells in the VEGF group secreted $8.91 \times 10^3 \pm 2.65 \times 10^3$ pg/ml mouse VEGF-A protein. Mouse VEGF-A protein was undetectable in the culture medium of no RNA or GFP (see Fig. 2b). At day 7 post transplantation, the graft vessel area over total graft area increased significantly in VEGF EndoC-bH3 grafts, by 2.85-fold (vs no RNA) and 2.48-fold (vs GFP) (0.085 ± 0.02 [$n = 4$] in VEGF vs 0.030 ± 0.004 [$n = 4$] in no RNA [$p < 0.05$] vs 0.034 ± 0.01 [$n = 5$] in

Fig. 1 *Vegf-A* mRNA transfection of mouse islets results in improved graft revascularisation. **(a)** Experimental design. Islets were isolated from C57BL/6 donor mice and 100 medium-sized islets were randomly selected per well for overnight culture. The peri-islet capsule was disintegrated and islets were non-transfected or transfected with mRNA before syngeneic transplantation under the kidney capsule. Kidney grafts were removed at day 7 post transplantation for vascularisation analyses. **(b)** *Vegf-A* mRNA transfection of mouse islets significantly increased mouse VEGF-A protein secretion ($2.05 \times 10^2 \pm 84.7$ pg/ml [$n = 5$] in no RNA and $2.46 \times 10^2 \pm 1.26 \times 10^2$ pg/ml [$n = 5$] in GFP vs $1.1 \times 10^3 \pm 56$ pg/ml [$n = 4$]

in VEGF). **(c–e)** Representative immunofluorescent staining of **(c)** non-transfected, **(d)** *Gfp* and **(e)** *Vegf-A* mRNA-transfected mouse islet grafts. **(f)** *Vegf-A* mRNA transfection of mouse islet grafts significantly increased graft vascularisation at 7 days post transplantation (vessel area/graft area: 0.056 ± 0.01 [$n = 3$] in no RNA and 0.063 ± 0.02 [$n = 4$] in GFP vs 0.118 ± 0.01 [$n = 3$] in VEGF). Insulin (green), lectin (red), Hoechst (blue). Scale bars, 200 μ m. One-way ANOVA with Holm–Šidák’s post hoc comparison: * $p < 0.05$ and *** $p < 0.0001$. d, day; INS, insulin; mVEGFA, mouse VEGF-A; ON, overnight

GFP [$p < 0.05$]) (Fig. 2c–f). Primary human islets in the VEGF group secreted $2.55 \times 10^3 \pm 1.40 \times 10^3$ pg/ml mouse VEGF-A protein compared with undetectable levels in the culture medium of no RNA or GFP (see Fig. 3b). In VEGF

grafts, the graft vessel area over total graft area significantly increased by 3.17-fold (vs no RNA) and 3.80-fold (vs GFP) at day 7 (0.048 ± 0.013 [$n = 3$] in VEGF vs 0.015 ± 0.0051 [$n = 4$] in no RNA [$p < 0.01$] vs 0.013 ± 0.0046 [$n = 4$] in GFP [$p <$

Fig. 2 *Vegf-A* mRNA transfection of EndoC-bH3 cells results in improved graft revascularisation. **(a)** Experimental design. EndoC-bH3 cells were expanded in culture and subjected to 4-hydroxy-tamoxifen-mediated excision of the immortalising transgenes. 1×10^5 cells were transfected per replicate and transplanted under the kidney capsule of SCID-beige mice. Kidney grafts were removed at day 7 post transplantation for vascularisation analyses. **(b)** The culture medium of EndoC-bH3 in the VEGF group contained high levels of mouse VEGF-A protein 24 h after transfection ($8.91 \times 10^3 \pm 2.65 \times 10^3$ pg/ml [$n = 5$] vs undetectable in GFP and no RNA). **(c–e)** Representative immunofluorescent staining of **(c)**

non-transfected, **(d)** *Gfp* and **(e)** *Vegf-A* mRNA transfected EndoC-bH3 grafts. **(f)** *Vegf-A* mRNA transfection significantly increased graft revascularisation at 7 days post transplantation (vessel area/graft area: 0.030 ± 0.004 [$n = 4$] in no RNA and 0.034 ± 0.01 [$n = 5$] in GFP vs 0.085 ± 0.02 [$n = 4$] in VEGF). Green, insulin; red, lectin; blue, Hoechst. Scale bars, 200 μ m. One-way ANOVA with Holm–Šidák’s post hoc comparison: * $p < 0.05$. 4-OH-Tam, 4-hydroxy-tamoxifen; d, day; INS, insulin; mVEGFA, mouse VEGF-A; ND, not detected; SCID-bg, SCID-beige mice

Fig. 3 *Vegf-A* mRNA transfection of human islets results in improved graft revascularisation and in a higher graft beta cell volume. **(a)** Experimental design. Human islets were isolated from cadaveric donor pancreases and cultured until shipment from Leiden. On arrival in Brussels, the human islet cells were plated at 1×10^5 cells per well for overnight culture. The peri-islet capsule was dissociated and islets were transfected with mRNA or non-transfected before transplantation under the kidney capsule of SCID-beige mice. Kidney grafts were removed at day 7 or day 30 post transplantation for vascularisation and beta cell volume analyses. **(b)** The culture medium of human islets in the VEGF group contained elevated levels of mouse VEGF-A protein 24 h after transfection ($2.55 \times 10^3 \pm 1.40 \times 10^3$ pg/ml [$n=3$]) vs undetectable in GFP and no RNA. **(c–h)** Representative immunofluorescent staining of **(c, f)** non-transfected, **(d, g)** *Gfp*- and **(e, h)** *Vegf-A* mRNA-transfected human islet grafts at 7 days **(c–e)** and 30 days **(f–h)** post transplantation. **(i)** *Vegf-A* mRNA transfection significantly increased graft

revascularisation at 7 days post transplantation (vessel area/graft area: 0.015 ± 0.0051 [$n=4$] in no RNA vs 0.013 ± 0.0046 [$n=4$] in GFP vs 0.048 ± 0.013 [$n=3$] in VEGF) and 30 days post transplantation (vessel area/graft area: 0.029 ± 0.0052 [$n=5$] in no RNA and 0.027 ± 0.0056 [$n=4$] in GFP vs 0.049 ± 0.0042 [$n=8$] in VEGF). **(j)** *Vegf-A* mRNA transfection resulted in a higher graft beta cell volume at 7 days post transplantation (0.0245 ± 0.0028 μ l [$n=4$] in no RNA and 0.0121 ± 0.0012 μ l [$n=3$] in GFP vs 0.0335 ± 0.0034 μ l [$n=4$] in VEGF) and 30 days post transplantation (0.0178 ± 0.0021 μ l [$n=5$] in no RNA and 0.0129 ± 0.0012 μ l [$n=4$] in GFP vs 0.0292 ± 0.0032 μ l [$n=7$] in VEGF). Green, insulin; yellow, glucagon; red, lectin; blue, Hoechst. Scale bars, 200 μ m. One-way ANOVA with Holm–Šidák’s post hoc comparison: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. d, day; GLU, glucagon; INS, insulin; mVEGFA, mouse VEGF-A; ND, not detected; ON, overnight; SCID-bg, SCID-beige mice

0.01]) and by 1.70-fold (vs no RNA) and 1.82-fold (vs GFP) at day 30 post transplantation (0.049 ± 0.0042 [$n=8$] in VEGF vs 0.029 ± 0.0052 [$n=5$] in no RNA [$p < 0.05$] vs 0.027 ± 0.0056 [$n=4$] in GFP [$p < 0.05$]) (see Fig. 3c–i). A significant 1.37-fold (vs no RNA) and 2.77-fold (vs GFP) higher beta cell volume was measured in VEGF at day 7 post transplantation (0.0335 ± 0.0034 μ l [$n=4$] in VEGF vs 0.0245 ± 0.0028 μ l [$n=4$] in no RNA [$p < 0.05$] vs 0.0121 ± 0.0012 μ l [$n=3$] in GFP [$p < 0.001$]). At day 30 post transplantation, the beta cell volume in VEGF remained 1.64 times

(vs no RNA) and 2.26 times higher (vs GFP) (0.0292 ± 0.0032 μ l [$n=7$] in VEGF vs 0.0178 ± 0.0021 μ l [$n=5$] in no RNA [$p < 0.01$] vs 0.0129 ± 0.0012 μ l [$n=4$] in GFP [$p < 0.001$]) (see Fig. 3j), suggesting a reduction in graft attrition associated with the increased early vascularisation.

A pilot experiment with marginal mass islet grafts in alloxan-induced diabetic mice showed no improvement of blood glucose levels in VEGF over no RNA (data not shown). This may be due to an immune reaction to the mRNA-transfected mouse implants engrafted in immunocompetent

hosts, as synthetic mRNA can elicit a type 1 interferon response. The expression of the intracellular mRNA receptor retinoic acid-inducible gene 1, *Rig1* (also known as *Ddx58*), and its downstream targets interferon, *Ifn1*, and myxovirus resistance, *Mx1*, was indeed strongly increased on mRNA transfection of islets in vitro. *Rig1* activation and *Ifn1* and *Mx1* induction could be efficiently avoided by using the synthetically modified *Vegf-A* mRNA (see ESM Fig. 3a–c and ESM Results).

modVEGF had enhanced graft revascularisation at day 7 post transplantation to a similar extent as VEGF in the set-up shown in Fig. 1a (data not shown). Next, the effect of mRNA transfection of marginal mass islet grafts on glycemic control in diabetic mice was compared between no RNA, VEGF and modVEGF (the experimental design is shown in ESM Fig. 4a). At 28 days post transplantation, no RNA mice had 2 h fasting blood glucose levels of 16.64 ± 2.85 mmol/l ($n = 10$), compared with 21.74 ± 3.63 mmol/l ($n = 7$) in VEGF and 14.02 ± 1.82 mmol/l ($n = 12$) in modVEGF (see ESM Fig. 4b–d). In the no RNA group, 40% of mice had at least two consecutive blood glucose readings below 14 mmol/l maintained over the metabolic follow-up period of 28 days, compared with 28.57% in VEGF and 58.33% in modVEGF (see ESM Fig. 4e). At day 29 post transplantation, overnight fasting glycaemia was 12.36 ± 2.00 mmol/l ($n = 10$) in no RNA, 13.07 ± 2.85 mmol/l ($n = 7$) in VEGF and 9.43 ± 0.61 mmol/l ($n = 12$) in modVEGF. Mean AUC during IPGTT results at day 29 post transplantation were 2730 ± 176 mmol/l/min ($n = 10$) in no RNA, 2758 ± 300 mmol/l/min ($n = 7$) in VEGF and 2457 ± 128 mmol/l/min ($n = 12$) in modVEGF (see ESM Fig. 4f). Taken together, these results indicate that transplantation of a marginal mass of modified *Vegf-A* mRNA-transfected mouse islets conferred a relevant, albeit not statistically significant, improvement in metabolic outcome.

Discussion

The success of engraftment of any cell-based implant critically depends on its rapid integration with the host vasculature [25]. In islet transplantation, the delay in graft revascularisation severely compromises graft survival and function [26]. Experimental delivery of proangiogenic signals has previously been shown as beneficial for islet transplantation [13]. However, the viral-vector-based methods that were used restricted its translational potential. Transfection of IVT mRNA represents an emerging gene therapeutic approach to which the anticipated risks of classic gene therapy do not apply. In the current study, we employed a novel mRNA-based approach to obtain transient VEGF-A overexpression in mouse and human islet cells, thereby preventing the side effects of chronic VEGF-A overexpression, including increased vascular permeability and inflammation [16, 17, 27].

Vegf-A mRNA transfection did not affect islet cell survival or glucose-stimulated insulin secretion in vitro. *Vegf-A* mRNA transfection immediately before transplantation significantly increased islet graft revascularisation in the early post-transplantation period of both mouse and human islet cell implants. Unmodified mRNA induced a type 1 interferon response in islets, which could be avoided with modified synthetic mRNA to confer mild glucometabolic benefits in vivo.

While our work pioneers the use of mRNA transfection in beta cell transplantation protocols, future studies need to evaluate the potential of (modified) *Vegf-A* mRNA transfection for emerging alternative beta(-like) cell sources, including those derived from embryonic or induced pluripotent stem cells. The renal subcapsular space allows easy graft retrieval, but being a highly vascularised site, the full benefit of accelerated graft revascularisation may not become apparent [6, 28]. Clinically relevant alternative sites, including the subcutis, that are shown to suffer from limited graft revascularisation should also be tested [6, 7, 28]. More rapid revascularisation will benefit the oxygen and nutrient supply of islet allografts, but could be associated with an earlier influx of inflammatory cells. Combination with effective immunomodulation will therefore remain essential to ensure optimal engraftment. Finally, the success of islet engraftment is determined by factors other than graft revascularisation and immunoprotection, including graft re-innervation [29]. This difficulty places the mRNA strategy in a unique position as it offers a platform by which combinations of therapeutic proteins can be easily delivered to tackle diverse hurdles simultaneously.

Taken together, our mRNA-based approach opens a broad range of experimental opportunities and serves as a proof of principle to improve the success of engraftment of cell-based implants in diabetes and regenerative medicine.

Acknowledgements The authors thank V. Laurysens, A. Demarré and E. Quartier (BENE, Vrije Universiteit Brussel, Brussels, Belgium) for technical help. We also thank J. van den Amele (Andrea Brand Lab, Gurdon Institute, University of Cambridge, Cambridge, UK) for revising the manuscript.

Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding The authors acknowledge support by grants from the Research Foundation Flanders (FWO), the VUB Research Council, Stichting Diabetes Onderzoek Nederland, the European Union Sixth and Seventh Framework Program, the Wetenschappelijk Fonds Willy Gepts (WFWG) of the UZ Brussel and the Belgian Federal Science Policy (IAPVII-07). WS is a PhD fellow of Research Foundation Flanders.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement WS, EdK, CG, CH, KT, LB, HH and NDL designed and conceived the experiments. WS, YV, YH, GL and SDG

acquired and analysed the data. EdK provided human islets. CH and KT provided *Gfp* mRNA, *Vegf-A* mRNA and modified *Vegf-A* mRNA. WS, YV, YH, CH, KT, CG, LB, HH and NDL interpreted the data. WS and NDL drafted the article. All authors revised the article and approved of the final version. NDL is the guarantor of this work.

References

- Ling Z, De Pauw P, Jacobs-Tulleneers-Thevissen D et al (2015) Plasma GAD65, a marker for early beta-cell loss after intraportal islet cell transplantation in diabetic patients. *J Clin Endocrinol Metab* 100:2314–2321
- Eriksson O, Eich T, Sundin A et al (2009) Positron emission tomography in clinical islet transplantation. *Am J Transplant* 9:2816–2824
- Biamés M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E (2002) β -cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* 51:66–72
- McCall M, James Shapiro AM (2012) Update on islet transplantation. *Cold Spring Harb Perspect Med* 2:a007823
- Keymeulen B, Gillard P, Mathieu C et al (2006) Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. *Proc Natl Acad Sci U S A* 103:17444–17449
- Staels W, De Groef S, Heremans Y et al (2016) Accessory cells for beta-cell transplantation. *Diabetes Obes Metab* 18:115–124
- Cantarelli E, Piemonti L (2011) Alternative transplantation sites for pancreatic islet grafts. *Curr Diab Rep* 11:364–374
- Stokes RA, Cheng K, Lalwani A et al (2017) Transplantation sites for human and murine islets. *Diabetologia* 60:1961–1971
- Pepper AR, Pawlick R, Bruni A et al (2017) Transplantation of human pancreatic endoderm cells reverses diabetes post transplantation in a prevascularized subcutaneous site. *Stem Cell Reports* 8:1689–1700
- Espes D, Lau J, Quach M, Ullsten S, Christofferson G, Carlsson PO (2016) Rapid restoration of vascularity and oxygenation in mouse and human islets transplanted to omentum may contribute to their superior function compared to intraportally transplanted islets. *Am J Transplant* 16:3246–3254
- Coppens V, Heremans Y, Leuckx G et al (2013) Human blood outgrowth endothelial cells improve islet survival and function when co-transplanted in a mouse model of diabetes. *Diabetologia* 56:382–390
- Carlsson PO, Palm F, Mattsson G (2002) Low revascularization of experimentally transplanted human pancreatic islets. *J Clin Endocrinol Metab* 87:5418–5423
- Zhang N, Richter A, Suriawinata J et al (2004) Elevated vascular endothelial growth factor production in islets improves islet graft vascularization. *Diabetes* 53:963–970
- Su D, Zhang N, He J et al (2007) Angiopoietin-1 production in islets improves islet engraftment and protects islets from cytokine-induced apoptosis. *Diabetes* 56:2274–2283
- Baum C, von Kalle C, Staal FJ et al (2004) Chance or necessity? Insertional mutagenesis in gene therapy and its consequences. *Mol Ther* 9:5–13
- De Leu N, Heremans Y, Coppens V et al (2014) Short-term overexpression of VEGF-A in mouse beta cells indirectly stimulates their proliferation and protects against diabetes. *Diabetologia* 57:140–147
- Brissova M, Aamodt K, Brahmachary P et al (2014) Islet microenvironment, modulated by vascular endothelial growth factor- α signaling, promotes beta cell regeneration. *Cell Metab* 19:498–511
- Sahin U, Kariko K, Tureci O (2014) mRNA-based therapeutics—developing a new class of drugs. *Nat Rev Drug Discov* 13:759–780
- Benazra M, Lecomte MJ, Colace C et al (2015) A human beta cell line with drug inducible excision of immortalizing transgenes. *Mol Metab* 4:916–925
- Van Lint S, Goyvaerts C, Maenhout S et al (2012) Preclinical evaluation of TriMix and antigen mRNA-based antitumor therapy. *Cancer Res* 72:1661–1671
- Kariko K, Muramatsu H, Welsh FA et al (2008) Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol Ther* 16:1833–1840
- Durbin AF, Wang C, Marcotrigiano J, Gehrke L (2016) RNAs containing modified nucleotides fail to trigger RIG-I conformational changes for innate immune signaling. *MBio* 7:e00833–e00816
- Staels W, Heremans Y, Leuckx G et al (2017) Conditional islet hypovascularisation does not preclude beta cell expansion during pregnancy in mice. *Diabetologia* 60:1051–1056
- D'Hoker J, De Leu N, Heremans Y et al (2013) Conditional hypovascularization and hypoxia in islets do not overtly influence adult beta-cell mass or function. *Diabetes* 62:4165–4173
- Mao AS, Mooney DJ (2015) Regenerative medicine: current therapies and future directions. *Proc Natl Acad Sci U S A* 112:14452–14459
- Jansson L, Carlsson PO (2002) Graft vascular function after transplantation of pancreatic islets. *Diabetologia* 45:749–763
- Agudo J, Ayuso E, Jimenez V et al (2012) Vascular endothelial growth factor-mediated islet hypervascularization and inflammation contribute to progressive reduction of beta-cell mass. *Diabetes* 61:2851–2861
- Pepper AR, Gala-Lopez B, Ziff O, Shapiro AM (2013) Revascularization of transplanted pancreatic islets and role of the transplantation site. *Clin Dev Immunol* 2013:352315
- Korsgren O, Andersson A, Jansson L, Sundler F (1992) Reinnervation of syngeneic mouse pancreatic islets transplanted into renal subcapsular space. *Diabetes* 41:130–135