Take a deep dive into the business of transplantation!

2021 Digital Liver & Kidney Transplant Financial Bootcamp

Access Online!

February 26 - March 19, 2021

Register at ASTS.org/bootcamps

ASTS
American Society of Transplant Surgeons®

PROFESSOR SHAREEN FORBES (Orcid ID : 0000-0002-9127-0641)

Article type : Original Article

Fibroblast Growth Factor 7 Releasing Particles Enhance Islet Engraftment and Improve Metabolic Control Following Islet Transplantation in Mice with Diabetes

Salamah M. Alwahsh[1,4], Omar Qutachi[2], Philip J. Starkey Lewis[1], Andrew Bond[3], June Noble[3], Paul Burgoyne[3], Nik Morton[3], Rod Carter[3], Janet Mann[1], Sofia Ferreira-Gonzalez[1], Marta Alvarez-Paino[2], Stuart J. Forbes[1], Kevin M. Shakesheff [2], Shareen Forbes[3]

1. Centre for Regenerative Medicine, University of Edinburgh, 5 Little France Dr, Edinburgh, EH16 4UU, UK
2. School of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, UK
3. BHF Centre for Cardiovascular Science, University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK
4. current affiliation: Joint MD Program, College of Medicine and Health Sciences, Palestine Polytechnic University, Hebron, Palestine

Salamah M. Alwahsh and Omar Qutachi contributed equally to this work.
Kevin M. Shakesheff and Shareen Forbes are joint senior authors.

Correspondence
Shareen Forbes
Email: Shareen.Forbes@ed.ac.uk

Abbreviations: ASGPR, Asioglycoprotein receptor, BrdU, 5-Bromo-2'-Desoxyuridine, FCS, Fetal Calf Serum, FGF7, Fibroblast Growth Factor 7, FGF7-GAL-PLGA, FGF7 loaded galactosylated poly(DL-lactide-co-glycolic acid), GFs, growth factors, H&E, Haematoxylin and Eosin, HGF, hepatic growth factor, HNF4α, hepatocyte nuclear factor 4α, HPV, Hepatic Portal Vein, HSA, human serum albumin, IAH, impaired awareness of hypoglycemia, PSR, picrosirius red, SH, severe hypoglycemia, STZ, streptozotocin, T3, tri-iodothyronine, T1D, Type 1 diabetes, VEGF, Vascular Endothelial Growth Factor.

Abstract

Transplantation of islets in Type 1 diabetes is limited by poor islet engraftment into the liver, with 2-3 donor pancreases required per recipient. We aimed to condition the liver to enhance islet engraftment to improve long-term graft function. Diabetic mice received a non-curative islet transplant (n=400 islets) *via* the hepatic portal vein (HPV) with Fibroblast Growth Factor 7 loaded galactoslyated poly(DL-lactide-co-glycolic acid) (FGF7-GAL-PLGA) particles; 26μm diameter particles specifically targeted the liver, promoting hepatocyte proliferation in short-term experiments: in mice receiving 0.1mg FGF7-GAL-PLGA particles (60ng FGF7) versus vehicle, cell proliferation was induced specifically in the liver with greater efficacy and specificity than subcutaneous FGF7 (1.25mg/kg ×2 doses; ~75μg FGF7). Numbers of engrafted islets and vascularisation were greater in liver sections of mice receiving islets and FGF7-GAL-PLGA particles *versus* mice receiving islets alone, 72 hours post-transplant. More mice (6 out of 8) that received islets and FGF7-GAL-PLGA particles normalised blood glucose concentrations by 30-days post-transplantation, *versus* 0 of 8 mice receiving islets alone with no evidence of increased proliferation of cells within the liver at this stage and normal liver function tests. This work shows liver targeted FGF7-GAL-PLGA particles achieve selective FGF7 delivery to the liver promoting islet engraftment to help normalise blood glucose levels with a good safety profile.

1. Introduction

Islets are clusters of polyhormonal cells including insulin secreting β-cells. In Type 1 diabetes (T1D), destruction of pancreatic β-cells by autoimmune processes leads to insulin deficiency requiring insulin replacement. Severe hypoglycemia (SH) is a side effect of exogenous insulin and affects over 10% of those with T1D [1]. Human islet allotransplantation stabilizes glycemic control and decreases the frequency of recurrent SH in T1D [1-8]. However >60% of transplanted islets fail to engraft into the liver [9,10] and islets from 2-3 pancreas donors per recipient are needed to impact glycemic control [11]. Islets are avascular and following islet transplantation, the blood vessel supply between islets and the liver starts to form by day 3. The majority of islet loss occurs within the first 3 days post-transplant [12]. Hypoxia due to a lack of a blood supply and inflammation contributes to islet loss [13]. We hypothesized that a hepatic microenvironment favouring islet retention and vascularisation in the early stages post-transplantation would ameliorate early islet loss and aid long-term function.

Preconditioning the host liver with growth factors (GFs) creates a receptive ‘‘niche’’ involving the re-modelling and proliferation of liver cells [14,15]. Systemic growth factors such as tri-iodothyronine (T3) [16,17], hepatocyte growth factor (HGF) [14], and Fibroblast Growth Factor 7 (FGF7) [18] have been used to increase rat liver cell proliferation and enhance the efficiency of retroviral gene delivery. FGF7 is a small polypeptide member of the FGF family that binds to the FGF7 receptor and has proliferative and anti-apoptotic effects on epithelial cells including hepatocytes [18,19].

GFs may promote islet engraftment through: 1) liver cell proliferation and immediate islet “trapping”; 2) up-regulation of VEGF, promoting vasculogenesis and early islet vascularization [20,21]; 3) anti-inflammatory activity, thereby aiding islet survival [22,23]; 4) inhibition of T-cell mediated immune effects[24] reducing islet rejection.

Administering GFs systemically is limited by their short half-life, low tissue penetration, and effects on multiple organs. Low GF concentrations in the targeted organ necessitates dose escalation with off-target effects [25]. We tested targeted GF delivery to the liver to promote short-term liver cell proliferation, enhance islet engraftment and improved metabolic control in a mouse model of T1D.

PLGA polymer is a biodegradable material used in medical devices. The polymer matrix achieves desirable release kinetics based on the polymer hydration profile [26]. For targeted delivery to the liver, the PLGA polymer may have galactose added to it, [27,28] exploiting asialoglycoprotein receptor (ASGPR)-mediated endocytosis. There are ~25,000 ASGPR in the hepatocyte plasma

membrane with a specific binding affinity toward the galactose moiety attached on the PLGA particles [28]. Based upon its ability to induce proliferation in the liver following systemic use, a GF was selected for use in an engineered polymer for targeted delivery to the liver.

Our aim was then to create a microenvironment in the liver suitable for early islet engraftment, by using the GF-PLGA polymer associated complex. In order to do this we: 1) characterized the biodistribution and release kinetics of several formulations and particle sizes *in vivo;* 2) identified an optimal particle size and dose; 3) co-transplanted GF-loaded galactosylated PLGA (GAL-PLGA) particles concurrently with a non-curative mass of islets *via* the clinically relevant hepatic portal vein (HPV) into diabetic mice and monitored glycemic control over a 6 week period with histological assessments of islet engraftment in the liver.

2. Materials and Methods

2.1 Animals

Male C57Bl/6 mice (8-10 weeks old, Harlan Laboratories, UK) were housed under standard conditions in a 14-hr light to 10-hr dark cycle and given standard chow and water *ad libitum*.

2.2 Injection of growth factors and proliferation of cells within liver

In short-term experiments, twelve week old C57Bl/6 mice (n=8/group) received the following GFs or vehicle: Group 1) recombinant FGF7 1.25mg/kg subcutaneously (s.c.) (Sobi Pharmaceuticals, Sweden), Group 2) HGF 250µg/kg i.v. (R&D systems™, UK), Group 3) T3 4mg/kg s.c. (R&D systems™, UK), Group 4) all three GFs and Group 5) 100µL saline (vehicle), at day -2 and day 0. These doses were based on previous studies [18] with FGF7 which demonstrated that at 24hrs following one injection of FGF7 at high dose (5mg/kg), 3 of 8 mice were anorectic and hypoglycemic (blood glucose levels between 3.0-3.9 mmol/L) with signs of distress (Suppl. Table 1). Therefore lower doses of FGF7 were used with no adverse effects. Mice were pulsed with BrdU (1mg dissolved in PBS) via intraperitoneal (*i.p.*) injection 48hrs later and culled 1hr afterwards. Liver lobes were processed for immunohistochemistry (see below) with the antibodies listed in Suppl. Table 2. The GF associated with the greatest liver cell proliferation was selected and given s.c. prior to administering islets *via* the HPV, to determine if glycemic control was improved. This GF was subsequently incorporated into galactosylated PLGA particles.

2.3 FGF7 and effects on insulin secretion and oxygen consumption rates of islets in vitro

Islets (n=20 islets per well in triplicate) were incubated in 0 (control), 5ng/mL and 30 ng /mL FGF7 for 24 hours. FGF7 concentrations were based on concentrations released from 1mg FGF7-GAL-PLGA particles. Glucose stimulated insulin secretion (GSIS) was measured with insulin quantified by ELISA (Mercodia, Sweden) [10] and Oxygen consumption rates (OCR) of islets were measured in triplicate as previously described [10].

2.4 PLGA particle preparation, assembly, and characterisation

PLGA 50:50 lactide:glycolide ratio (52 kDa, DL-lactide, Lakeshore Biomaterials, USA) was functionalised with lactobionic acid (LB, Sigma Aldrich, UK) and fabricated from 5.5% PLGA in dichloromethane (DCM, Fisher, UK) by a double emulsion method [25]. The polymer solution, containing 0.1% w/v FGF7 and 0.9% human serum albumin (HSA, Sigma Aldrich, UK) was

homogenised stirred, filtered and freeze dried. Particle size distribution (Coulter LS230, Beckman, UK) was measured. Protein release (HSA+FGF7) was measured by bicinchoninic acid assay (Sigma Aldrich, UK) for 21 days.

2.5 Biodistribution of particles *via* the HPV or tail vein

PLGA particles were rhodamine-labelled. Mice (n=3/group) received an injection of 1 mg PLGA particles in 100µL 30% fetal calf serum (FCS). Non-galactosylated PLGA particles (2, 10, 22 µm mean diameter) and GAL-PLGA particles (2, 10, 26 µm mean diameter) were injected with a 30G needle *via* the HPV and *via* the tail vein (*i.v.*); vehicle injections were also run as controls. Mice were culled 24hrs post-injection, blood samples collected by cardiac puncture and the liver, lung, kidney, heart and spleen harvested for analysis.

2.6 Safety and efficacy studies with FGF7-GAL-PLGA particles

Safety and efficacy studies were performed with FGF7-GAL-PLGA particles (26µm) in increasing doses. Twelve week old C57Bl/6 mice (6 groups; n= 3-4 / group) received an HPV injection of FGF7-GAL-PLGA particles (0.01, 0.1, 1 or 5 mg in 100µL 30% FCS), 30% FCS or FGF7 s.c. (1.25 mg/kg; 100µL, once daily for 2 days). Terminal serum samples were collected for LFTs, FGF7 and VEGF-A, 72hrs post first injection; livers were processed for immunohistochemistry (H+E staining). Liver homogenates were further analysed for VEGF-A concentrations.

Based on the data above, the effect of 26µm FGF7-GAL-PLGA particles (0.1mg) on liver cell proliferation 72 hours post-transplant was determined. C57Bl/6 mice (12 week old; 4 groups; n= 3-4 per group) were injected *via* the HPV with: 1) FGF7-GAL-PLGA particles (0.1mg), 2) GAL-PLGA particles (0.1mg), 3) 30% FCS ×2 (100µL) and 4) FGF7 s.c. (1.25 mg/kg once daily for 2 days). Mice were pulsed with BrdU 1 mg *i.p* 1hr pre-cull and livers processed for immunohistochemistry (cell proliferation) as described.

2.7 Mouse islet isolation

Pancreatic islets were isolated from 12 week-old male C57Bl/6 mice by a collagenase digestion method [10], islet purity was ≥90%.

2.8 Induction of diabetes in mice

C57Bl/6 mice (n=8-10/group) received streptozotocin (STZ) (Sigma-Aldrich) at 16-17 weeks old by administration of 180 mg/kg *i.p.* and classed as hyperglycemic if non-fasted glucose levels

were ≥17.0mmol/L (306mg/dL) for two consecutive days. Islet transplantations took place within 10 days of STZ.

2.9 Transplantation of islets with subcutaneous FGF7 and FGF7-GAL-PLGA particles

Diabetic C57Bl/6 mice (n=6-8/group) were transplanted with: 1) 400 islets (in 200 μL RPMI 1640 medium), and 2) 400 islets plus FGF7 1.25 mg/kg s.c. ×2 doses, (48hr pre-transplant and at time of transplant). Control experiments included non-diabetic mice (n=4/group) given 3) FGF7 1.25mg/kg s.c. (100μL) ×2 doses and 4) vehicle (saline (100μL) s.c.×2 doses). Body weight and venous blood glucose (OneTouch Verio, LifeScan) were monitored daily and mice sacrificed 6 weeks post-transplantation.

Further experiments included diabetic C57Bl/6 mice (n=8-10/group) transplanted with: 1) 400 islets, 2) 400 islets plus 0.1mg FGF7-GAL-PLGA particles *via* the HPV, 3) vehicle injected mice. Mice were monitored and at 6 weeks post-transplant, mice were fasted overnight given 1mg BrdU *i.p*. and administered a 2g/kg IPGTT with glucose measurements at 15min, 30min, 60min, 90min and 120min afterwards and a plasma insulin at 60min. Mice were subsequently culled and livers (n=8-10/group) analysed for cell proliferation, vascularization, VEGF-A and fibrosis. Pancreases (n=4/group) were analysed for beta cell proliferation. The insulin content of the remaining pancreases were extracted and analysed.

In short-term experiments mice (n=3/group) were transplanted with 0.1mg FGF7-GAL-PLGA particles plus 400 islets versus a group transplanted with 400 islets alone (n=3/group) and sacrificed 72hr post-transplantation. Livers were analysed using immunohistochemistry for islet numbers (insulin and glucagon) and vascularization (CD31 and VEGF-A).

2.10 Biochemical analysis

ALT, albumin and bilirubin (Alpha Laboratories Ltd., Eastleigh, UK) were analysed on the Cobas Fara centrifugal analyser (Roche), human FGF7 and insulin concentrations by ELISA (Thermo Scientific, USA; Mercodia, Sweden, respectively); insulin content of the pancreas was measured following weighing, homogenisation and sonication [29]. VEGF-A was quantified by ELISA in serum samples and in liver homogenates at 72hrs and 6 weeks post-transplant (U-PLEX Mouse VEGF-A Assay, MSD).

2.11 Histological analysis

We quantified liver cell proliferation, islet engraftment, liver fibrosis, necrosis and vascularization. Tissue was either fixed, embedded in paraffin and cut serially (5μm) or processed using cryosections (8-30 μm). In brief to analyse: (i) proliferation-liver sections were immunostained with BrdU and hepatocyte nuclear factor 4α (HNF4α) to hepatocyte proliferation and total hepatocyte population respectively (ii) PLGA particle detection-rhodamine labelled particles were detected using fluorescence microscopy and quantified from an average of eleven ×40 fields per organ. To detect rhodamine-labelled PLGA particles in Kupffer cells, the Kupffer cells were detected using a rat anti-F4/80 immunostaining followed by an anti-rat Alexa Fluor 488 secondary antibody. (iii) islet engraftment-β-cells were quantified in ≥8 formalin fixed paraffin embedded (FFPE) sections of liver from all lobes (> 50 μm apart [in Z orientation]). More than 15 non-overlapping fields per section were evaluated, using ×20 magnification (Nikon Eclipse E600 fluorescent microscope). The average number of β-cells detected per FFPE section was standardized to the total analyzed fields [30]. (iv) Necrosis and fibrosis-H&E stains were produced using a Shandon Varistain Automated Slide Stainer. Collagen fibres in the liver tissues, were detected with picrosirius red (PSR) staining [31]. (v) Vascularization- was determined in paraffin-embedded liver sections using immunofluorescence for CD31 [32] and later the ETS-related gene (ERG): a transcription factor specific for endothelial cells[33]; VEGF-A quantification was attempted in liver sections using an immunofluorescence method [10,33]. For each immunostain, control procedures included isotype-matched rabbit monoclonal antibodies. DAPI staining was performed to label nuclei. Slides were mounted using an aqueous medium and imaged using an Operetta High-Content System (PerkinElmer).

2.12 Statistical Analysis

Results are expressed as mean ±SEM unless otherwise stated. Significance was determined by unpaired *t* tests or one-way ANOVA with Tukey's post-hoc testing using Prism 6.0 software (GraphPad Software, La Jolla, CA). A $p < 0.05$ was considered significant.

Results

3.1 Subcutaneous FGF7 enhanced liver cell proliferation more than other GFs in short-term experiments

FGF7 1.25mg /kg s.c. was associated with the greatest total proliferation of liver cells (parenchymal and non-parenchymal) *versus* HGF 250µg/ kg *i.v.*, T3 4mg/ kg s.c., and all three growth factors in combination; all mice were given 2 injections of GFs, the first at the time of transplant and the second 48hrs later with liver cell proliferation assessed 48hrs following this (Figure 1A). Mice receiving FGF7 s.c. exhibited pronounced cell proliferation in all organs including the lungs, pancreas, heart and spleen, as demonstrated by BrdU immunofluorescence staining versus controls (Figure 1B and Figure 1C). Therefore, FGF7 was selected for further studies.

3.2 Subcutaneous FGF7 with islets did not control blood glucose levels more effectively than transplantation of islets alone

Mice with diabetes transplanted with 400 islets plus FGF7 1.25mg/kg s.c (×2 injections), did not demonstrate improved glycemic control compared with mice transplanted with islets alone by 6 weeks, with no mice cured from their diabetes: glucose concentrations at 6 weeks: (mean±SEM): 18.1±2.2 mmol/L *vs*. 19.2±1.8 mmol/L, respectively (p=0.80). Control normoglycemic mice receiving FGF7 (1.25mg s.c. x2 doses) *vs.* vehicle treated mice showed no difference in glucose concentrations over a 6 week period (mean±SEM): 8.6±0.4 mmol/L *vs.* 8.3±0.6 mmol/L, respectively (p=0.91).

3.3 FGF7 has no effect on insulin secretion or OCR in short-term *in vitro* studies with islets

FGF7 at a dose of 5ng/mL or 30ng/mL had no effect on insulin secretion or OCR (Suppl. Fig. 1).

3.4 FGF7-GAL-PLGA particles released FGF7 predominantly in the first 48 hours over a 21 day period

Fabricated GAL-PLGA particles were regular and spherical with porous surfaces (Figure 2A). The average diameter of the galactosylated particle was (mean±SD) 26±6 µm with 57±2% FGF7 loading efficiency (Figure 2B).

The release kinetics showed an initial burst release phase, releasing ~one-third of the FGF7 payload on day 1, declining to 8% release on day 2 and 3% on day 3. Release was maintained at 1% between days 4 to 6 increasing to ~8% release from day 9 to 21 (Figure 2C). Cumulative *in vitro* release profiles of the particles and FGF7 delivery dose (ng per mg particles) is shown over 3

weeks (Figure 2D; Table 1). For 0.1mg PLGA particles the FGF7 content was 60ng and a 70% release of FGF7 over 21 days was ~40ng FGF7.

3.5 GAL-PLGA particles administered via HPV injection specifically target the liver

Non-galactosylated PLGA particles (mean diameter ~22μm) were found exclusively in lung (Figure 3A). In contrast, the galactosylated PLGA (GAL-PLGA) targeted FGF7 delivery to the liver; 26μm diameter particles conferred exclusive hepatic localisation. Smaller GAL-PLGA particles (~2μm and 10μm diameters) showed hepatic retention but not exclusively (Figure 3A-C). The smallest GAL-PLGA particles (diameter ~2μm), were engulfed by F4/80-positive liver resident macrophages (Figure 3D). In parallel, the biodistribution of PLGA-particles after injection into a tail vein was determined. A large proportion of the GAL-PLGA-particles (10μm diameter) were retained in the lung. Therefore larger sizes (26μm diameter) of GAL-PLGA particles were not tested for hepatic localisation as it was reasoned that these too would be trapped in the lung (Suppl. Fig 2A-C).

3.6 A FGF7-GAL-PLGA particle dose of 0.1mg injected via the HPV was associated with a stable body weight and normal liver function tests

Mice receiving 0.01mg and 0.1mg FGF7-GAL-PLGA particles *via* the HPV remained well with no demonstrable weight loss and no difference in serum albumin (marker of hepatocyte function), ALT or bilirubin (markers of liver injury) at 72hrs after transplant versus vehicle (Fig. 4A-C), an effect that was still apparent 6 weeks post-transplant (Suppl. Fig. 3). Human FGF7 serum levels were detected at 24hrs in mice transplanted with 0.1mg FGF7-GAL-PLGA particles (Fig. 4D). In the group administered 0.1mg FGF7-GAL-PLGA particles blood vessels in the liver appeared macroscopically milky white 24 hours after injection via the HPV and H&E staining exhibited occasional small necrotic areas (Fig. 4E and Fig. 4F).

Higher doses of FGF7-GAL-PLGA particles (1mg and 5mg) were not associated with significant weight loss 24 hours post injection (Suppl. Fig. 4A) but an increase in liver injury markers including ALT, bilirubin and albumin was observed (Suppl. Fig. 4B-D) with patchy liver necrosis (Suppl. Fig. 4E-F).

3.7 FGF7-GAL-PLGA particles (0.1mg) significantly increased proliferation of liver cells at 72 hours post-transplant with no increased proliferation at 6 weeks post-transplant

Based on the dose response studies, 0.1mg PLGA-GAL-FGF7 particles were administered via the HPV in mice and liver cell proliferation (total and hepatocyte) examined at 72hrs. Co-localisation of $BrdU^+$ and $HNF4\alpha^+$ cells in liver sections of mice that received FGF7-GAL-PLGA particles (Fig. 5A) was 1.5-fold greater than in mice receiving FGF7 1.25mg/kg s.c. ×1 dose per day for 2 days. The greatest cell proliferation overall was observed in the liver of mice treated with FGF7-GAL-PLGA particles (Fig. 5B, proportion (%) of proliferating cells in the liver (1.7±0.1% *vs*. 0.9±0.1% in mice received FGF7 s.c.; p=0.03) and 55 % of proliferating cells were hepatocytes (p=0.04, Fig. 5C).

At 6 weeks, the % proliferating cells in the liver of mice treated with FGF7-GAL-PLGA particles was not significantly different *vs*. control hyperglycemic mice: (0.009 ± 0.002 % *vs.* 0.015±0.001 %; n=4, p=0.13).

3.8 FGF7-GAL-PLGA particles (0.1mg) transplanted with islets promoted early islet engraftment with improved long-term glycemic control with no evidence of liver fibrosis

Greater numbers of islets were seen in the livers of hyperglycemic mice transplanted intraportally with islets and FGF7-GAL-PLGA particles *vs* islets alone as evidenced by greater numbers of dual insulin-glucagon positive cells in the liver 72hrs post-transplant (Fig. 6A and Fig. 6B, p=0.02). At 72hrs post-transplant, the percentage area of liver that was CD31 positive was greatest in mice treated with FGF7-GAL-PLGA particles (0.1mg) plus islets *vs*. islets alone and *vs*. GAL-PLGA particles alone: 6.8±0.9% *versus* 5.6±0.6% versus 2.2±0.06% (p=0.04; Fig 7A and 7B). At 6 weeks ERG and $CD31^+$ staining was non-significantly greatest in the livers of mice co-transplanted with FGF7 particles with islets (Fig. 7C and Fig. 7D, p=0.12). There was no VEGF-A staining in the livers quantifiable over the background compared to the isotype control in mice at 72hr and 6 weeks post-transplant (Suppl. Fig 5A and 5B). VEGF-A concentrations were detectable in serum and liver homogenates at 72hr and 6 weeks post-transplant with non-significantly greater concentrations in mice transplanted with 5mg FGF7 particles (Suppl. Fig 5C and D).

Mice receiving islets and FGF7-GAL-PLGA particles had tighter glycemic control *vs.* those receiving islets alone with blood glucose levels normalising by day 30 post-transplant (Fig. 8A, p=0.03) and with a greater proportion achieving a cure from their diabetes (75% *vs*. 0%; p<0.001).

Stimulated insulin concentrations at 60min post *i.p*.GTT were not significantly different between the islet alone *versus* islet+FGF7-GAL-PLGA groups: (median(IQR)): 150(143-170) *vs*. 155(148-182) pmol/L, p=0.37. When glucose concentrations were expressed in relation to insulin concentrations at 60mins post 2g/kg *i.p*.GTT, however, mice co-transplanted with FGF7 had greater insulin:glucose ratios (Fig 8B).

There was no difference in collagen content in the liver tissues between the groups at day 42 (Fig. 8C-D). Serum biomarkers for liver injury at 6 weeks post-transplant were not different to controls (Suppl. Fig. 4A-C).

3.9 No evidence of pancreas regeneration 6 weeks post-transplantation of islets ± galactosylated FGF7-GAL-PLGA particles (0.1mg)

No proliferating ß-cells were detected in the pancreases of mice treated with FGF7-GAL-PLGA particles (Suppl. Fig 6A). There was no significant difference in pancreatic insulin content between groups (Suppl. Fig 6B).

4. Discussion

Transplantation of islets into patients with T1D stabilizes glycemic control, reducing SH [1,5,34] but due to poor engraftment of islets into the liver [35,36] islets from 2 to 3 donor pancreases, a scarce resource, are required.

In diabetic rodents, partial hepatectomy preceding intra-portal islet transplantation is associated with improved glycemic outcomes versus islet transplant alone, likely due to GF release, remodelling of the liver niche and liver cell proliferation, improving islet engraftment and revascularization [20,30,37]. However, partial hepatectomy is not a clinically applicable adjuvant therapy for intraportal islet transplantation in man. FGF7 RNA is expressed in most organs throughout the human body, with moderate expression in the pancreas and no expression in the islet in adulthood [38]. FGFR2 is the cognate receptor for FGF7 and has been localised to the beta cells of the islets of Langerhans [39] but is absent from the alpha cells and the exocrine pancreas [40]. Islets are mainly derived from the cells of the bud epithelium and FGF7 treatment activates ductal cell proliferation and their subsequent differentiation into β-cells in human fetal pancreatic cell preparations [41]. In an adult islet however exogenous FGF7 causes the ductal epithelium to proliferate but there is no evidence of endocrine differentiation or β-cell proliferation [42]. In our experiment with adult mouse donor islets, it is unlikely that adult intra-islet ductal cells differentiated into β-cells.

In our studies FGF7 promoted proliferation of cells within the liver and it seems likely that this is one of the dominant mechanism promoting islet engraftment within the liver.

Of note the doses of growth factors used were based on safety and efficacy from our own experiments and the published literature and were not administered in equimolar amounts. The combination of FGF7, HGF and T3 was less efficacious with respect to proliferation of liver cells versus FGF7 alone. We hypothesize that there is a balance between metabolic demand of the tissue and proliferation of cells in the liver–with all three GFs metabolic demand may be greater than with FGF7 alone impacting negatively on proliferation of liver cells.

Our results demonstrate increased vascularization of islets in the liver, with increased endothelial marker staining (CD31) in the liver at an early stage, coupled with improved glycemic control when the liver is targeted by FGF7 contained in galactosylated PLGA particles: the numbers of islets in the liver were greater in the mice receiving a portal injection of 0.1mg PLGA-GAL-FGF7 particles of 26μm diameter plus islets versus those receiving islets alone. Importantly the dose of FGF7 received was approximately 60ng over a 4 week period representing approximately a 1000-

fold lower dose than the dose of 1.25mg/kg FGF7 (×2 doses) administered subcutaneously to mice.

Furthermore, the majority of mice administered these particles were cured following islet transplantation. Importantly, there was no β-cell regeneration in the native pancreas and FGF7 did not augment insulin secretion in our *in vitro* experiments, consistent with the beneficial effects being mediated by improved hepatic islet engraftment. Of note, there was no evidence of diminished OCRs from islets exposed to FGF7 and therefore no evidence of an adverse effect of FGF7 on islet function in the short-term. Subcutaneous FGF7 increased hepatic non-parenchymal cell proliferation, however glycemic control was not improved following islet transplantation. FGF7 was not detectable systemically 24hrs following subcutaneous administration and these short-term effects may not be sufficient to increase islet engraftment. Islet engraftment, where blood vessels form between the islets and the liver, occurs largely between days 3 to 28 [43]. Subcutaneous FGF7 also causes cell proliferation in other organs including the lungs, pancreas, kidney, heart and spleen which limits clinical applicability.

When GAL-PLGA particles were studied over a 21 day period, the route of administration, particle size and galactosylation influenced its sequestration within organs. The 26µm GAL-PLGA particles delivered via the HPV route were sequestered solely in the liver. Smaller galactosylated particles (2µm and 10µm) delivered via the HPV route, were sequestered in the liver and the capillary beds of the lungs, spleen and other organs. Control PLGA particles of 22µm without the galactosylated moiety were sequestered in the lung only, demonstrating that galactosylation is required for localisation of the particle in the liver *via* ASGPR-mediated endocytosis. When the 10µm GAL-PLGA particles were delivered peripherally, a large proportion were trapped in the lung with only some reaching the liver; hence this would not be a feasible way to translate this therapy into man.

In our short-term experiments, FGF7 60ng packaged into 0.1mg PLGA particles caused liver proliferation with no change in liver serum markers although a minute patch of necrosis was seen which requires further exploration. With this dose of FGF7, 55% of proliferating cells were hepatocytes, contrasting with 22% when two doses of 1.25mg/kg FGF7 were administered subcutaneously. Liver injury including patchy necrosis of the liver was demonstrable with 1mg and 5mg FGF7-GAL-PLGA particles, suggesting dose response studies in larger animal models may be useful before clinical studies are undertaken. Importantly in the studies at 6 weeks there

was no evidence of hepatocyte proliferation, suggesting that modulation of the liver niche in the short-term is sufficient for islet engraftment with no long-term deleterious effects in the liver.

Numbers of islets transplanted [44], along with younger donor age [45] impact transplant outcome. This study demonstrates that modulation of the liver niche by FGF7-GAL-PLGA particles is a potential therapeutic strategy for increasing islet engraftment and islet transplant outcomes in man. FGF7 may increase engraftment of islets by stimulating angiogenesis *via* VEGF induction directly [21] or indirectly [46,47] in keeping with increased hepatic CD31 staining at 72hrs post transplant in recipient mice. We did not detect VEGF-A in liver, the immunoflouresence technique may not be sensitive enough to detect VEGF-A at low levels or it may be upregulated at a different time point. In this study 0.1mg of FGF7-GAL-PLGA particles would release ~40ng of FGF7 in a mouse over 21 days leading to increased islet engraftment in the liver. FGF7 has FDA approval and held a license for severe oral mucositis in patients with hematologic malignancies receiving myelotoxic therapy. The recommended dose is ~25mg intravenously for a 70kg person over a 6 day period [48]. Extrapolating the dose of FGF7 administered in a mouse via particles direct to the liver to humans on a weight for weight basis, the dose used in man via the HPV would be >250 fold lower than the licensed dose for treating oral mucositis. We believe this treatment can potentially be translated into man. Such a strategy would mean that islets isolated from just one donor pancreas may be sufficient to diminish hypoglycemia and stabilize glycemic control in patients with T1D enabling more patients to be transplanted.

Author Contributions

SF, KS and SJF conceptualised study and obtained funding for the study. OQ – formulated FGF7 particles and drafted FGF7-particles methods; SA, PSL, AB and JN performed animal experiments and laboratory assays. PSL performed immunohistochemistry for vascularizton markers and performed additional statistical analyses. SA helped draft the manuscript and performed statistical analyses; JM, PB and SFG – gave technical laboratory assistance. SA, NM, and RC performed and analysed oxygen consumption rate assays. SJF was PI for the liver regeneration studies, KMS was PI for the FGF7-particle formulation studies and SF was PI for the FGF7 and metabolic studies; SF drafted and revised the manuscript and figures and performed statistical analyses for the in vivo transplant studies. All authors critically reviewed the manuscript. SF is the guarantor of this work and as such had full access to all the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

Diabetes UK Grant number 13/0004682, UK Regenerative Medicine Platform Grant number: MR/K026666/1, MRC Computational & Chemical Biology of the Stem Cell Niche (Capital grant): MR/L012766/1, MRC Regenerative Medicine Grant: MR/S03692X/1, Chief Scientist Office ETM/325, Wellcome Trust–University of Edinburgh Institutional Strategic Support Fund, Edinburgh and Lothian's Health Foundation Award, Wellcome Trust New Investigator Award (100981/Z/13/Z), Royal Society Funding (RSG\R1\180079). Sobi Pharmaceuticals donated FGF7 under a material transfer agreement for use in subcutaneous injections only in mice in the Diabetes UK funded grant. The University of Nottingham and The University of Edinburgh have a joint UK Patent Application: No. 1818977.9. Date of Filing: 21 November 2018.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

ORCID ID:

Shareen Forbes - https://orcid.org/0000-0002-9127-0641

Kevin Shakesheff - https://orcid.org/0000-0003-3236-2439

Figure Legends

Figure 1

Cell proliferation in liver and other organs after injection of GFs. Mice received ×2 injections of the following GFs or vehicle 48 hours apart (day 0 at Transplant and day 2) and culled 48 hours following the last injection of growth factor or vehicle. One hour before cull, BrdU was injected *i.p*. to detect cell proliferation. The number of BrdU positive cells (=proliferating cells) was evaluated by the Operetta system and Columbus software.

(A) vehicle (100 µL saline) s.c., HGF 250 µg/kg *i.v*., T3 4 mg/kg s.c., FGF7 1.25 mg/kg s.c, or combination of all three GFs. **(B)** Immunofluorescence staining for BrdU in various organs in mice receiving FGF7 1.25 mg/kg s.c. ×2 doses or **(C)** vehicle 100 µL saline s.c ×2 doses.

Arrows indicate BrdU positive cells. DAPI (blue) indicate cell nuclei. Data represent the mean ± SEM.

Figure 2

Characterisation of GAL-PLGA particles. (A) Representative scanning electron micrographs of particles. **(B)** Size distribution of particles with average size diameter and entrapment efficiency (%) for total protein. **(C)** Individual daily release percentage, and **(D)** Cumulative release kinetics for the total protein payload over a three week period. Data represent mean±SD. EE – entrapment efficiency.

Figure 3

Hepatic portal vein injection of 26 µm GAL-PLGA particles via the HPV provides specific hepatic localisation. PLGA particles non-galactosylated (22µm) and GAL-PLGA (2, 10 and 26µm) were injected into mice (1mg, HPV) to determine particle distribution in organs. **(A)** Representative fluorescent images (×40) of organs extracted 24 hours after injection of particle formulations. Cryosections (30µm) were fixed and stained: DAPI (blue, cell nuclei); fluorescent particles (red epifluorescence) are highlighted (white arrows). **(B)** Mean particle counts (logarithm, from 11 slides) quantified per tissue grouped in formulations indicated. Bars represent the mean ± SD, n=3 mice per group. **(C)** Percentage of GAL-PLGA (26 µm) and non-galactosylated (22µm) PLGA-particle biodistribution in various organs. **(D)** Small particles (2 µm, white arrows) localise with F4/80 positive cells (green) in liver tissue suggesting phagocytic uptake. The insert represents an expanded image. Scale bars 60 µm.

Figure 4

Dose response study shows that particles do not cause overt hepatotoxicity

(A) Serum ALT activity, **(B)** albumin **(C)** bilirubin and **(D)** human FGF7 levels from mice transplanted via HPV receiving FGF7-GAL-PLGA particles (26 μm; 0.01mg and 0.1mg) *versus* vehicle (30%, 100 μL FCS); additional groups in **D** mice receiving FGF7 s.c. 1.25mg/kg s.c. ×2 doses. **(E)** Macroscopic view of the liver pre- and post-transplant with FGF7-GAL-PLGA particles **(F)** H-E staining liver (the black arrow indicates a necrotic patch). Mice were culled 72hrs post-transplant.

Data are mean ± SEM, n=3 mice per group. HPV: hepatic portal vein.

Figure 5

Effects of targeted FGF7-GAL-PLGA delivery via HPV *versus* FGF7 s.c. on liver cell proliferation. (A) FGF7 1.25 mg/kg s.c. ×2 doses, 0.1 mg FGF7-GAL-PLGA (HPV) or GALPLGA alone (HPV), 30% FCS (HPV), 72hr following first injection. BrdU was administered 1mg i.p before cull. Representative micrographs of dual immunostaining applied on liver sections for BrdU (green, cell proliferation), HNF4α (red, hepatocytes) and DAPI (blue, nucleus staining). In the upper panels, white arrows show $BrdU^+$ non-parenchymal cells ($HNF4\alpha^-$), as magnified in the inset. In the middle row, mainly dual positive nuclei are observed (orange-yellow), while in the lower panel (PLGA alone treated mice), the proliferating cells were mainly non-parenchymal. Inset (×400) show higher magnified regions of liver sections of different treatments. **(B)** Percentage of the proliferating ($BrdU^+$) cells in each mouse group. **(C)** Fraction of proliferating hepatocytes ($BrdU^+$, $HNF4\alpha^+$) to the total proliferating cells ($BrdU^+$).

Cell counting by Operetta system and Columbus. Scale 100 μm. * $P<0.05$ using one-way ANOVA Tukey's post-hoc.

Figure 6

Islet detection in liver tissue 72hrs post islet-transplantation. Intraportal islet transplants islets alone (n=400), and islets (n=400) co-transplanted with FGF7-GAL-PLGA particles (0.1mg) were performed and mice (n=3 per group) culled 72hrs post-transplant. **(A)** Dual immunofluorescence staining for islets (insulin- β cells and glucagon- α cells) in liver tissues. Scale 100 μm. (Note - islets are exposed to sheer stress during transplantation accounting for fragmented appearance.)

(B) Average area of β-cells from ≥ 8 FFPE sections from all 4 lobes of the liver were quantified from ≥ 15 non-overlapping fields and expressed in terms of graft area (%). Each data point represents the average β-cell to total graft area from 120 fields under 20X magnification. Mean ± SEM is shown. p-value assessed by unpaired t-test.

Figure 7

CD31 detection at 72hrs and ERG detection at 6 weeks post-transplant in whole liver sections

At 72hrs post-transplant groups of mice were studied (n=4/gp): 1) vehicle –hyperglycemic, 2) intraportal islet transplants alone (n=400); 3) islets (n=400) co-transplanted with FGF7-GAL-PLGA particles (26um diameter, 0.1mg).

(A) Immunofluorescence staining for CD31 nuclei in liver tissues. **(B)** Quantification of CD31 nuclei in groups. p=0.04; one-way ANOVA.

At 6 weeks post-transplant three groups of mice were studied (n=5-8/gp): hyperglycemic-vehicle, intraportal islet transplants alone (n=400), and islets (n=400) co-transplanted with FGF7-GAL-PLGA particles (26um diameter, 0.1mg). **(A)** Immunofluorescence staining for ERG nuclei in liver tissues. **(B)** Quantification of ERG nuclei in all groups. p=0.12 ; one-way ANOVA.

Figure 8

Biochemical assays and assessment of liver fibrosis in diabetic C57Bl/6 mice transplanted with a marginal islet mass ± FGF7-GAL-PLGA particles (0.1mg) monitored for 6 weeks. **(A)** Non-curative mass intraportal islet transplantation ± FGF7-GAL-PLGA particles (0.1mg). Daily blood glucose concentrations are shown. Islets alone *vs*. islets + FGF7-GAL-PLGA (0.1mg) particles * *P*=0.03 one-way ANOVA. **(B)** Insulin:glucose ratio was significantly increased in the islet+FGF7-GAL-PLGA group. **(C)** Picrosirius red (PSR)-stained liver tissues for fibrosis, **(D)** Percentage of collagen (red pixels) to liver tissue (yellow). Each value represents the mean of 8 (×10) fields per mouse liver section. Data were generated using an automated slide Scanner with InForm software.

References

1. Forbes S, McGowan NW, Duncan K, et al. Islet transplantation from a nationally funded UK centre reaches socially deprived groups and improves metabolic outcomes. Diabetologia. 2015;58(6):1300-1308.
2. Qi M, Kinzer K, Danielson KK, et al. Five-year follow-up of patients with type 1 diabetes transplanted with allogeneic islets: the UIC experience. Acta diabetologica. 2014;51(5):833-843.
3. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. The New England journal of medicine. 2000;343(4):230-238.
4. Forbes S, Oram RA, Smith A, et al. Validation of the BETA-2 Score: An Improved Tool to Estimate Beta Cell Function After Clinical Islet Transplantation Using a Single Fasting Blood Sample. Am J Transplant. 2016;16(9):2704-2713.
5. Lablanche S, Vantyghem MC, Kessler L, et al. Islet transplantation versus insulin therapy in patients with type 1 diabetes with severe hypoglycaemia or poorly controlled glycaemia after kidney transplantation (TRIMECO): a multicentre, randomised controlled trial. The lancet Diabetes & endocrinology. 2018;6(7):527-537.
6. Forbes S, Senior PA, Shapiro AMJ. Islet transplantation in type 1 diabetes: moving forward. The lancet Diabetes & endocrinology. 2018;6(7):516-517.
7. Hering BJ, Clarke WR, Bridges ND, et al. Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia. Diabetes care. 2016;39(7):1230-1240.
8. Brennan DC, Kopetskie HA, Sayre PH, et al. Long-Term Follow-Up of the Edmonton Protocol of Islet Transplantation in the United States. Am J Transplant. 2016;16(2):509-517.
9. Barshes NR, Wyllie S, Goss JA. Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts. Journal of leukocyte biology. 2005;77(5):587-597.
10. Forbes S, Bond AR, Thirlwell KL, et al. Human umbilical cord perivascular cells improve human pancreatic islet transplant function by increasing vascularization. Sci Transl Med. 2020;12(526).
11. Rother KI, Harlan DM. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. The Journal of clinical investigation. 2004;114(7):877-883.
12. Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. Diabetes. 2002;51(1):66-72.
13. Kanak MA, Takita M, Kunnathodi F, Lawrence MC, Levy MF, Naziruddin B. Inflammatory response in islet transplantation. International journal of endocrinology. 2014;2014:451035.
14. Hammond JS, Gilbert TW, Howard D, et al. Scaffolds containing growth factors and extracellular matrix induce hepatocyte proliferation and cell migration in normal and regenerating rat liver. Journal of hepatology. 2011;54(2):279-287.

15. Shimoda M, Chen S, Noguchi H, Matsumoto S, Grayburn PA. In vivo non-viral gene delivery of human vascular endothelial growth factor improves revascularisation and restoration of euglycaemia after human islet transplantation into mouse liver. Diabetologia. 2010;53(8):1669-1679.
16. Forbes SJ, Themis M, Alison MR, Selden C, Coutelle C, Hodgson HJ. Retroviral gene transfer to the liver in vivo during tri-iodothyronine induced hyperplasia. Gene therapy. 1998;5(4):552-555.
17. Alwahsh SM, Rashidi H, Hay DC. Liver cell therapy: is this the end of the beginning? Cellular and molecular life sciences : CMLS. 2018;75(8):1307-1324.
18. Forbes SJ, Themis M, Alison MR, Sarosi I, Coutelle C, Hodgson HJ. Synergistic growth factors enhance rat liver proliferation and enable retroviral gene transfer via a peripheral vein. Gastroenterology. 2000;118(3):591-598.
19. Takase HM, Itoh T, Ino S, et al. FGF7 is a functional niche signal required for stimulation of adult liver progenitor cells that support liver regeneration. Genes Dev. 2013;27(2):169-181.
20. Saito Y, Chan NK, Hathout E. Partial hepatectomy improves the outcome of intraportal islet transplantation by promoting revascularization. Islets. 2012;4(2):138-144.
21. Xiong Y, Scerbo MJ, Seelig A, et al. Islet vascularization is regulated by primary endothelial cilia via VEGF-A-dependent signaling. Elife. 2020;9.
22. Liu S, Zhang L, Cheng J, Lu Y, Liu J. Sustained release of hepatocyte growth factor by cationic self-assembling peptide/heparin hybrid hydrogel improves beta-cell survival and function through modulating inflammatory response. Int J Nanomedicine. 2016;11:4875-4890.
23. Fiaschi-Taesch N, Stewart AF, Garcia-Ocana A. Improving islet transplantation by gene delivery of hepatocyte growth factor (HGF) and its downstream target, protein kinase B (PKB)/Akt. Cell biochemistry and biophysics. 2007;48(2-3):191-199.
24. Panoskaltsis-Mortari A, Taylor PA, Rubin JS, et al. Keratinocyte growth factor facilitates alloengraftment and ameliorates graft-versus-host disease in mice by a mechanism independent of repair of conditioning-induced tissue injury. Blood. 2000;96(13):4350-4356.
25. Huang Z, Zhu G, Sun C, et al. A novel solid-phase site-specific PEGylation enhances the in vitro and in vivo biostabilty of recombinant human keratinocyte growth factor 1. PloS one. 2012;7(5):e36423.
26. Fredenberg S, Wahlgren M, Reslow M, Axelsson A. The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems--a review. Int J Pharm. 2011;415(1-2):34-52.
27. Ashwell G, Morell AG. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. Adv Enzymol Relat Areas Mol Biol. 1974;41(0):99-128.
28. Li Y, Huang G, Diakur J, Wiebe LI. Targeted delivery of macromolecular drugs: asialoglycoprotein receptor (ASGPR) expression by selected hepatoma cell lines used in antiviral drug development. Curr Drug Deliv. 2008;5(4):299-302.
29. Labs J. Insulin Content by Acid-Ethanol Extraction. 2009.

30. Sudo T, Hiyama E, Murakami Y, Yokoyama Y, Takesue Y, Sueda T. Hepatic regeneration promotes engraftment of intraportally transplanted islet cells. Surgery. 2005;137(6):612-619.
31. Raven A, Lu WY, Man TY, et al. Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. Nature. 2017;547(7663):350-354.
32. Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. J Histochem Cytochem. 2006;54(4):385-395.
33. Haber MA, Iranmahboob A, Thomas C, Liu M, Najjar A, Zagzag D. ERG is a novel and reliable marker for endothelial cells in central nervous system tumors. Clin Neuropathol. 2015;34(3):117-127.
34. Brooks AM, Walker N, Aldibbiat A, et al. Attainment of metabolic goals in the integrated UK islet transplant program with locally isolated and transported preparations. Am J Transplant. 2013;13(12):3236-3243.
35. Gala-Lopez BL, Neiman D, Kin T, et al. Beta Cell Death by Cell-free DNA and Outcome After Clinical Islet Transplantation. Transplantation. 2018;102(6):978-985.
36. Lehmann-Werman R, Neiman D, Zemmour H, et al. Identification of tissue-specific cell death using methylation patterns of circulating DNA. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(13):E1826-1834.
37. Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. The American journal of pathology. 2010;176(1):2-13.
38. Proteinatlas.org. Protein atlas FGF7 and tissue. https://wwwproteinatlasorg/ENSG00000140285-FGF7/tissue
39. Proteinatlas.org. proteinatlas FGF2R in pancreas. https://wwwproteinatlasorg/ENSG00000066468-FGFR2/tissue/pancreas.
40. Hart AW, Baeza N, Apelqvist A, Edlund H. Attenuation of FGF signalling in mouse beta-cells leads to diabetes. Nature. 2000;408(6814):864-868.
41. Percival AC, Slack JM. Analysis of pancreatic development using a cell lineage label. Exp Cell Res. 1999;247(1):123-132.
42. Yi ES, Yin S, Harclerode DL, et al. Keratinocyte growth factor induces pancreatic ductal epithelial proliferation. The American journal of pathology. 1994;145(1):80-85.
43. Hathout E, Chan NK, Tan A, et al. In vivo imaging demonstrates a time-line for new vessel formation in islet transplantation. Pediatric transplantation. 2009;13(7):892-897.
44. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. Diabetes. 2005;54(7):2060-2069.
45. Niclauss N, Bosco D, Morel P, et al. Influence of donor age on islet isolation and transplantation outcome. Transplantation. 2011;91(3):360-366.

46. Beer HD, Gassmann MG, Munz B, et al. Expression and function of keratinocyte growth factor and activin in skin morphogenesis and cutaneous wound repair. J Investig Dermatol Symp Proc. 2000;5(1):34-39.
47. Frank S, Hubner G, Breier G, Longaker MT, Greenhalgh DG, Werner S. Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. The Journal of biological chemistry. 1995;270(21):12607-12613.
48. Kepivance in oral Mucositis - https://reference.medscape.com/drug/kepivance-palifermin-342271 PR.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Tables

Table 1 Release of FGF7 (% and ng/ 1mg particle) at the selected time points up to day 21

Day	Release (%)	Release in ng per 1mg particles
1	28.4 (±2.1)	163.3 (±5.5)
2	7.8 (±1.3)	45.4 (±9.5)
3	2.5 (±1.5)	14.5 (±1.5)
4	1.6 (±0.2)	9.3 (±2.0)
5	1.3 (±0.07)	7.9 (±0.7)
6	1.1 (±0.2)	6.8 (±1.4)
7	1.3 (±0.1)	7.7 (±0.9)
9	3.0 (±0.7)	17.5 (±4.9)
11	3.1 (±0.1)	18.0 (±1.7)
13	2.9 (±0.06)	16.9 (±0.7)
17	5.9 (±0.9)	33.9 (±4.0)
21	7.2 (±2.0)	44.7 (±9.7)

Microparticles (FGF-GAL-PLGA - 25 mg) were set up in triplicate and suspended in 1 ml PBS, gently rocked on a 3-dimensional shaker (Gyrotwister, Fisher Scientific UK Ltd) at 5 rpm in a humidified incubator at 37°C and supernatant collected at specified time points. (Mean±SEM).

Fig. 1

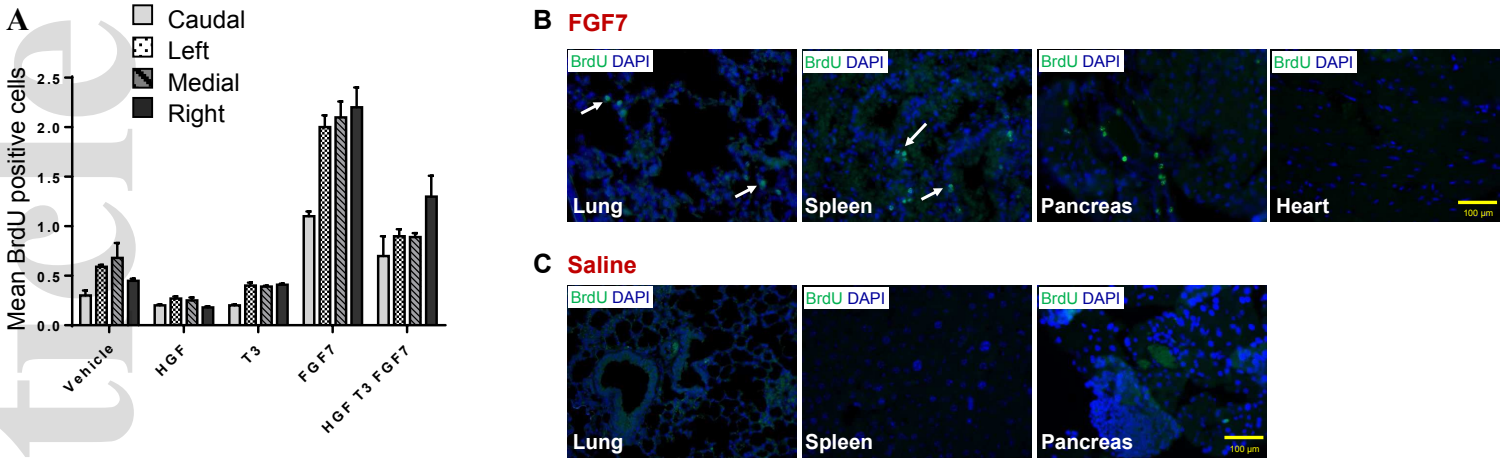


Fig. 2

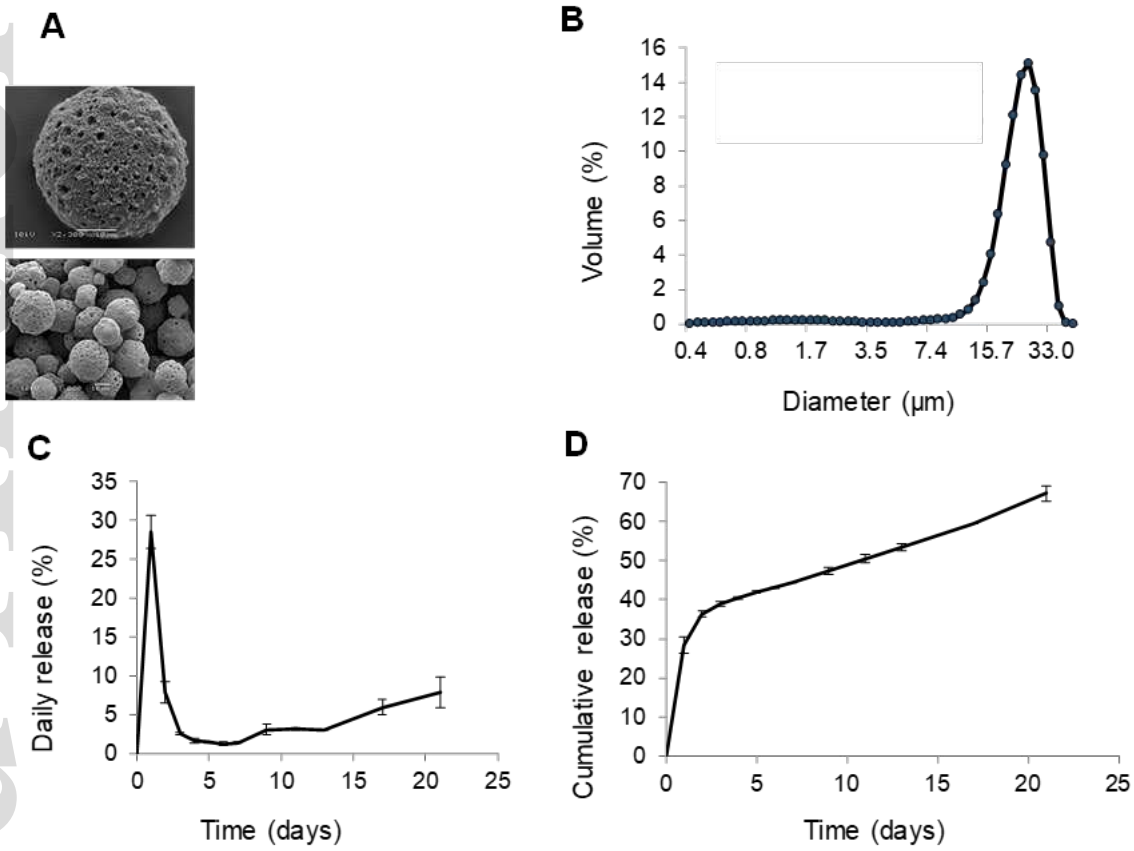


Fig. 3

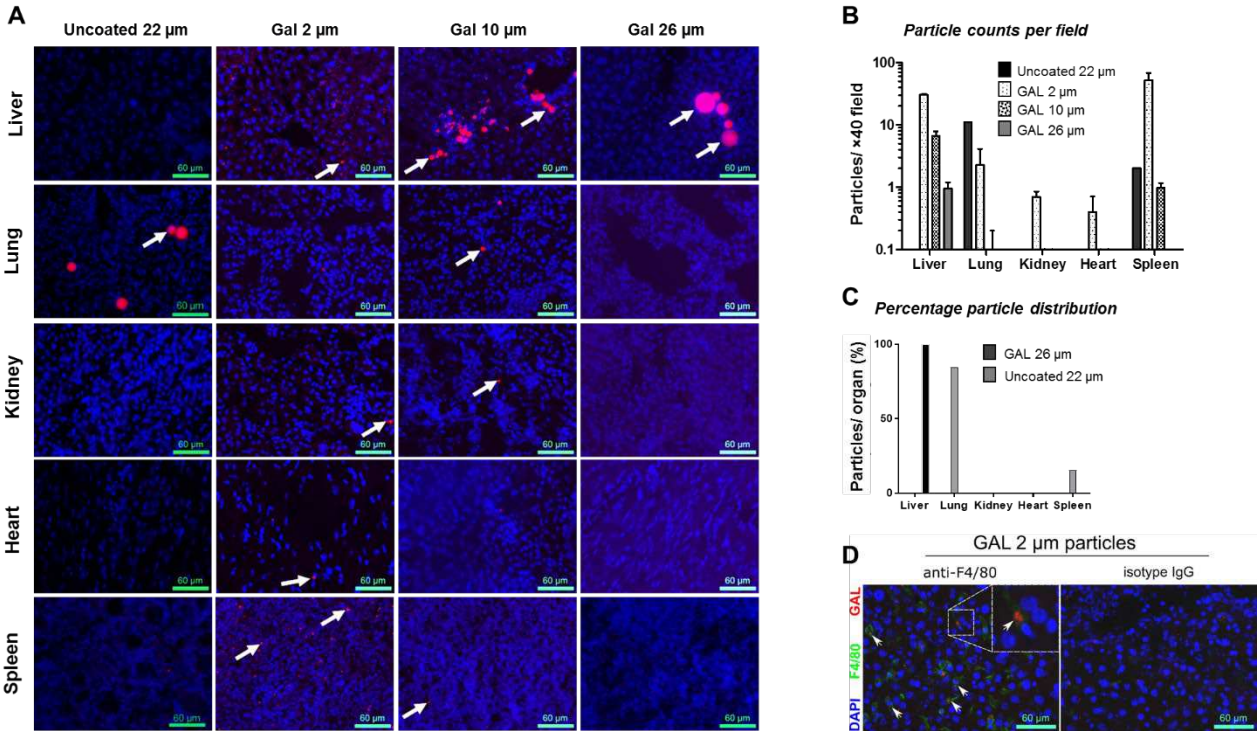
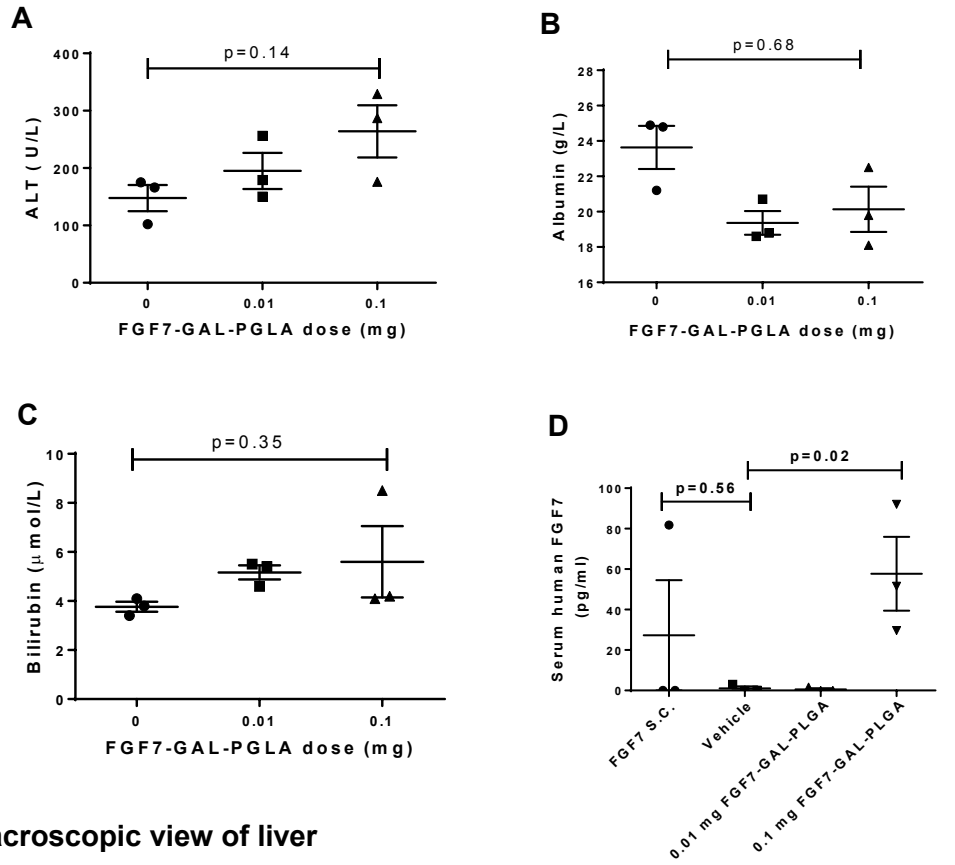
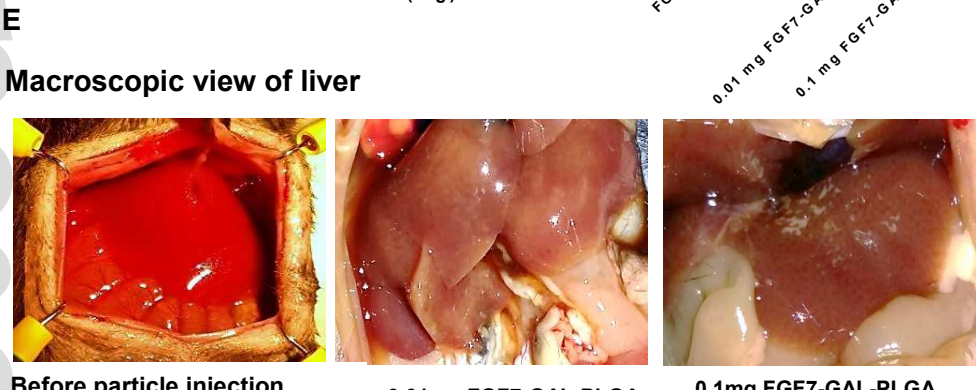


Fig. 4





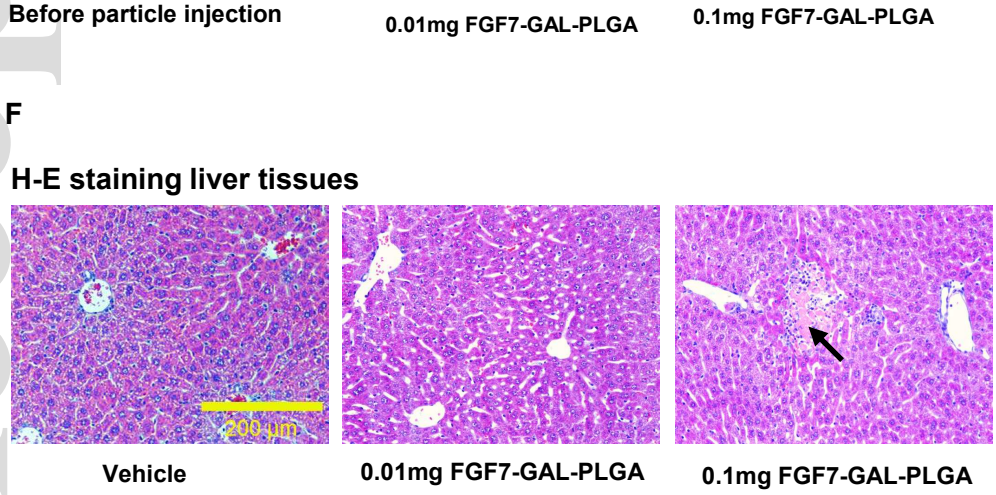


Fig. 5

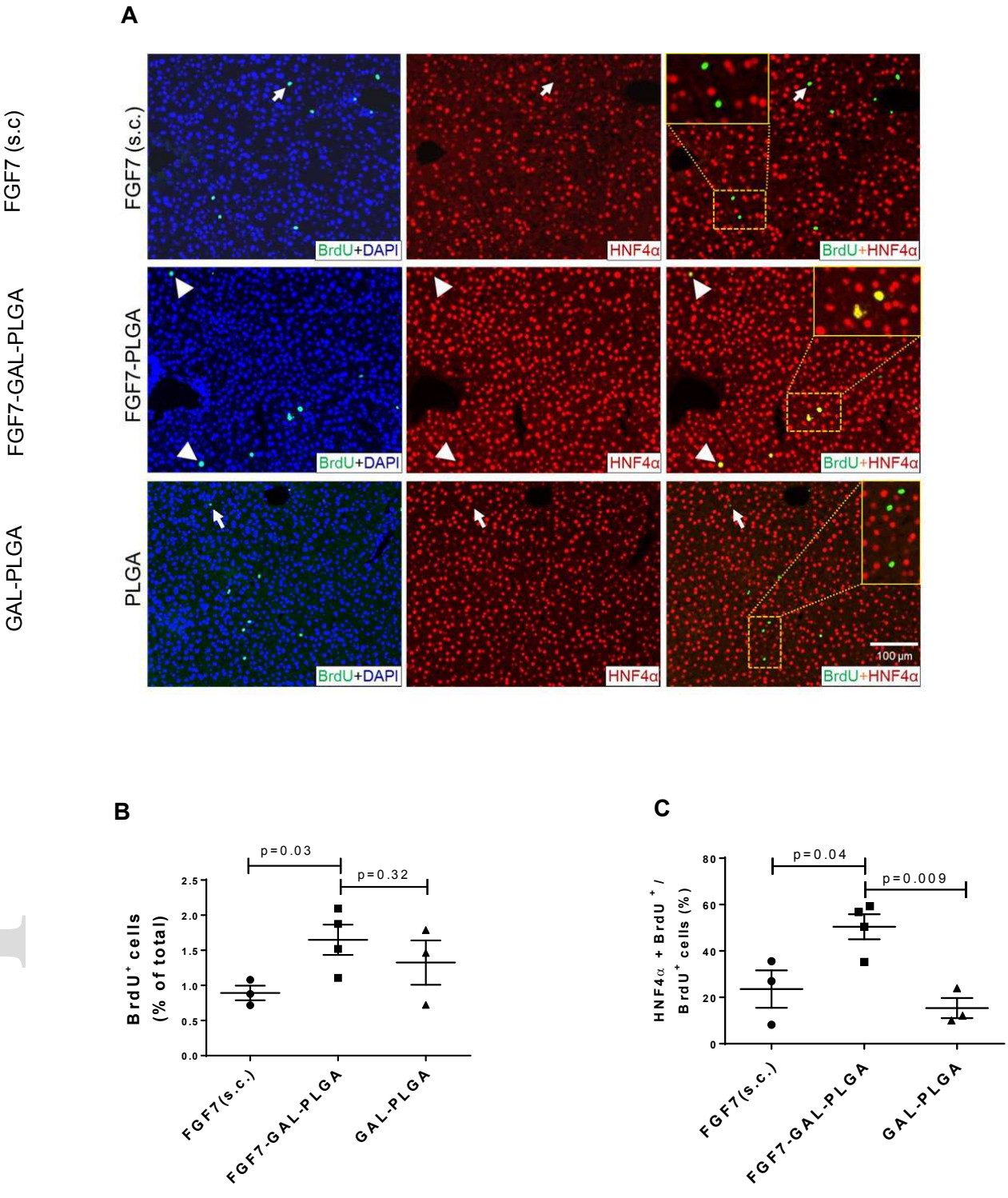
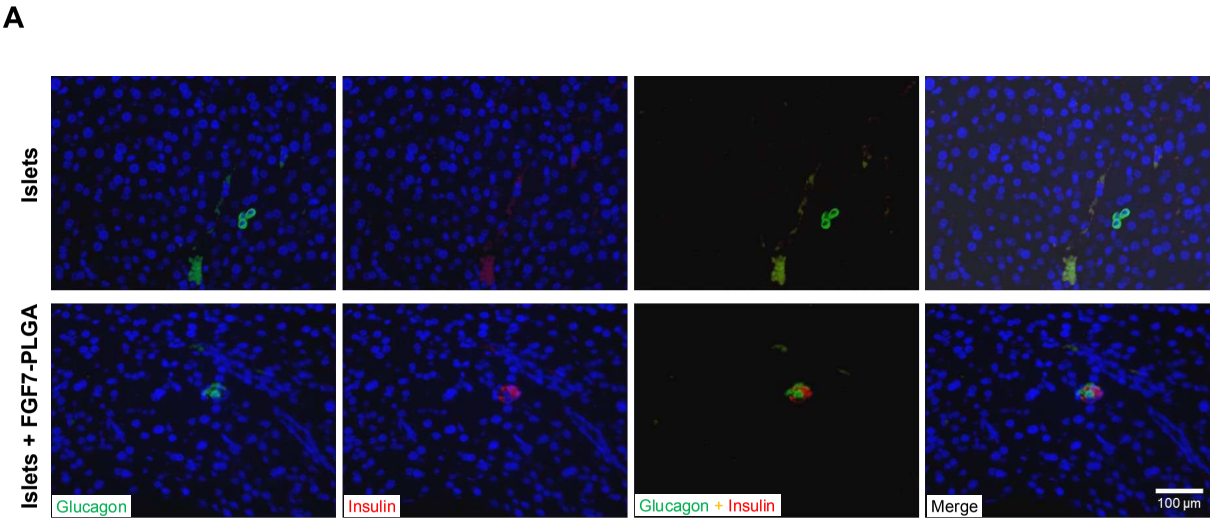


Fig. 6



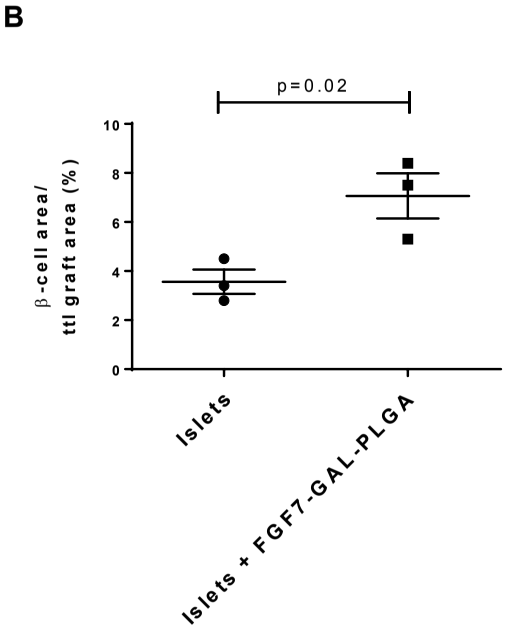
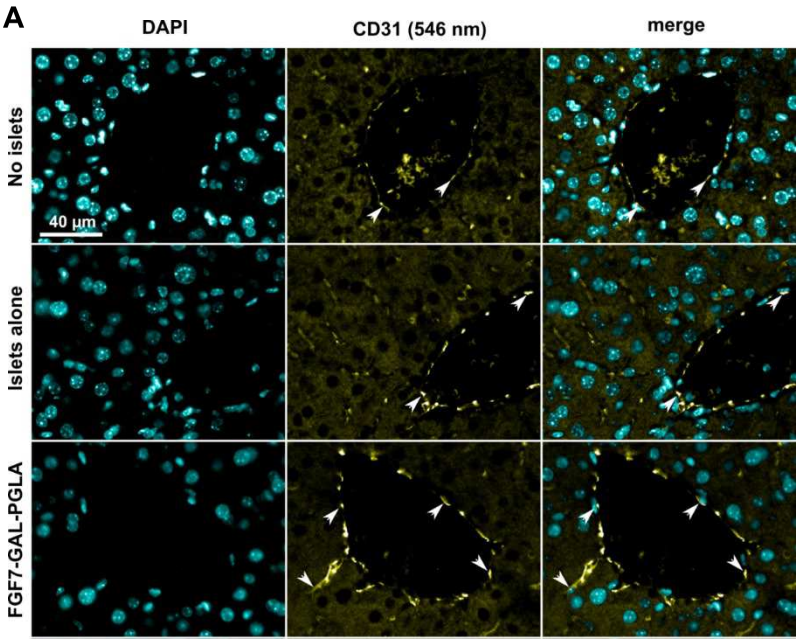
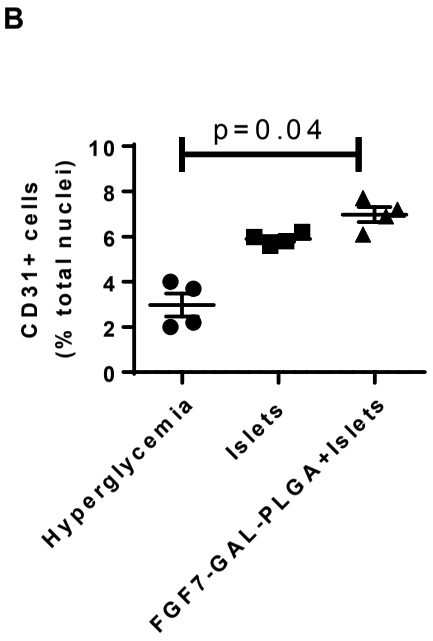
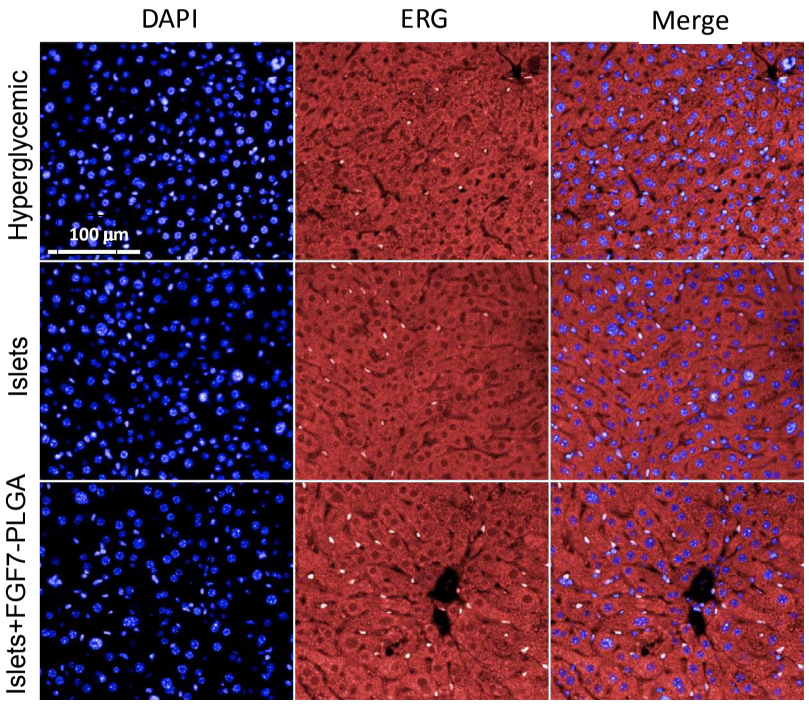


Fig 7





C



D

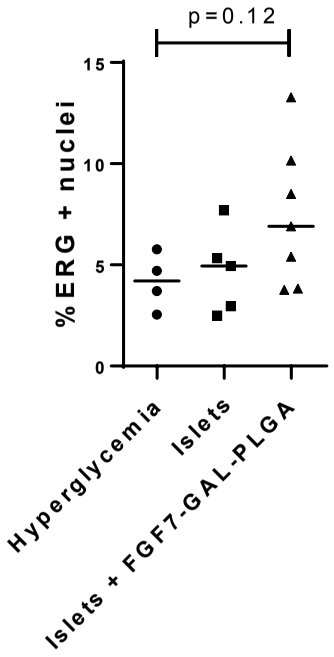


Fig. 8