The Cytoprotection of Chitosan Based Hydrogels in Xenogeneic Islet Transplantation: An *In Vivo* Study in Streptozotocin-Induced Diabetic Mouse

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ABSTRACT

Immune rejection and scarcity of donor tissues are the restrictions of islets transplantation. In this study, the cytoprotection of chitosan hydrogels in xenogeneic islet transplantation was demonstrated. Wistar rat islets encapsulated in chitosan hydrogels were performed glucose challenge test and live/dead cell staining *in vitro*. Islets/chitosan hydrogels were transplanted into the renal subcapsular space of diabetic C57BL/6 mice. Non-fasting blood glucose level (NFBG), body weight, intraperitoneal glucose tolerance test (IPGTT), and glucose disappearance rate were determined perioperatively. The serum insulin level was analyzed, and the kidney transplanted with islets/chitosan hydrogels were retrieved for histological examination after sacrifice. The present results showed that islets encapsulated in chitosan hydrogels secreted insulin in response to the glucose stimulation as naked islets with higher cell survival. The NFBG of diabetic mice transplanted with islets/chitosan hydrogels decreased from 487±46 to 148±32 at one day postoperation and maintained in the range of 201±36 mg/dl for four weeks with an increase in body weight. IPGTT showed the glucose disappearance rate of mice transplanted with islets/chitosan hydrogels was significant faster than that of mice transplanted with naked islets; the serum insulin level increased from 0.29±0.06 to 1.69±0.65 μg/dl postoperatively. Histological examination revealed that the islets successfully engrafted at renal subcapsular space with positive insulin staining. The immunostain was negative for neither the T-cell lineages nor the monocyte/macrophages. This study indicates that the chitosan hydrogels deliver and protect encapsulated islets successfully in xenotransplantation.

Keywords: Chitosan, Encapsulation, Hydrogels, Type 1 diabetes, Xenotransplantation

INTRODUCTION

Pancreatic islet transplantation normalizes the metabolic control of blood glucose to prevent the development of chronic complications is an efficacious treatment for type 1 diabetes [1]. Although limited donor sources prevent islet transplantation from becoming a major treatment option. A diabetic patient often requires two or even three donor pancreases to achieve successful transplantation which makes the problem of donor shortage even more serious [2]. Using xenogeneic species as donor sources may solve the problem of an insufficient donor supply [3]. However, xenogeneic islets are subject to immune rejection in humans because of the extensive antigenic differences [4]. Therefore, immune rejection still remains a major restriction to xenotransplantation.

Immunoisolation is an effective approach which can facilitate the use of xenogeneic cell source to solve the problem of insufficient donor supply, and can minimize or eliminate the need of systemic immunosuppression [5]. The approach is utilizing a semi-permeable barrier to enclose the transplanted cells which can protect enclosed cells from the attack of recipient's immune system. The enclosed cells can secrete therapeutic molecules and diffuse through the semi-permeable barrier to recipient's body. Several immunoisolative systems have been developed that include islets enclosed in a semi-permeable membrane [6], diffusion chamber [7], microencapsulation [8] and macroencapsulation system [9].

Injectable hydrogels is of great interest in cell encapsulation and tissue engineering [10]. Chitosan based hydrogels, a thermosensitive material composing of chitosan and glycerol 2-phosphate disodium salt hydrate, is liquid at room temperature and gelation as hydrogels at 37 °C [11]. The densely reticulate structure of hydrogels may serve as a barrier to protect encapsulated tissues. In addition, the

mass diffusion of small molecules within the hydrogels is fast due to the property of high water content [12]. Moreover, this hydrogels even has cytoprotective effect to against cytokine-mediated cytotoxicity [13]. These characteristics make the hydrogels as a promising material for immunoisolation.

In this study, a concordant animal model was applied to evaluate the cytoprotection of chitosan hydrogels in xenogeneic islet transplantation. Our hypothesis was that islets could be protected from the attack of recipient immune system when encapsulated in chitosan hydrogels and the assumption was demonstrated.

MATERIAL AND METHOD

Preparation of chitosan hydrogels

2.5 % chitosan (417963, Sigma, USA) solved in 0.1 M acetic acid (017-00256, Wako, Japan) was autoclaved for sterilization. Glycerol 2-phosphate disodium salt hydrate (β-GP, G6251, Sigma, USA) solved in deionized water (0.8 W/V) was filtered by a 0.22 μm filter (Millex-HA, Millipore, USA) for sterilization. The β-GP solution was added dropwise into the chitosan solution under stirring and until the pH value of the mixed solution became 7.4. The chitosan/β-GP solution was preserved in a 4 °C refrigerator before further study.

Islet isolation and evaluation

The animal experiment was reviewed and approved by the Institutional Animal Care and Use Committee for Frontier Medical Sciences, Kyoto University. Wistar rats (male, aged 9-10 weeks, Shimizu laboratory supplies, Japan) were used as donors.

Islet isolation was done according to methods in the previous study [14]. Briefly, through mid-line laparotomy, 10 ml of type XI collagenase solution (1200 CDU/ml, C9407, Sigma, USA) was infused into the common bile duct that legated at the hepatic side before the inflow to the duodenum. The pancreases were removed and digested in a water bath set at 37 °C for 18 mins. The digested pancreases were filtered by stainless steel mash to separate the islets, and purified by the discontinuous gradient solution (Dextran 70, 17-0280-02, Amersham, Sweden).

The purity of islet was assessed with dithizone staining (D5130, Sigma, USA) and >85 %. Islet quantity was determined as islet equivalents (IEQ) using a formula to convert islet populations of differing sizes to islet volume [15]. The yield of islet was 700-800 IEQ per rat. The harvested islets were cultured in CRML-1066 medium (11530, Gibco, USA) supplemented with 1 % antibiotic-antimycotic solution (15240-062, Gibco, USA) and 10 % fetal bovine serum (12103-78P, JRH, USA) in a incubator set at 5 % CO_2, 37 °C.

In vitro glucose challenge

Islets suspended in chitosan/β-GP solution at the density of 500IEQ/1000μl solution were injected into a 24-well culture plate; each well had 200 μl of chitosan solution with 100 IEQ. The culture plate was placed in a 37 °C incubator for 10 mins for gelation, and the CMRL-1066 medium was added into each well. After cultured for 1, 3 and 7 days, the medium was removed and the hydrogels with islets were washed twice by phosphate buffered saline (PBS); then 1 ml of CMRL-1066 medium with 3.3 mmole glucose was added and cultured for 1 hr. The medium was then replaced with CMRL1066 medium with 16.7 mmole glucose and further incubated for 1 hr. Finally, the medium was replaced with CMRL-1066 medium with 3.3 mmole

glucose and cultured for another 1 hr. At the end of each incubation period, the media were collected and analyzed by rat insulin enzyme-linked immunosorbent assay (rat insulin ELISA assay, AKRIN-010T, Shibayagi, Japan) with a microplate reader (iMark^{TM}, BIO-RAD, CA, USA) at the wavelength of 450 nm. The same quantity of IEQ without hydrogels was performed identical procedure as a control group. The static glucose stimulation index (SI) was calculated by dividing the insulin secretion from the high glucose incubation by the insulin secretion during low glucose incubation.

Live-dead staining and live/dead cell ratio

The islets encapsulated in chitosan hydrogels underwent live-dead staining (Live-Dead double staining kit, QIA76, Calbiochem, Merek, Darmstadt, Germany) to analyze the cell survival after being cultured for 7 days. After treatment, islets/chitosan hydrogels was observed with a fluorescent microscope (IX70, Olympus, Japan). The live/dead cell ratio was determined by dividing the number of live cells (green) by the sum of live (green) and dead cells (red). Six images of dependant islets were chosen randomly to estimate the average live/dead cell ratio. The control group was naked islets cultured in a 24-well culture plates for the same managements.

Recipient preparation and transplantation

C57BL/6 mice (Shimizu Laboratory Supplies, Japan), aged 9-10 weeks, weighing 23-25 g were used as recipients. Mice were induced to diabetic by single intraperitoneal injection of 190 mg/kg streptozotocin (S0130, Sigma, USA) solved in a pH=4.5 citrate buffer (09127-61, Nacalai, Japan) 14 days preoperatively. Mouse

with non-fasting blood glucose (NFBG) level higher than 400 mg/dl on two consecutive measurements was chosen as recipient.

The animal study was divided into four groups, the first one was normal mice (Normal mice group, n=8); the second group was diabetic mice without any treatment (Diabetic mice group, n=8); the third group was diabetic mice transplanted with naked islets (Naked islets group, n=8); the fourth group was diabetic mice transplanted with chitosan/β-GP solution containing islets (Islets/hydrogels group, n=12). Diabetic mice induced with STZ were randomly grouped into the second, third and fourth groups.

The operation was performed under general anesthesia of isoflurane inhalation. After adequate skin preparation and sterilization, a longitudinal skin incision was made on the lumbar dorsum of mouse, and the left kidney was exposed. For the third group, 500 IEQ suspended in 30 μl of PBS were transplanted into the renal subcapsular space. For the fourth group, 500 IEQ were suspended in 30 μl of chitosan/β-GP solution and transplanted. The kidney was then carefully placed back and the incision was closed with absorbable and non-absorbable sutures in turn. No immunosuppressive or anti-inflammatory therapies were treated after surgery. The NFBG of mice was measured by tail vein puncturing three times in the first week postoperatively and once a week thereafter for totally four weeks (DRI-CHEM 3000 with GLU-W, Fujifilm, Japan). The body weights of mice were recorded perioperatively.

Intraperitoneal glucose tolerance test

Intraperitoneal glucose tolerance test (IPGTT) was performed after overnight fasting at two weeks postoperation. A glucose solution (2 g/10ml/kg body weight) was intraperitoneally injected to induce hyperglycemia, and the blood glucose level

was measured every 15 mins for the first 60 mins and every 20 mins for the following 60 mins for totally 120 mins. Glucose disappearance rate (K value) for each group was calculated using the equation $K=70/t_{1/2}$, where $t_{1/2}$ is the time in minutes for the blood glucose level decrease to 50 % of its level at 15 mins [16].

Serum insulin level

Mice were sacrificed to collect blood samples by cardiac puncturing at four weeks postoperation. The serum was separated for the determination of insulin with a rat insulin ELISA assay.

Histological and immunohistochemical examination

The kidney transplanted with naked islets or islets/chitosan hydrogels was removed after sacrifice for histological examination. The sections were stained with hematoxylin and eosin (H&E, 3008-1&3204-2, Muto, Japan). For the immunohistochemical staining, sections were incubated with primary anti-insulin antibody (monoclonal anti-rat insulin antibody, Spring bioscience, USA). The localization of antigen was indicated by a red color with the aminoethylcarbazole (AEC, Histostain-Plus kit, Invitrogen, USA). Other sections were incubated with primary anti-CD3 antibody (CD3^{+} T-cell lineages, monoclonal anti-mouse CD3 antibody, eBioscience, USA) or anti-CD68 antibody (CD68^{+} monocyte/macrophages, monoclonal anti-mouse CD68 antibody, AbD serotec, USA) and indicated by a brown color with the 3, 3'-Diaminobenzidine (DAB, Histostain-Plus kit, Invitrogen, USA). Sections were further counter-stained with the hematoxylin and examined by optical microscope (BX51, Olympus, Japan). The pancreas of mouse was also harvested and performed the H&E and IHC staining.

Statistical analysis

Data was expressed as mean ± standard error of the mean (SEM). Statistical analyses of NFBG and body weight were analyzed by Two-way analysis of variance (ANOVA). *In vitro* glucose challenge test, live/dead cell survival, serum insulin level and *K* values of IPGTT were analyzed by One-way ANOVA. Difference was considered significant if *p*-value was less than 0.05.

RESULT

In vitro **glucose challenge**

The results of *in vitro* glucose challenge are shown in Fig. 1. Insulin secretion increased significantly in response to the stimulation of high level glucose followed by decreased to basal secretion in low glucose level for both the Naked islets group (SI=1.99±0.49) and Islets/hydrogels group (SI=2.18±0.23) after cultured for one day.

Naked islets group lost function to response the glucose stimulation progressively after cultured for 3 (SI=1.35±0.46) and 7 days (SI=1.29±0.33). The SI on day 3 and 7 of Naked islets group were significant lower than that of day 1 (*p<0.05*). On the contrary, islets encapsulated in chitosan hydrogels maintained the function of insulin secretion and responded to the glucose stimulation. The static glucose stimulation index of Islets/hydrogels group was 2.28±0.14 for day 3 and 1.89±0.23 for day 7, respectively; that was significant higher than that of Naked islets group (*p<0.05*).

Live-dead staining and live/dead cell ratio

Fig. 2 is the live-dead staining for the islets encapsulated in chitosan hydrogels. The cell survival ratio for islets encapsulated in hydrogels (Fig. 2(a), 84.2±4.9 %) was significant higher than that of naked islets (Fig. 2(b), 28.9±7.3 %) (n=6, *p>0.01*).

Animal study

The NFBG of Naked islets group decreased from 472±34 to 163±40 mg/dl one day post-operatively and maintained in the range of 160-213 mg/dl for 3 days (Fig. 3(a)). However, it returned to 311±110 mg/dl at day 7 and restored to hyperglycemic status thereafter. The NFBG of Islets/hydrogels group decreased from 487±56 to 148±32 mg/dl and maintained in the range of 148-242 mg/dl for four weeks with a significant difference (*p<0.05*) with that of Naked islets group or Diabetic mice group.

The changes in body weights are shown in Fig. 3(b). The body weights of Naked islets group decreased from 24.3±1.4 to 18.7±1.2 g that did not has a significant difference with that of Diabetic mice group (*p>0.05*). The body weight of Islets/hydrogels group increased progressively from 21.8±1.3 to 24.8±1.0 g which was significantly higher than that of Naked mice group (*p<0.01*).

Intraperitoneal glucose tolerance test

Fig. 3(c) shows the blood glucose variation for the IPGTT. Mice of Islets/hydrogels group showed significantly lower blood glucose level when compared with that of Naked islets group. The *K* value of Normal mice group was 0.85±0.11; that of Naked islets group could not be calculated since the blood glucose level did not decreased to 50% of its level at 15 mins nor the decreasing fitting line was not obtained in the group. The *K* value of Islets/hydrogels group was 0.76±0.25

which has no significant different with that of Normal mice group (*p>0.05*).

Serum insulin level

The serum insulin level of Normal mice group was 1.97±0.16 μg/dl; and that of Diabetic mice group has a significantly lower level (0.29±0.06 μg/dl, *p<0.05*, Fig. 3(d)). The level of Naked islets group was 0.31±0.04 μg/dl which did not differ from that of Diabetic mice group (*p>0.05*). The insulin level of Islets/hydrogels group was 1.69±0.65 μg/dl which was higher than that of Diabetic mice and Naked islets group significantly (*p<0.01*); although it was lower than the level of Normal mice group, there was no significant difference between them (*p>0.05*).

This study was not designed to compare the mortality of each group; however, one mouse found dead one week post-operatively, one mouse dead at week three and other two mice dead at week four for Naked islets group. All mice in Islets/hydrogels group were survival during this study.

Histological examination

The histological sections of Islets/hydrogels group revealed that the islets grafted at the renal subcapsule space of mouse (Fig. 4(a), H&E, 40X). The immunohistochemical staining showed the islets had insulin production (Fig. 4(b), red color, immunostain of insulin antibody, 100X). The sections stained for CD3 or CD68 antibody show no evidence of immune cell infiltration or accumulation (Fig. 4(c), immunostain of CD3 antibody, 40X; Fig. 4(d), immunostain of CD68 antibody, 100X). Pancreases harvested from the diabetic mice contained shrunken islets with a decreased number of islets per section. Most islets were dissociated from the surrounding acinar tissues and negative for insulin staining (data not shown).

DISCUSSION

The cytoprotection of chitosan hydrogels for xenogeneic islet transplantation is demonstrated in this study. Insulin secretion of islets encapsulated in chitosan hydrogels was tested first. Islets encapsulated in chitosan hydrogels sensitized the glucose stimulation and secreted insulin to response as naked islets at day one. The insulin content of Islets/hydrogels group was similar to that of Naked islets group. This result indicates the insulin secreting function of islets was not influenced by the chitosan hydrogels. Islets encapsulated in hydrogels still maintained the insulin secreting at day 3 and 7; however, the naked islets lost function gradually. The lost of secreting function can be contributed to that islets require extracellular matrix for survival and function, and this matrix is destroyed during the isolation procedure. Therefore, the hydrogels serves as an extracellular matrix that potentially increases islet survival and function [17]. This assumption is further proved by the results of live/dead ratio that reveals islets encapsulated in hydrogels had higher survival.

Regarding the results of the animal study, the NFBG of mice decreased to euglycemia and maintained for 7 days after naked islet transplantation. However, it returned to hyperglycemia which attributed to the rejection of grafts [18]. The NFBG of mice transplanted with islets/hydrogels also decreased to euglycemia one day postoperatively indicating the transplanted islets within hydrogels quickly released insulin into diabetic mice. Euglycemia was maintained for four weeks proving the continuous function of transplanted islets. Concerning the naked islets rejected within one week, these results indicate that chitosan hydrogels protects islets from the recipient's immune system efficaciously.

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Body weight is a common indicator for type 1 diabetes [19]. The body weight of diabetic mice transplanted with islets/chitosan hydrogels increased gradually. On the contrary, the mice transplanted with naked islets lost weight continuously in a similar fashion to that of the diabetic mice. Increased body weight indicates recovery of the blood glucose metabolism of diabetic mice, through it still lower than normal mice. The *K* value of glucose disappearance rate can be considered as an indicator to monitor the function of transplanted islets [20]. *K* value for the Islets/hydrogels group was much better than that of Naked islets group, and similar to the Normal mice group without a significant difference. These results suggest that the function of transplanted islets/hydrogel was comparable with native pancreatic islets.

The sustaining insulin secretion of transplanted islets/hydrogels was further confirmed by the detection of serum insulin. The serum insulin level of mice decreased significantly after injection of STZ that reveals the native islets were destroyed completely. The diabetic mice transplanted with naked islets also had similar insulin level that reveals the transplanted islets lost its function at this time point. The serum insulin level of diabetic mice transplanted with islets/hydrogels increased significantly that means transplanted islets compensated for the native pancreatic islets and the insulin level was comparable with normal mice.

In this study, STZ-induced diabetic mice had high mortality (4/8, 50 %) that was also reported in other experiences [21]. The mortality of Naked islets group was 50 % (4/8), which was higher than that of Islets/chitosan hydrogels group (0 %, 0/12). This finding might indicate that hydrogels protected islets effectively to improve the metabolism of severe diabetics that can cause about 50% mortality. All of above results shall be attributed to the transplanted islets in hydrogels releasing insulin continually to compensate the endocrine dysfunction of the pancreas in diabetic mice.

The histological sections reveal that the islets grafted at the renal subcapsular space; the transplanted islets were well-granulated with insulin production indicating the viability and maintained function of the transplanted islets. The immunostain was negative for neither the T-cell lineages (CD3$^+$) nor the monocyte/macrophages (CD68$^+$), two common infiltrating cells for implantable biomaterials [22]. This cytoprotection of chitosan hydrogels may attribute to the glycerol moieties in β-GP have positive charges which may interfere with infiltrating cells [23]. Another possible mechanism is the interactions of chitosan-chitosan hydrophobic which expected to play a major role in the gelation process, the hydrophobic property of chitosan hydrogels may also against infiltrating cells [24].

Although several kinds of gels are applied in cell encapsulation, such as agarose, alginate, PVA and some derivatives, chitosan gel with the thermosensitive property is an injectable carrier. In spite of encapsulated islets may suspend in an isotonic solution and inject into animal's body, the chitosan hydrogels with proper viscosity shall facilitate the manipulation. In addition, it's easier to remove the islet/chitosan hydrogels when compared with the microencapsulated islets since the microspheres may disperse widely and difficult to be retrieved. Furthermore, compared with the implantable BAPs such as membrane forms or diffusion chambers, the injectable islets/chitosan solution could be transplanted with a minimal invasive surgery under fluoroscopic guidance. Since the chitosan is a well-known and safe biomaterial, this hydrogels could be an alternative for immunoisolation in the autoimmune animal models, large mammals and, eventually, could be applied safely for clinical application. Further studies, such as the longevity of transplanted islets and a discordant animal model, should be demonstrated to ensure the application is suitable for treating type 1 diabetes.

CONSLUSION

Islets encapsulated chitosan hydrogels responded glucose stimulation to secrete insulin as naked islets. The NFBG of diabetic mice transplanted with islets/chitosan hydrogels decreased to euglycemia with a progressive increased in body weight. The performances of transplanted islets extended from 7 days to four weeks when chitosan hydrogels applied. IPGTT shows that the glucose disappearance rate of mice transplanted with islets/chitosan hydrogels was faster than that transplanted with naked islets, the serum insulin level also increased postoperatively. Histological sections reveal the islets grafted at renal subcapsular space with positive insulin staining; the immunostain was negative for neither the T-cell lineages nor the monocyte/macrophages. This study indicates that the chitosan hydrogels deliver and protect encapsulated islets successfully in xenotransplantation.

REFERENCE

[1] H. Ichii, C. Ricordi, Current status of islet cell transplantation. J. Hepatobiliary Pancreat. Surg. 16 (2009) 101-112.

[2] E.A. Ryan, J.R. Lakey, R.V. Rajotte, G.S. Korbutt, T. Kin, S. Imes, A. Rabinovitch, J.F. Elliott, D. Bigam, N.M. Kneteman, G.L. Warnock, I. Larsen, A.M. Shapiro, Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. Diabetes 50 (2001) 710-719.

[3] D.A. MacKenzie, D.A. Hullett, H.W. Sollinger, Xenogeneic transplantation of porcine islets: an overview. Transplantation 76 (2003) 887-891.

[4] N. Tonomura, A. Shimizu, S. Wang, K. Yamada, V. Tchipashvili, G.C. Weir, Y.G. Yang, Pig islet xenograft rejection in a mouse model with an established human immune system. Xenotransplantation 15 (2008) 129-135.

[5] A.S. Narang, R.I. Mahato, Biological and biomaterial approaches for improved islet transplantation. Pharmacol. Rev. 58 (2006) 194-243.

[6] S. Sakai, T. Ono, H. Ijima, K. Kawakami, *In vitro* and *in vivo* evaluation of alginate/sol-gel synthesized aminopropyl-silicate/alginate membrane for bioartificial pancreas. Biomaterials 23 (2002) 4177-4183.

[7] K.C. Yang, C.C. Wu, S.S. Lin, S. Sumi, F.H. Lin, The *in vivo* performance of bioartificial pancreas in bone marrow cavity: A case report of a spontaneous diabetic feline. Biochem. Biophys. Res. Commun. (2010) doi:10.1016/j.bbrc.2009.12.152.

[8] S. Bohman, A.J. King, Islet alpha cell number is maintained in microencapsulated islet transplantation. Biochem. Biophys. Res. Commun. 377 (2008) 729-733.

[9] Z. Qi, Y.N. Shen, G. Yanai, K.C. Yang, Y. Shirouzua, Y. Hiura, S. Sumi. The *in vivo* performance of polyvinyl alcohol macro-encapsulated islets. Biomaterials (2010) in press.

[10] L. Yu, J. Ding, Injectable hydrogels as unique biomedical materials. Chem. Soc. Rev. 37 (2008) 1473-1481.

[11] J. Berger, M. Reist, A. Chenite, O. Felt-Baeyens, J.M Mayer, R. Gurny, Pseudo-thermosetting chitosan hydrogels for biomedical application. Int. J. Pharm. 288 (2005) 197-206.

[12] J.L. Drury, D.J. Mooney, Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 24 (2003) 4337-4351.

[13] K.C. Yang, C.C. Wu, F.H. Lin, Z. Qi, Y.H. Cheng, M.P. Chen, S. Sumi, Chitosan/gelatin hydrogels as immunoisolative matrix for injectable bioartificial

pancreas. Xenotransplantation 15 (2008) 407-416.

[14] N. Sakata, Y. Gu, M. Qi, C. Yamamoto, A. Hiura, S. Sumi, M. Sunamura, , S. Matsuno, K. Inoue, Effect of rat-to-mouse bioartificial pancreas xenotransplantation on diabetic renal damage and survival. Pancreas 32 (2006) 249-257.

[15] T. Yamamoto, A. Horiguchi, M. Ito, H. Nagata, H. Ichii, C. Ricordi, S. Miyakawa, Quality control for clinical islet transplantation: organ procurement and preservation, the islet processing facility, isolation, and potency tests. J. Hepatobiliary Pancreat. Surg. 16 (2009) 131-136.

[16] R.P. Robertson, Consequences on beta-cell function and reserve after long-term pancreas transplantation. Diabetes 53 (2004) 633-644.

[17] N. Nagata, Y. Gu, H. Hori, A.N. Balamurugan, M. Touma, Y. Kawakami, W. Wang, T.T. Baba, A. Satake, M. Nozawa, Y. Tabata, K. Inoue, Evaluation of insulin secretion of isolated rat islets cultured in extracellular matrix. Cell Transplant .10 (2001) 447-451.

[18] Z. Qi, C. Yamamoto, N. Imori, A. Kinukawa, K.C. Yang, G. Yanai, E. Ikenoue, Y. Shen, Y. Shirouzu, A. Hiura, K. Inoue, S. Sumi, Immunoisolation effect of polyvinyl alcohol (PVA) macro-encapsulated islets in type 1 diabetes therapy. Cell Transplant. (2010) in press.

[19] I.R. Sweet, O. Yanay, L. Waldron, M. Gilbert, J.M. Fuller, T. Tupling, A. Lernmark, W.R. Osborne, Treatment of diabetic rats with encapsulated islets. J. Cell Mol. Med. 12 (2008) 2644-2650.

[20] J. Jin, Y. Yu, H. Yu, C. Wang, X. Zhang, Effects of pioglitazone on beta-cell function in metabolic syndrome patients with impaired glucose tolerance. Diabetes Res. Clin. Pract. 74 (2006) 233-241.

[21] M. Perez-Basterrechea, R.M. Briones, M. Alvarez-Viejo, E. Garcia-Perez, M.M.

Esteban, V. Garcia, A.J. Obaya, L. Barneo, A. Meana, J. Otero, Plasma-Fibroblast Gel as Scaffold for Islet Transplantation. Tissue Eng. Part A 15 (2009) 569-577.

[22] L. Inverardi, N.S. Kenyon, C. Ricordi, Islet transplantation: immunological perspectives. Curr. Opin. Immunol. 15 (2003) 507-511.

[23] C. Jarry, J.C. Leroux, J. Haeck, C. Chaput, Irradiating or autoclaving chitosan/polyol solutions: effect on thermogelling chitosan-beta-glycerophosphate systems. Chem. Pharm. Bull. (Tokyo) 50, (2002) 1335-1340.

[24] M. Lavertu, D. Filion, M.D. Buschmann, Heat-induced transfer of protons from chitosan to glycerol phosphate produces chitosan precipitation and gelation. Biomacromolecules 9 (2008) 640-650.

Figure Legend

Fig. 1(a) Insulin secretion increased significantly in response to the stimulation of high level glucose followed by decreased to basal secretion in low glucose level for both the Naked islets group (SI=1.99±0.49) and Islets/hydrogels group (SI=2.18±0.23) after cultured for one day. (b) Naked islets lost function to response the glucose stimulation at day 3 (SI=1.35±0.46) and (c) 7 days (SI=1.29±0.33). Islets encapsulated in chitosan hydrogels maintained the function of insulin secretion and responded to the glucose stimulation during this test.

Fig. 2 The green color represents live cell and the red color represents dead cell. The cell survival ratio for (a) islets encapsulated in hydrogels was higher than that of (b) naked islets.

Fig. 3(a) The NFGB of Islets/chitosan hydrogels group was lower than that of Diabetic mice group and Naked islets group significantly; and (b) body weights of Islets/chitosan hydrogels group was significantly higher than that of Diabetic mice group and Naked islets group. (c) The IPGTT test showed that the *K* value of Normal mice group was 0.85±0.11; that of Naked islets group could not be calculated. The *K* value for Islets/hydrogels group was 0.76±0.25 that has no significant different with that of Normal mice group (*p>0.05*). (d) The serum insulin level Diabetic mice group was lower than that of Normal mice group significantly (*p<0.01*). The level of Islets/hydrogels group was significantly higher than Naked islets group (*p<0.01*), and no significant difference with Normal nice group(*p>0.05*).

Fig. 4 The histologic sections of Islets/hydrogels group reveal that (a) the islets were

grafted at the renal subcapsule space of mouse (H&E, 40X). (b) Immunohistochemical staining shows the islets had positive insulin staining (red color, immunostain of insulin, 100X). Sections stained with antibody specific to immune cells show there was no immune cell infiltration or accumulation. (c) Negative of CD3^{+} T-cell lineages (40X); (d) Nor of CD68^{+} monocyte/macrophages (100X).

Glucose challenge (day 1)

Glucose challenge (day 3)

Glucose challenge (day 7)
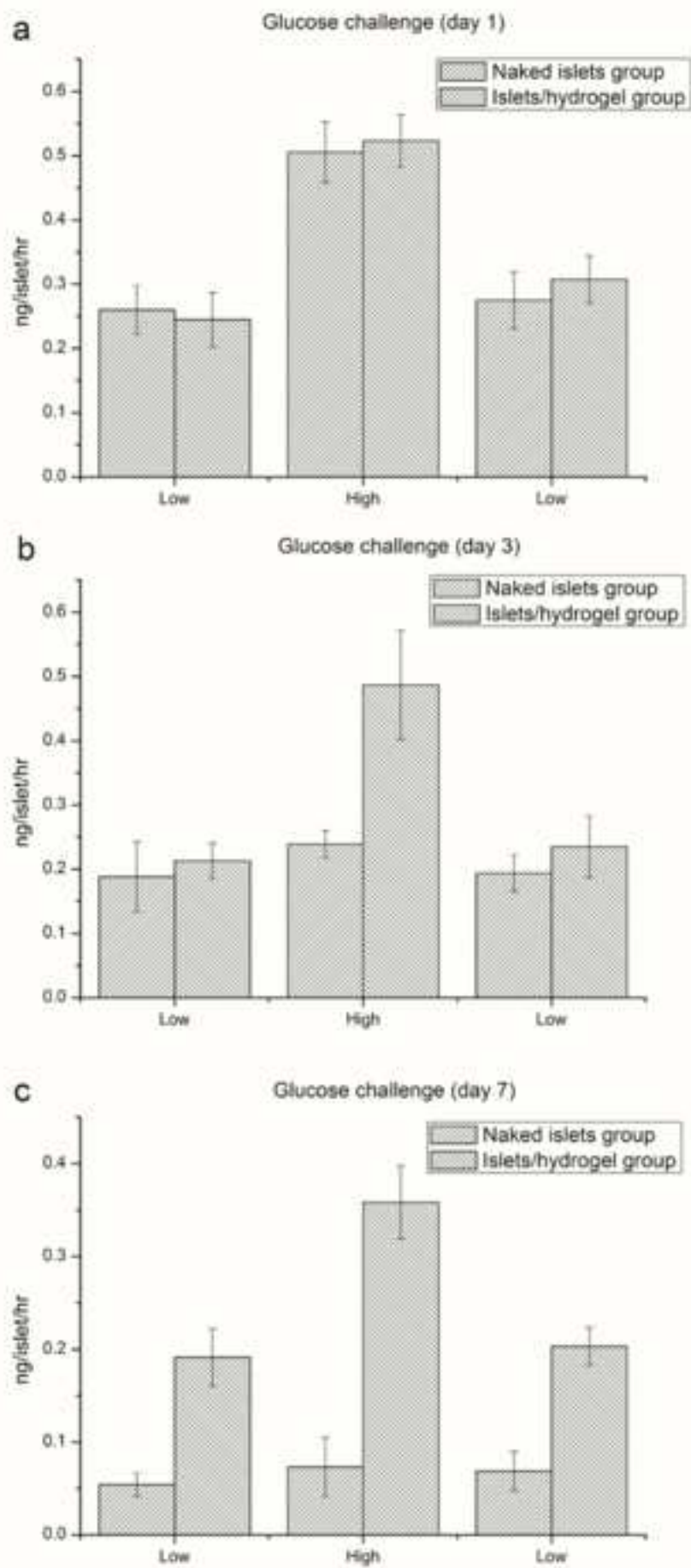
Figure 2
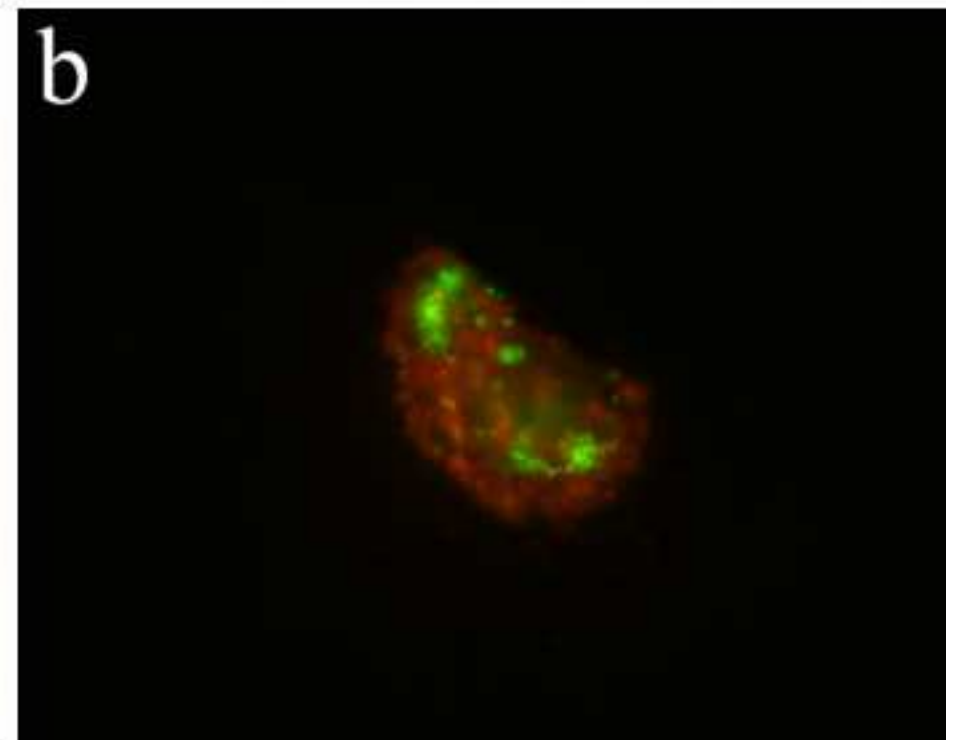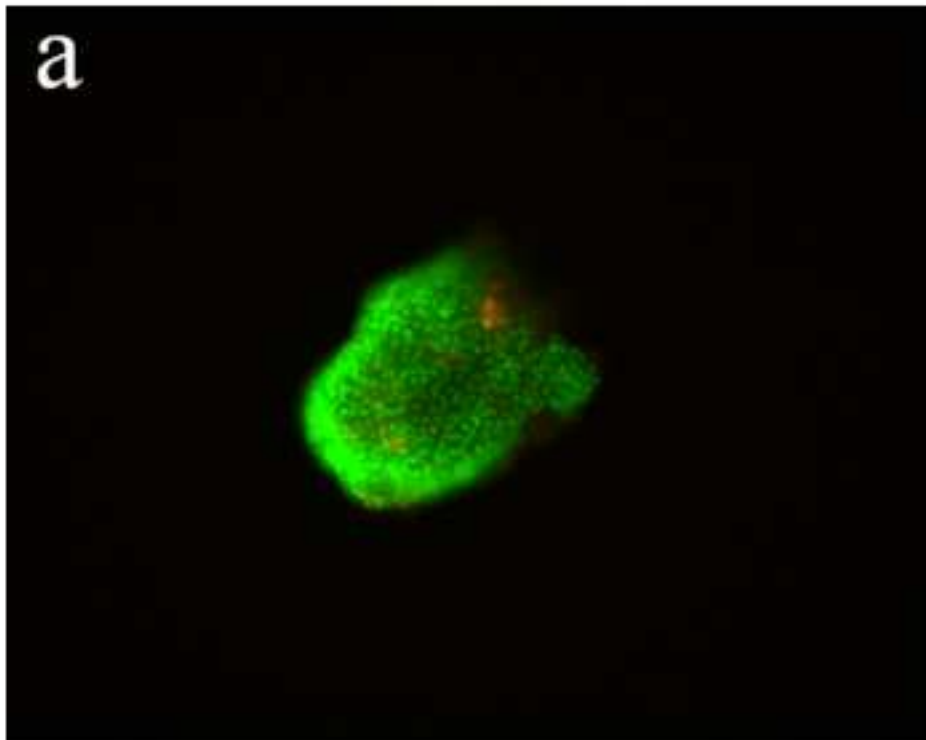**Click here to download high resolution image**
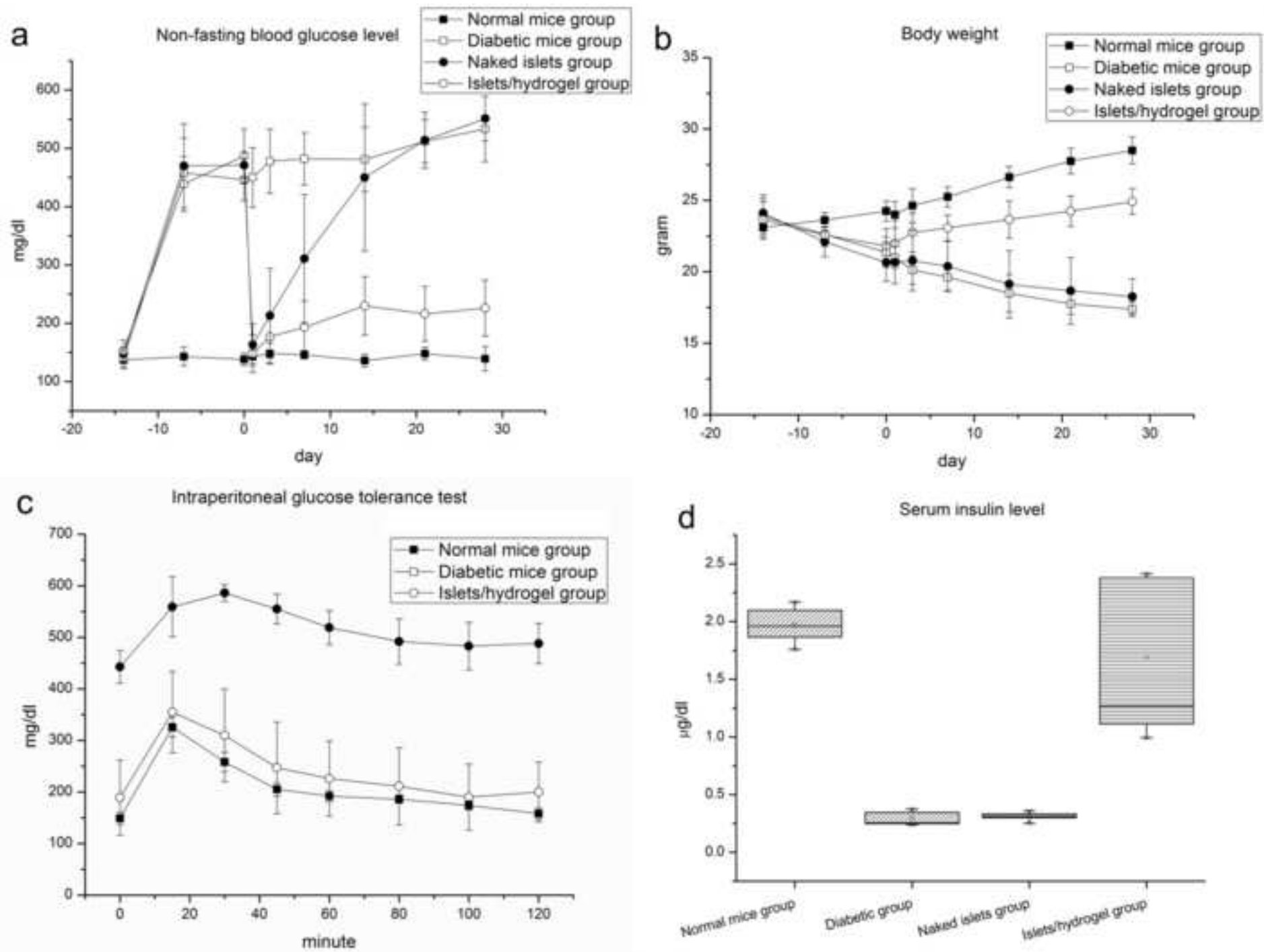
Figure 3
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Figure 4
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