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RESEARCH ARTICLE

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Necrostatin-1 releasing nanoparticles: In vitro and in vivo efficacy for supporting immunoisolated islet transplantation outcomes

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Abstract

Immunoisolation of pancreatic islets in alginate microcapsules allows for transplantation in the absence of immunosuppression but graft survival time is still limited. This limited graft survival is caused by a combination of tissue responses to the encapsulating biomaterial and islets. A significant loss of islet cells occurs in the immediate period after transplantation and is caused by a high susceptibility of islet cells to inflammatory stress during this period. Here we investigated whether necrostatin-1 (Nec-1), a necroptosis inhibitor, can reduce the loss of islet cells under stress in vitro and in vivo. To this end, we developed a Nec-1 controlled-release system using poly (D,L-lactide-co-glycolide) (PLGA) nanoparticles (NPs) as the application of Nec-1 in vivo is limited by low stability and possible side effects. The PLGA NPs stably released Nec-1 for 6 days in vitro and protected beta cells against hypoxia-induced cell death in vitro. Treatment with these Nec-1 NPs at days 0, 6, and 12 post-islet transplantation in streptozotocin-diabetic mice confirmed the absence of side effects as graft survival was similar in encapsulated islet grafts in the absence and presence of Nec-1. However, we found no further prolongation of graft survival of encapsulated grafts which might be explained by the high biocompatibility of the alginate encapsulation system that provoked a very mild tissue response. We expect that the Nec-1-releasing NPs could find application to immunoisolation systems that elicit stronger inflammatory responses, such as macrodevices and vasculogenic biomaterials.

KEYWORDS
islet transplantation, nanoparticles, necrostatin-1, type 1 diabetes

1 | INTRODUCTION

Pancreatic islet transplantation can provide minute-to-minute regulation of glucose levels in Type 1 Diabetes (T1D) patients. This improves the quality of life, prevents severe hypoglycemic episodes, and protects against the development of secondary T1D complications, such as nephropathy, cardiovascular diseases, and retinopathy. With non-encapsulated islets but under heavy immunosuppression, 50%–80% of the patients remain insulin independent 5 years after islet transplantation.[1] Although the long-term efficacy of islet

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transplantation has improved over the years, continued advancements are needed to make this therapy available for all T1D patients.

The use of chronic immunosuppression is one of the factors that severely limit the broad clinical adoption of islet transplantation. Chronic immunosuppression negatively impacts the long-term survival of islets and is also associated with many side effects such as the repeated occurrence of pathogenic infections and nephrotoxicity.[2] For these reasons, many efforts are being undertaken to develop technologies that allow islet transplantation without the need for life-long immunosuppression. Immunoisolation by islet encapsulation is an example of such a technique. Encapsulation of islets in a semi-permeable membrane, made of for example alginate, protects the islets from direct contact with immune cells but permits diffusion of oxygen, nutrients, and hormones.[3]

Although recent in vivo studies with immunoisolated islets have shown the function of grafted islets for months up to a year, it has also been shown that survival is still limited.[4,5] Recent studies show that this limited survival can be attributed to the significant loss of islets in the immediate period after transplantation, which may be up to 60% of the originally transplanted cells.[5–7] In this immediate period after transplantation, islets are very susceptible to inflammation in the vicinity of the graft, which might be induced by the mandatory implantation surgery or by danger-associated molecular patterns (DAMPs) released from the islets.[8,9] During this acute period, islets are rebuilding their extracellular matrix, adapting to their new micro-environment, and are vulnerable to stress.[10] Many insulin-producing cells die in this immediate period after transplantation by several cell death processes including necroptosis.

Necrostatin-1 (Nec-1) is a well-known necroptosis inhibitor with proven in vitro efficacy in preventing islet cell death.[11–13] Nec-1 is a low molecular weight alkaloid that was investigated, for the first time, in 2005 by Degterev et al.[14] Nec-1 has been reported to support in vitro maturation and functional islet survival.[12] Furthermore, the incorporation of Nec-1 in alginate-based immunoisolating capsules protects insulin-producing cells in vitro against cytokine-induced inflammatory stress.[11] In addition, Paredes-Juarez et al. showed that Nec-1 reduces the production of DAMPs by islets when mimicking in vitro the low-oxygen and low-nutrient conditions in the immediate post-transplantation period.[13] These DAMPs activate an inflammation cascade leading to reduced graft survival, and therefore Nec-1 is proposed to be a promising candidate to improve transplantation outcomes. In addition, by culturing islets in Nec-1-containing media before transplantation, diabetic mice became normoglycemic in a shorter period, with faster glucose clearance and higher levels of insulin than islets that were not pre-exposed to Nec-1.[15]

However, the therapeutic application of Nec-1 is limited by metabolic instability (short half-life time) and its possible side-effect as it, for example, also inhibits indoleamine 2,3-dioxygenase, an important component of the response to inflammation.[16,17] To enhance its stability in vitro and in vivo we here studied the incorporation of Nec-1 into poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles (NPs). The application of these Nec-1 NPs was tested on hypoxia-exposed beta cells in vitro and in a streptozotocin-induced diabetes mouse model after the transplantation of alginate-encapsulated islets. Nec-1 is expected to only act locally at the site of implantation, in this case in the peritoneal cavity, without severe side effects.

2 | MATERIALS AND METHODS

2.1 | Fabrication of Nec-1 and control particles

To fabricate PLGA NPs, 10–20 mg of PLGA (acid terminated, lactide:glycolide 50:50, MW 24,000–38,000; Sigma-Aldrich, St. Louis, USA) was dissolved in dichloromethane (DCM; Sigma-Aldrich) and vortexed with 1 mg of either Nec-1 (Abcam, Waltham, USA) or Nec-1 inactive control (N-demethylated thiohydantoin analog of Nec-1; Abcam). The drug-PLGA mixture was subsequently ultrasonicated with 4 mL polyvinyl alcohol (PVA; Sigma-Aldrich) for 3 min at maximum power. The ultrasonicated drug-PLGA-PVA mixture was then poured into a beaker with 20 mL PVA and put to stir at 400 rpm for 4 h inside a fume hood. Excess DCM was left to evaporate. The NPs were then washed 3 times with ultrapure water at 16,000×*g* for 10 min. Subsequently, the NPs were resuspended in 2 mL of ultrapure water and lyophilized using a FreeZone 4.5 L Freeze Dry System (Labconco, Kansas City, USA). The diameter of the nanoparticles was determined by dynamic light scattering (Zetasizer Nano ZS System; Malvern Panalytical, Westborough, USA).

2.2 | Nec-1 loading efficiency

To quantify the loading efficiency of the NPs, 2 mg of lyophilized NPs were mixed with 1 mL of ultrapure methanol (Thermo Fisher Scientific, Norcross, USA) for 2 days to allow for Nec-1 or Nec-1 analog to dissolve completely in methanol. This mixture was spun down for 5 min at 20,000×*g* and the supernatant (which should only contain Nec-1/Nec-1 analog in methanol) was then withdrawn completely, only leaving a pellet of empty NPs. Methanol was completely evaporated using a Savant SpeedVac high-capacity concentrator (Thermo Fisher Scientific). Dried Nec-1 or Nec-1 analog was then dissolved in 100 µL of acetonitrile (ACN; Thermo Fisher Scientific) and analyzed by high-performance liquid chromatography (HPLC).

To determine drug loading, 100 µL of Nec-1 or inactive Nec-1 analog in ACN was mixed with 900 µL of 0.1% trifluoroacetic acid (TFA; Thermo Fisher Scientific) in water to make a 90:10 mixture of Nec-1 in 0.1% TFA. Of this sample, 10 µL was then loaded onto our HPLC setup with the following parameters: Synergi 4 µm Fusion-RP 80 Å, LC 250 × 4.6 mm column (Phenomenex, Torrance, USA) at 25°C, a mobile phase of 10%–100% ACN + 0.1% TFA in 20 min and held for 10 min at 100% ACN with 0.1% TFA, flow rate of 1 mL/min, wavelength 220 nm. Sample concentrations were calculated using standard curves created for each HPLC run.

2.3 | In vitro release kinetics of Nec-1 from PLGA NPs

To determine release kinetics, 6 mg of Nec-1-encapsulating PLGA NPs were dissolved at 1 mg/mL in phosphate-buffered saline (PBS) and shaken at 37°C. At different time points, dissolved PLGA samples were spun down for 10 min at 16,000×*g*, and 250 µL of supernatant was collected and replaced with 250 µL PBS. Nec-1 was extracted from the supernatant with DCM. DCM was evaporated using a Savant SpeedVac high-capacity concentrator. Extracted Nec-1 was reconstituted in 100 µL of ACN and ran on HPLC as described above.

2.4 | Released Nec-1 bioactivity

To investigate whether the released Nec-1 was still functional, rat insulinoma INS-1 cells (passage 5–10) were seeded in 24-well plates at 50,000 cells per well and co-incubated under hypoxic conditions (5% O_2) for 48 h with either 150 µM soluble Nec-1, Nec-1 NPs (presented on a transwell), empty NPs (presented on a transwell), or with no treatment. A normoxic control was also cultured in parallel. Metabolic activity was measured via CCK-8 assays (Abcam).

2.5 | Pancreatic islet isolation

All described animal procedures were approved by the Dutch Central Committee Animal Experiments and the Animal Welfare Body of the University of Groningen (AVD1050020185726). Islets are isolated from male Sprague Dawley rats (Envigo, Horst, The Netherlands), weighing between 250 and 320 gm. The islet isolation procedure has been described previously by others.[18,19] Briefly, collagenase V solution (1 mg/mL; Sigma-Aldrich, Zwijndrecht, The Netherlands) was injected into the bile duct of these animals to distend the pancreas. After removing the pancreas, it was placed in a shaking water bath of 37°C for 18 min to complete the digestion. Subsequently, a density gradient (gradient stock solution; Corning BV Life Sciences, Amsterdam, The Netherlands) was used to separate the islets from the exocrine tissue. The islet batch was further purified by handpicking and overnight culture in CMRL (Life Technologies; Thermo Fisher Scientific, Groningen, The Netherlands) supplemented with 8.3 mM glucose (Sigma-Aldrich), 20 mM HEPES (Gibco; ThermoFisher Scientific), 2 mM Glutamax (Gibco), 50 U/mL/50 µg/mL penicillin–streptomycin (Gibco), and 10% fetal calf serum (Thermo Fisher Scientific) at 37°C and 5% CO_2.

2.6 | Islet transplantation procedure

Male C57BL/6NCrl mice (Charles River, Den Bosch, The Netherlands) weighing 20–22 gm were used as transplant recipients. Diabetes was induced in these animals by an intraperitoneal injection of 200 mg/kg streptozotocin (Sigma-Aldrich). Diabetes was confirmed by two consecutive measurements of blood glucose above 20 mmol/L within 7 days using a Bayer Health Care (Whippany, USA) blood glucose measurement device.

At the day of transplantation, the islets were microencapsulated in purified alginate (3.4% intermediate-G, 44% G-chains, 56% M-chains, 21% GM-chains, 37% MM-chains; ISP Alginates Ltd, Girvan, UK) as described previously by others.[20–22] Briefly, an islet/alginate mixture was placed into our custom-made air-driven droplet generator. Encapsulated islets were collected in 100 mM $CaCl_2$ and allowed to gel for at least 10 min, after which the capsulated islets were washed with Krebs Ringer HEPES buffer (pH 7.4; 133 mM NaCl, 4.69 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4{\cdot}7H_2O$, 25 mM HEPES, 2.52 mM $CaCl_2{\cdot}2H_2O$).

Under anesthesia, a small incision was made through the skin and muscle layer to place the encapsulated rat islets in the intraperitoneal space in the diabetic mice. Each mouse received 1000 encapsulated islets and a dose of buprenorphine (0.1 mg/kg Buprecare Multidose; Ecuphar NV, Breda, The Netherlands) as post-surgery analgesic. The incision in the skin and muscle layer was closed using 5.0 Vicryl sutures (Ethicon; Johnson & Johnson Medical N.V., Machelen, Belgium).[23]

2.7 | Particle injections

Immediately after islet transplantation, the mice received their first intraperitoneal injection of NPs. Control mice received NPs with the inactive analog of Nec-1 ($n = 7$) and the Nec-1 mice received NPs containing Nec-1 ($n = 7$). To this end, 0.65 mg of nanoparticles were dissolved in 500 µL Krebs Ringer HEPES buffer (Nec-1 concentration 100 µM). On days 6 and 12 post-transplantation, the mice received another injection of either control or Nec-1 NPs. The dosage of the particles was the same on all three injection days.

2.8 | Monitoring glucose homeostasis

To monitor graft function and health, nonfasting blood glucose levels and weight were measured weekly. Animals were considered to be normoglycemic when blood glucose levels were below 10 mmol/L. In addition, an intraperitoneal glucose tolerance test (IPGTT) was performed 4 and 8 weeks post-transplantation. Briefly, mice were fasted for 4 h before intraperitoneally injecting a glucose solution (3 g/kg; Sigma-Aldrich). Glucose levels were measured from blood sampled from the tail vein at 5 min before the glucose injection and at 0, 5, 10, 20, 30, 60, 90, and 120 min post-injection.

Rat C-peptide concentrations were measured in the plasma of the blood samples using ELISA (Crystal Chem Europe, Zaandam, The Netherlands). The ELISA was performed according to the manufacturer's instructions. The optical density was measured at 450 nm within 20 min after adding the stop solution using the VersaMax microplate reader (Molecular Devices, Berkshire, United Kingdom).

2.9 | Sacrifice and histology

When two consecutive measurements of blood glucose were above 20 mmol/L within 1 week the animal was euthanized and the encapsulated islets were collected for histology. At 100 days post-transplantation, $n = 2$ of each group were sacrificed for histology as well as the mice still being normoglycemic after 300 days. The encapsulated islets were embedded in paraffin using the Cellient™ processor (Hologic, Marlborough, Massachusetts). Paraffin sections (4 μm thick) were stained with hematoxylin and eosin and images were taken using a Leica DM 2000 LED microscope with a Leica DFC 450 camera (Leica Microsystems B.V., Amsterdam, The Netherlands).

2.10 | Statistical analysis

The statistical analysis was performed using GraphPad Prism (version 10.0.0; GraphPad Software, Inc, La Jolla, USA). A Shapiro–Wilk test was performed to test the data for normality. For statistical analysis of the cell viability in the CCK-8 metabolic assay, a one-way ANOVA with a Dunnett post-hoc test ($n = 4$) was applied where the mean of each group was compared to the mean of the normoxic control condition. Differences in mean survival time were tested by a two-sided t-test ($n = 5$) and a log-rank (Mantel-Cox) test was used for the Kaplan–Meier curves ($n = 5$). p-Values <.05 were considered significant. Data are presented as mean ± standard deviation.

3 | RESULTS

3.1 | Material characterization

Using dynamic light scattering, we confirmed that the synthesized NPs had an average diameter of 336.0 nm ± 52.0 nm (Figure 1). Using HPLC, the Nec-1 loading efficiency of the PLGA NPs was shown to be 85.0%, whereas the loading efficiency of the inactive Nec-1 analog was 60.4%. This discrepancy is likely because of changes in solubility due to chemical modifications in the inactive analog. This result indicates that the PLGA NPs were efficient at encapsulating Nec-1.

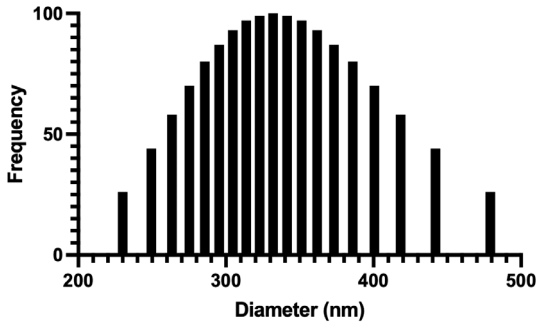

FIGURE 1 Size distribution of the poly (D,L-lactide-co-glycolide) nanoparticles. Dynamic light scattering measurement of nanoparticle diameter. The figure shows the size distribution of one batch of NPs, an equivalent size distribution was obtained for independent batches.

3.2 | Nec-1 release and bioactivity

Before testing the NPs in vivo, we investigated in vitro the release kinetics of Nec-1 from the NPs and the bioactivity of the released Nec-1. The release assay showed a burst release of approximately 75% of the Nec-1 in the first 48 h, followed by slower release for up to 6 days (Figure 2A). To determine the bioactivity of the released Nec-1, we cultured beta cell line INS-1 under hypoxic conditions in the presence and absence of Nec-1-containing NPs, empty NPs, and soluble Nec-1. After 48 h, the viability of the INS-1 cells was assessed (Figure 2B). Under hypoxia, the viability of untreated cells significantly decreased ($p < .0001$) compared to INS-1 cells cultured in normoxic conditions. The presence of empty NPs did not prevent this decrease, the viability of these cells was also significantly lower ($p < .0005$) than that of normoxic cells. In contrast, no significant differences were found between the normoxic control group and the Nec-1-containing groups under hypoxic conditions. This result indicates that Nec-1 released from NPs protected INS-1 cells against hypoxia-induced cell death to levels equivalent to soluble Nec-1.

3.3 | Long-term blood glucose levels

An islet transplantation mouse model was used to investigate the in vivo effects of the Nec-1 NPs. To this end, 1000 encapsulated rat islets were transplanted into the peritoneal cavity of diabetic C57BL/6 mice, and they received intraperitoneal injections of NPs with Nec-1 or the Nec-1 inactive analog (control) at day 0, 6, and 12 post-transplantation. This time interval between the injections was based on the in vitro sustained release of Nec-1 for up to 6 days (Figure 2A). Mice of both groups became normoglycemic within days after transplantation (Figure 3). The results of the IPGTTs at 4 and 8 weeks post-transplantation confirm that in the first period after transplantation, no differences can be observed between the groups in glucose control (Figure 4). At 4 weeks, the glucose levels and the concentration of C-peptide in the plasma samples are not significantly different between the groups. This also applies to the 8-week time point. Some recipients from both groups started to exhibit destabilized blood glucose control after day 50 (Figure 3) and no significant difference in graft mean survival time was observed between groups (mean survival time control 205 days and Nec-1175 days, $p = .6567$). For the Nec-1 group, 40% (2/5) of recipients maintained normoglycemia over 300 days, whereas only 20% (1/5) of control recipients remained normoglycemic (Figure 5). However, these differences were not significant.

3.4 | Fibrotic overgrowth of capsules

To investigate tissue responses to implanted capsules, hematoxylin and eosin staining was performed 100 days after transplantation and

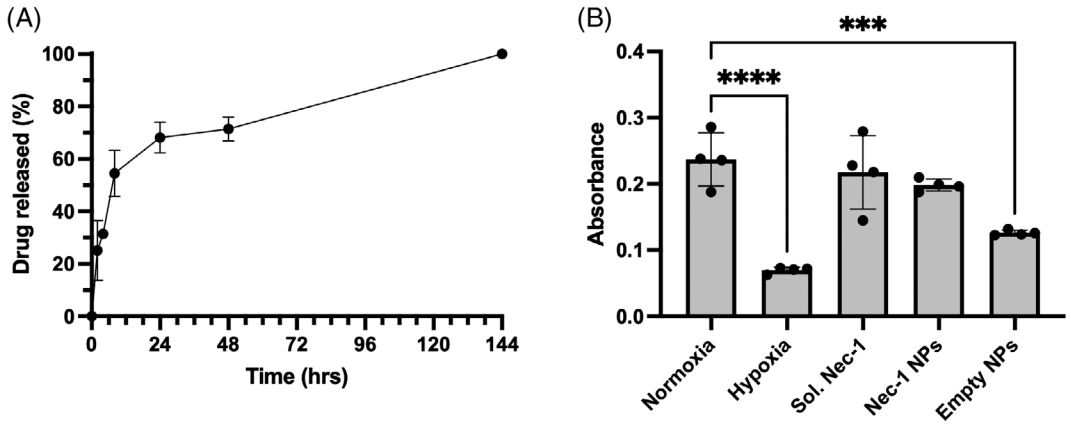

FIGURE 2 Functional release of Nec-1 from the poly (D,L-lactide-co-glycolide) particles. (A) Release of Nec-1 from PLGA nanoparticles (NPs) was measured over 6 days (144 h; biological replicates $n = 3$). (B) The bioactivity of the released Nec-1 was tested in a CCK-8 metabolic assay (biological replicates $n = 4$) by culturing INS-1 cells under hypoxic conditions. The absorbance is a measure of cell viability. Mean and standard deviation are plotted, and statistical analysis was carried out using a one-way ANOVA with a Dunnett post-hoc test comparing the groups with the normoxic control condition (***$p < .0005$; ****$p < .0001$). Similar results were obtained in another independent run.

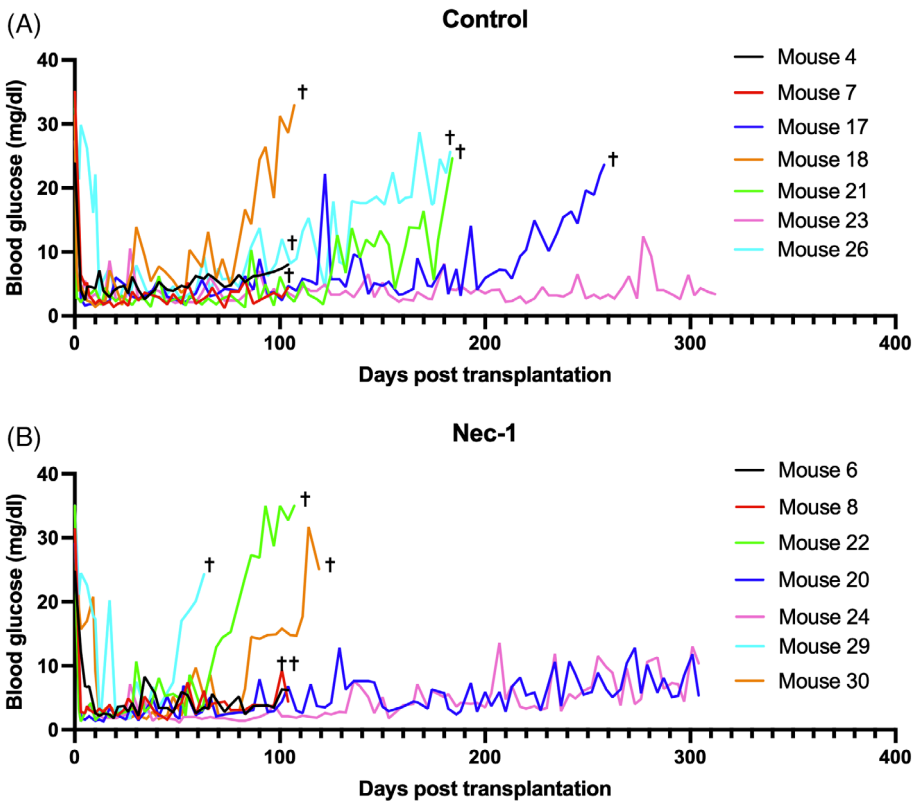

FIGURE 3 Long-term non-fasting blood glucose levels. Blood glucose levels after transplantation of 1000 encapsulated rat islets into the intraperitoneal cavity of diabetic C57BL6 mice. Control mice ($n = 7$) received intraperitoneal injections of PLGA nanoparticles with the Nec-1 inactive analog on days 0, 6, and 12 post-transplantation. On the same days, Nec-1 mice ($n = 7$) received PLGA particles containing Nec-1. Control mice 4 and 7 and Nec-1 mice 6 and 8 were sacrificed at 100 days post-transplantation for histology.

at the end of the study. Similar results were observed for both time points. The capsules were intact and no cellular overgrowth was found on the surface of the alginate capsules retrieved from control mice (Figure 6). The capsules from the Nec-1 mice were also intact and showed no signs of cellular overgrowth. In addition, no differences were observed in the histology of rejected and non-rejected grafts of both groups (data not shown).

4 | DISCUSSION

A significant loss of transplanted islet cells, involving cell death processes such as necroptosis, has been shown to reduce long-term graft survival.[24,25] For microencapsulated/immunoisolated islets, this loss mainly occurs in the immediate period after transplantation.[5–7] To reduce this, we studied the impact of Nec-1 on islet cell survival

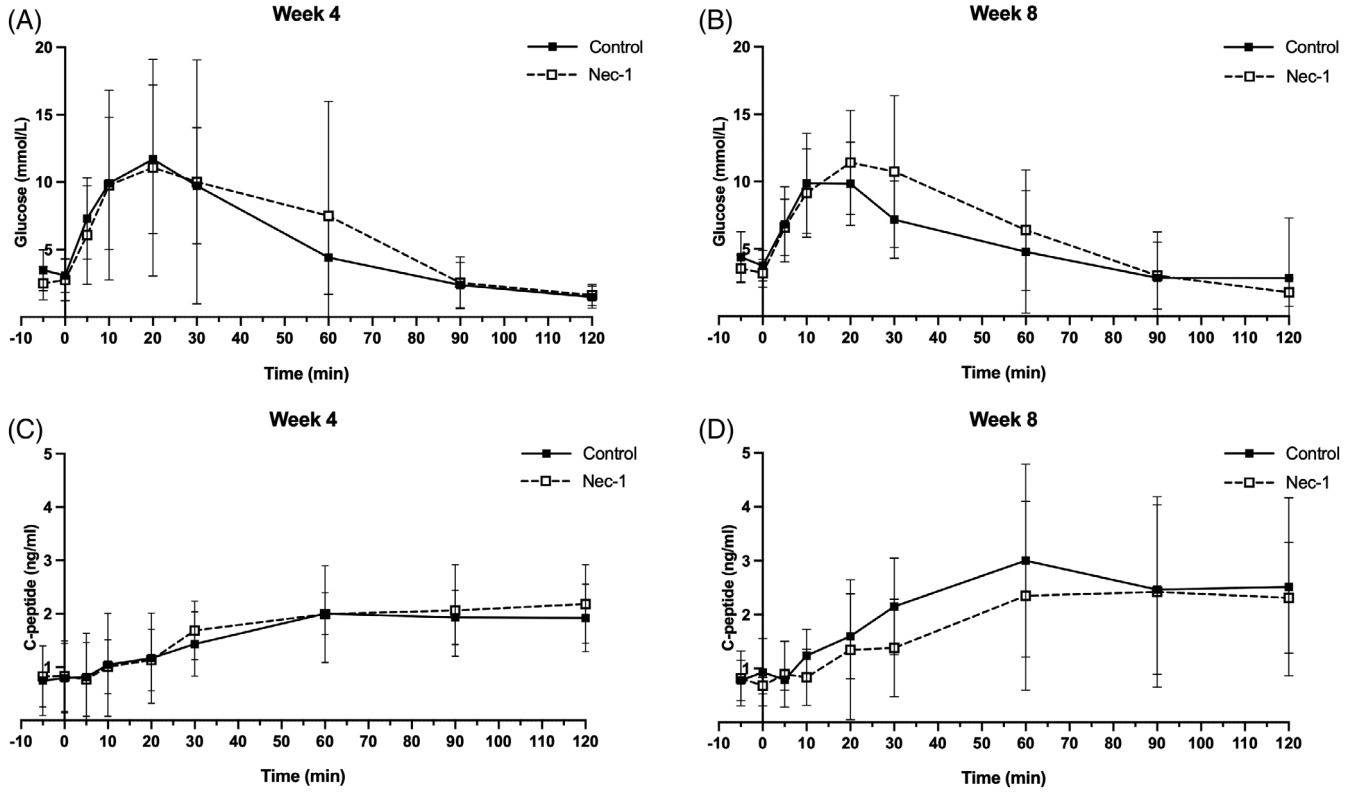

FIGURE 4 Intraperitoneal glucose tolerance test at 4 and 8 weeks post-transplantation. Blood glucose and C-peptide levels after an intraperitoneal glucose injection at 4 and 8 weeks after islet transplantation comparing the control treatment ($n = 6$) with Nec-1 treatment ($n = 5$). For mouse 26 (control), 29, and 30 (Nec-1) no IPGTTs were performed. Mean and standard deviation are plotted.

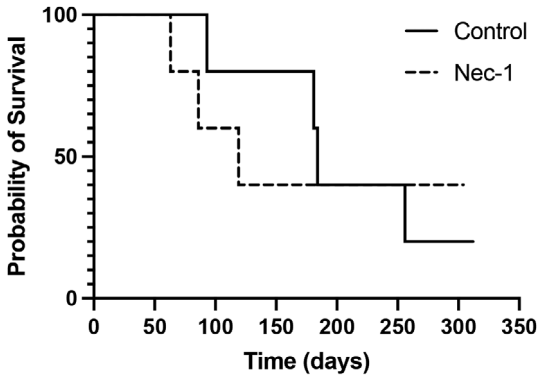

FIGURE 5 Long-term transplantation efficacy. Kaplan–Meier graph for graft survival rates after intraperitoneal transplantation of 1000 encapsulated rat islets with Nec-1 ($n = 5$) or the inactive Nec-1 analog (control; $n = 5$) treatment in diabetic C57BL6 mice. Control mice 4 and 7 and Nec-1 mice 6 and 8 were sacrificed, while still being normoglycemic, at 100 days post-transplantation for histology. These mice were excluded from this long-term transplantation efficacy graph.

in vitro and in vivo. To enhance the stability and efficacy of Nec-1, a PLGA-based controlled-release system was developed that releases Nec-1 in a sustained manner over 6 days. The released Nec-1 retained its bioactivity as demonstrated by a strong protective effect against beta cell death under hypoxic conditions, which is a potent inducer of necroptosis in islets. However, in vivo, no significant differences were found in the functional survival of encapsulated islets in a streptozotocin-induced diabetes mouse model with a highly biocompatible alginate-based encapsulation system.

The promising results in vitro but the inability to demonstrate a statistically prolonged survival of encapsulated islets after treatment with the Nec-1 NPs in vivo should not be interpreted as a lack of efficacy of Nec-1 NPs. Several reasons may explain the lack of long-term efficacy. Islet cell loss in encapsulated islets has been reported to occur predominantly in the first two weeks after implantation.[5–7] First, the current regimen comprising three Nec-1 NPs injections resulting in 18 days of Nec-1 release might not be sufficient and might be too short to impact the long-term survival of the grafts. Second, our alginate capsules might not provoke strong enough inflammatory responses to demonstrate an effect of Nec-1. The applied capsules are highly biocompatible as shown in our previous studies,[4,5] and also demonstrated here, in the animals that were sacrificed during the study, that capsules were free of any inflammatory cell adhesion irrespective of Nec-1 treatment or not. In the past, many different alginates with a large variation of chemical modifications have been tested to increase biocompatibility.[26] Notably, alginate capsule surface properties, alginate capsule particle size, and the alginate purification process all influence the tissue response against these

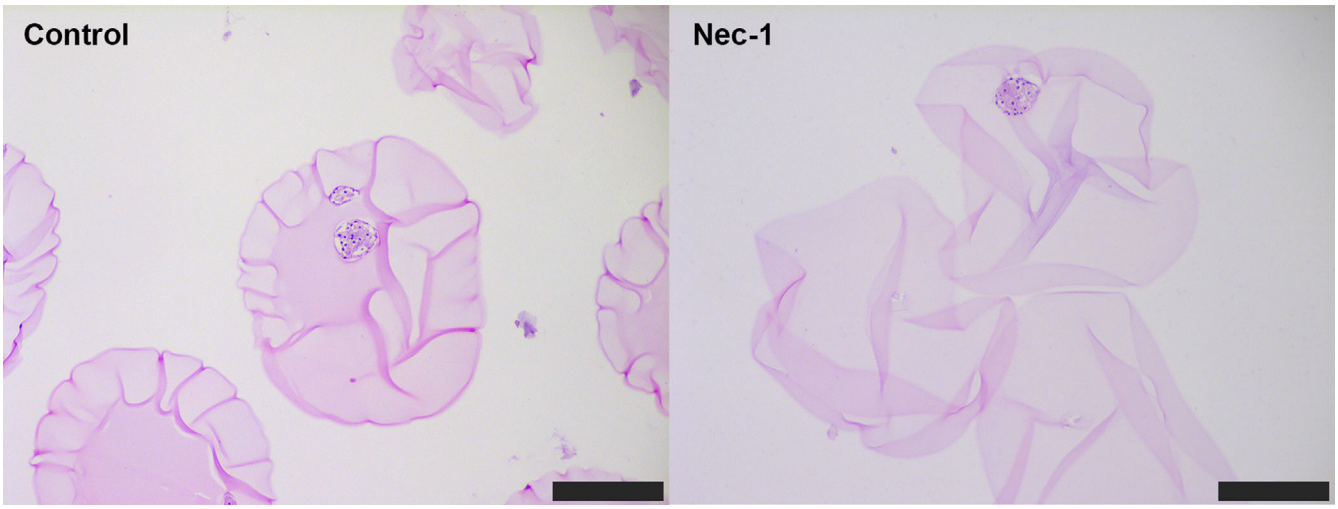

FIGURE 6 Histology of encapsulated islets from mice treated with control or Nec-1-releasing nanoparticles. Hematoxylin and eosin staining on paraffin sections. The scale bar is 200 μm.

microcapsules. Interestingly, some investigators report more and stronger tissue responses than others with seemingly similar strategies. This makes it very difficult to compare the studies of one group with another group. In most of the studies performed up to now, the efficacy of Nec-1 in vitro was only shown under high inflammatory stress or hypoxia.[11,13] With the low presence of these stressors in our well-defined encapsulation system, the effect of Nec-1 might be hard to demonstrate in vivo.

Although we did not observe a prolongation of survival of the grafts, we also did not observe any negative impact nor any change in the well-being of the animals suggesting that treatment with the Nec-1-releasing NPs had any negative side effects. The Nec-1 released from the NPs was able to protect against hypoxia-induced cell death in INS-1 beta cells. A mechanism that could be involved in this protective effect is the reduction of DAMP release. When encapsulated islets are exposed to severe stress as might occur in the immediate post-transplant period, islet cells may undergo necroptosis and release DAMPs, such as double-stranded DNA, uric acid, or high-mobility group box 1.[27] Also, beta cells have been shown to secrete these DAMPs under stressful conditions.[13] DAMPs activate the immune system via Toll-like receptors, leading to the loss of beta cells. Nec-1 is known to inhibit essential proteins within the necroptosis pathway, such as the receptor-interacting serine/threonine-protein kinase 1 and 3.[28] In this way, the release of DAMPs might be prevented by Nec-1 when beta cells are exposed to hypoxia or inflammatory stress. Especially in encapsulation systems where significant inflammation is to be expected such as approaches where vascularization of the membrane or macrodevice is favored[29–31] a significant impact of our system on beta cell survival might be expected. Follow-up studies could also consider using a lower number of islets or another transplantation site. A marginal dose of islets will increase the stress on the graft and Nec-1 might have a significant impact on graft survival. The same accounts for another transplantation site, such as the subcutaneous site, this has shown different responses in the past and might therefore lead to larger differences between the control and Nec-1 groups.

5 | CONCLUSION

We engineered Nec-1-releasing NPs as a controlled-release system that improves the metabolic stability and specificity of Nec-1 delivery. In vitro these Nec-1 NPs prevented hypoxia-induced cell death. In vivo, we found no side effects of Nec-1 but also no prolonged survival of islets in highly biocompatible alginate-based capsules. This might be explained by the low inflammatory response induced by these capsules. A better efficacy in preventing beta cell death and prolonging encapsulated islet grafts is expected in immunoisolating systems that provoke stronger responses in the immediate period post-transplantation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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