Biochemical and Biophysical Research Communications 463 (2015) 1084–1090

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Collagen esterification enhances the function and survival of pancreatic β cells in 2D and 3D culture systems

Jae Hyung Ko [a, 1], Yang Hee Kim [a, b, 1], Seong Hee Jeong [b], Song Lee [b], Si-Nae Park [a], In Kyong Shim [b, **], Song Cheol Kim [b, c, *]

[a] Regenerative Medicine Research Center, Dalim Tissen Co., LTD., 383-93, Yonnam-Dong, Mapo-gu, Seoul, South Korea
[b] Asan Institute for Life Science, 388-1 Pungnap-2 Dong, Songpa-gu, Seoul, South Korea
[c] Department of Surgery, University of Ulsan College of Medicine & Asan Medical Center, 388-1 Pungnap-2 Dong, Songpa-gu, Seoul, South Korea

ARTICLE INFO

Article history:
Received 4 June 2015
Accepted 9 June 2015
Available online 12 June 2015

Keywords:
Extracellular matrix
Islet
Diabetes
Esterified collagen

ABSTRACT

Collagen, one of the most important components of the extracellular matrix (ECM), may play a role in the survival of pancreatic islet cells. In addition, chemical modifications that change the collagen charge profile to a net positive charge by esterification have been shown to increase the adhesion and proliferation of various cell types. The purpose of this study was to characterize and compare the effects of native collagen (NC) and esterified collagen (EC) on β cell function and survival. After isolation by the collagenase digestion technique, rat islets were cultured with NC and EC in 2 dimensional (2D) and 3 dimensional (3D) environments for a long-term duration *in vitro*. The cells were assessed for islet adhesion, morphology, viability, glucose-induced insulin secretion, and mRNA expression of glucose metabolism-related genes, and visualized by scanning electron microscopy (SEM). Islet cells attached tightly in the NC group, but islet cell viability was similar in both the NC and EC groups. Glucose-stimulated insulin secretion was higher in the EC group than in the NC group in both 2D and 3D culture. Furthermore, the mRNA expression levels of glucokinase in the EC group were higher than those in the NC group and were associated with glucose metabolism and insulin secretion. Finally, SEM observation confirmed that islets had more intact component cells on EC sponges than on NC sponges. These results indicate that modification of collagen may offer opportunities to improve function and viability of islet cells.

1. Introduction

Type I diabetes is caused by autoimmune destruction of insulin-secreting pancreatic β cells, which play a critical role in glucose metabolism [1,2]. For islet transplantation to be considered successful, the functional efficiency of the transplanted islet graft must be retained. However, islet isolation procedures can disrupt the extracellular matrix (ECM) around islets and the internal microvasculature in the isolated islet, leading to apoptosis and early graft failure [3,4]. Previous studies show that collagens can have a significant impact on β-cell development and function [5–9]. Other studies have shown that hydrogels composed of collagen can promote β-cell proliferation [10] or maintain long-term islet viability and insulin secretion [5,11–13].

Chemical collagen modifications, such as acetylation, succinylation, esterification can alter the net charge and structure of collagen molecules by masking primary amino groups or carboxyl groups within the molecule. Esterification of collagen molecules induced by methylation or ethylation allows collagen molecules to have a net positive charge, and the degree of esterification of collagen affects the ability of cells to attach and proliferate as shown in baby hamster kidney cells, rat pheochromocytoma cells, and human hepatocellular carcinoma cell models [14,15]. These positive surface charges may attract oppositely charged cellular membrane proteins, thereby mediating the attachment of cells. To the best of our knowledge, there has been no previous report on the effects of positively charged collagens on islet survival or function. In our present study, we investigated the effect of positively charged collagen on long-term culture of islet cells and on islet

* Corresponding author. Department of Surgery, University of Ulsan College of Medicine & Asan Medical Center, 388-1 Pungnap-2 Dong, Songpa-gu, Seoul, South Korea.
** Corresponding author.
E-mail addresses: shimiink@gmail.com (I.K. Shim), drksc@amc.seoul.kr (S.C. Kim).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.06.062
0006-291X/

adhesion, release of insulin, and mRNA expression levels of genes related to glucose metabolism. In addition, we performed seeding of islets onto biocompatible 3D scaffold to verify the improvement in the functional capacity of islets.

2. Materials and methods

2.1. Islet isolation and culture

Pancreatic islets of inbred Lewis rats (Charles River Laboratory, Yokohama, Japan) were isolated by a collagenase type XI (Sigma, St Louis, MO) digestion method and cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS; Invitrogen), 100 U/mL penicillin, and 100 U/mL streptomycin (Invitrogen). All animal experiments were performed according to the guidelines of the Institute of Laboratory Animal Resources, Asan Institute for Life Sciences, Asan Medical Center (Seoul, Republic of Korea; No. 2011-13-193).

2.2. Preparation of NC or EC-coated plates and NC or EC sponges

Type I atelo-collagen (Dalimtissen Co. Ltd., Seoul, Korea) was used in this study. For esterification of collagen, lyophilized atelocollagen was immersed and agitated in ethanol, and this collagen suspension was then neutralized with 0.5M NaOH and centrifuged. The pellet was subsequently dissolved in autoclaved distilled water, dialyzed, and freeze-dried. Next, 24-well plates (BD, Franklin Lakes, NJ) were coated with 300 μL of 1.5% (w/v) NC and EC. NC or EC sponges were prepared by lyophilization of 1.5% (w/v, pH 7.4) EC and NC solution, respectively. Freeze-dried collagen sponges were cross-linked with 200 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in 95% ethanol for 24 h. Rinsed EC and NC sponges were freeze-dried and kept at −4 °C until further use.

2.3. Surface charge measurement of NC or EC

The zeta (ζ) potential is the electrostatic potential that exists at the shear plane of a particle and is related to both surface charge and the local environment of the particle. NC and EC were homogenized at 1 mg/mL in ethanol (Merck) using an automated homogenizer (Precellys 24, Bertin Technologies, Bretonneux, France) before zeta potential analysis. The determination of the ζ potential of NC and EC was performed using a ζ potential analyzer (Zetaplus, Brookhaven Instruments Corporation, Holtsville, NY) in three replicates.

2.4. Cell adhesion assay

To determine the effect of collagen manipulation on the strength of adhesion, islets were seeded onto collagen-coated culture dishes. Analysis was then performed after 24 h of culture using a centrifugation assay [16]. In this assay, the attached islets are subjected to a centrifugal force of 500 rcf for 2 min.

2.5. Cell proliferation assay

We investigated the effects of the positively charged collagen on the growth of anchored (L929 and rat MSC) and unanchored (K562) cells. After 3 days or 1 week of culture, 20 μL of 5 mg/mL 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dissolved in PBS was added to each well and the cells were incubated for 4 h at 37 °C and 5% CO_2. Crystallized formazan was dissolved by adding 1 mL of DMSO with gentle tapping after the removal of culture medium. Aliquots of 200 μL of dissolved formazan were transferred onto a 96-well plate and the absorbance at 540 nm was measured.

2.6. Islet viability assay

The viability of intact islets in NC or EC was assessed using fluorescein diacetate and propidium iodide (FDA/PI, Sigma) staining under a fluorescence microscope (TCS SP2, Leica, Wetzlar, Germany). Briefly, FDA and PI stock solutions were added to the sample at a final concentration of 0.67 and 75 μM, respectively. After 30 s of incubation, the sample was placed under fluorescence microscopy. Dead cells were stained red, and viable cells were stained green. Islet single cell viability was determined by a colorimetric MTT assay.

2.7. Glucose challenge test of islets

To exclude insulin added in the culture medium, cultured cells were washed three times with PBS, and subsequently washed with Krebs–Ringer buffer. Krebs–Ringer buffer with a low glucose concentration (3.3 mM) was added and the cells cultured for 1 h. This low glucose buffer was then collected, and the cells washed with Krebs–Ringer buffer. Krebs–Ringer buffer with a high glucose concentration (20 mM) was added and the cells cultured for 1 h. This high glucose buffer was also collected. The collected buffers were used to measure insulin concentrations by ELISA (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions.

2.8. Quantification of mRNA using real-time RT-PCR

To evaluate the expression of specific genes in the cultured islets, mRNA levels were evaluated by RT-PCR. Following RNA extraction, as per the manufacturer's protocol, cDNA was reverse transcribed using an Omniscript® RT kit (Qiagen, Mississauga, ON). Quantitative real–time PCR (Applied Biosystems SYBR Green Master Mix kit) was used to explore the expression levels of insulin, glucagon, pancreatic and duodenal homeobox 1 (PDX-1), glucokinase (GCK), sulfonylurea receptor 1 (SUR1), and glucose transporter 2 (GLUT2) genes, which are critical for glucose metabolism. Data were calculated using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference.

2.9. SEM analysis of islets in NC or EC sponges

Approximately 100 islets were loaded onto EC or NC sponges and cultured for 2 weeks. The sponges were fixed in Karnovsky's fixation solution (2% glutaraldehyde, 2% paraformaldehyde, and 0.5% $CaCl_2$) for 6 h and washed 3 times with 0.1M PBS. Samples were postfixed with 1% OsO_4 in PBS for 1 h and then washed with 0.1M PBS. The samples were dehydrated through a graded series of ethanol and critical-point dried. Dried samples were than cross-sectioned and coated with an ultrathin layer (300 Å) of gold/Pt in an ion sputter (E1010, Hitachi, Tokyo). The ultrastructure of islets was observed using a scanning electron microscope (S-800, Hitachi).

2.10. Statistical analysis

Data are presented as the mean ± the standard error of the mean (SEM) of three independent experiments. One way ANOVA with Fisher's LSD method was used with $^{*}p < 0.05$ indicating a statistically significant difference.

3. Results

3.1. ζ potential measurement of NC and EC

The zeta potential is the electric potential across all phase boundaries between solids and liquids and is widely used for the

quantification of an electric charge. The surface charges of NC and EC, as measured by zeta potential analyzer, are shown in Fig. 1A. The zeta potential of NC and EC were 19.51 ± 0.93 mV and 28.38 ± 1.19 mV, respectively ($p < 0.05$). Although surface charge modification can improve cell interaction and proliferation [17], this process simultaneously changes the characteristics of biological materials and consequently their biocompatibility.

3.2. Effect of EC on proliferation of various cell types

To determine the effect of modified collagen on the proliferation of various cell types, anchored and unanchored cells were seeded onto NC and EC-coated plates. After culturing for the indicated periods of time, the cells were analyzed by MTT assay. The results indicated that all cultured cells proliferated over time. After 3 days of culture, L929 cells exhibited a higher proliferation rate when cultured on EC than when cultured on NC ($p < 0.05$). In contrast, there was no significant difference in the proliferation rate of rat MSCs grown on EC or NC on day 3. However, after one week of culture, rat MSCs showed a higher proliferation rate when cultured on EC than on NC. K562 cells, which are anchorage independent, showed no statistically significant difference in proliferation rate according to culture conditions (Fig. 1B).

3.3. Effect of EC on islet adhesion

Several studies have provided insight into the integrin/ECM interactions that induce appropriate signaling for islet cell adhesion, proliferation, and differentiation. We studied the link between islet-substrate interactions and islet functionality in this study. To determine the effect of EC on the strength of adhesion, islets were seeded on NC or EC. Cells were analyzed after 24 h of culture using a centrifugation assay. As shown in Fig. 1C, culture on NC resulted in stronger islet adhesion than culture on EC. Our present data suggest that strong adhesion may affect insulin secretion.

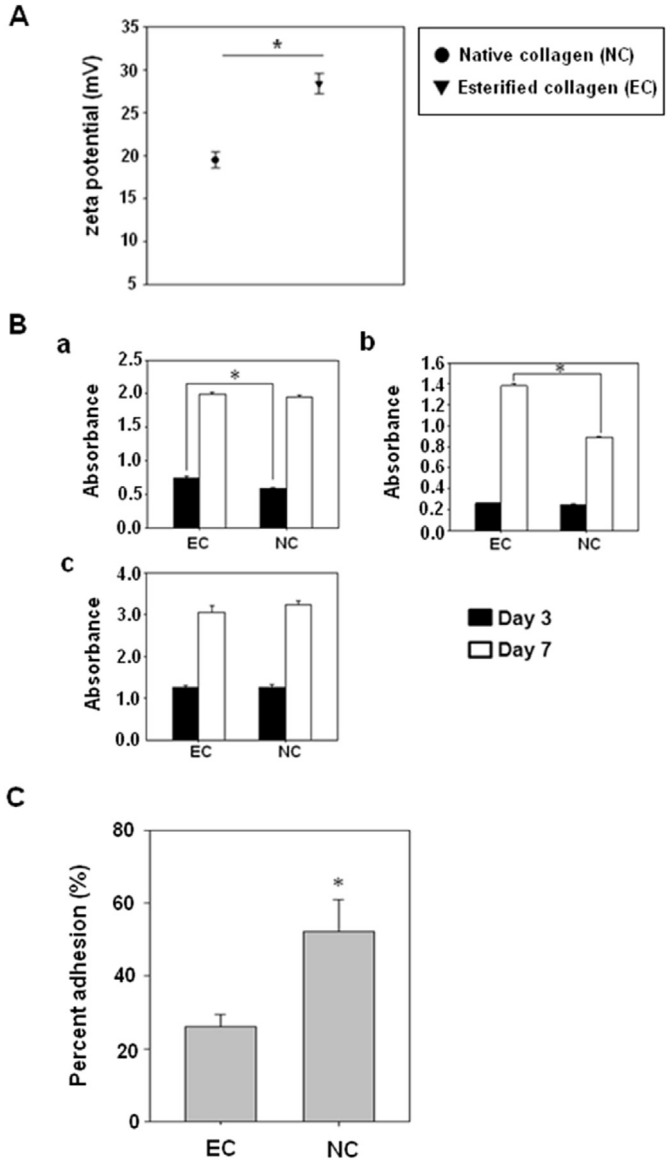

Fig. 1. (A) Zeta potential analysis of NC and EC. NC and EC were treated using an automated homogenizer and tested using a ζ potential analyzer. (B) Effect of EC on the proliferation of L929 cells (a), rat MSCs (b), and K562 cells (c). L929 cells and rat MSCs showed a high proliferation rate in EC group at day 3 and one week, respectively. (C) Percent adhesion of islets to different collagen-coated surfaces (*$P < 0.05$).

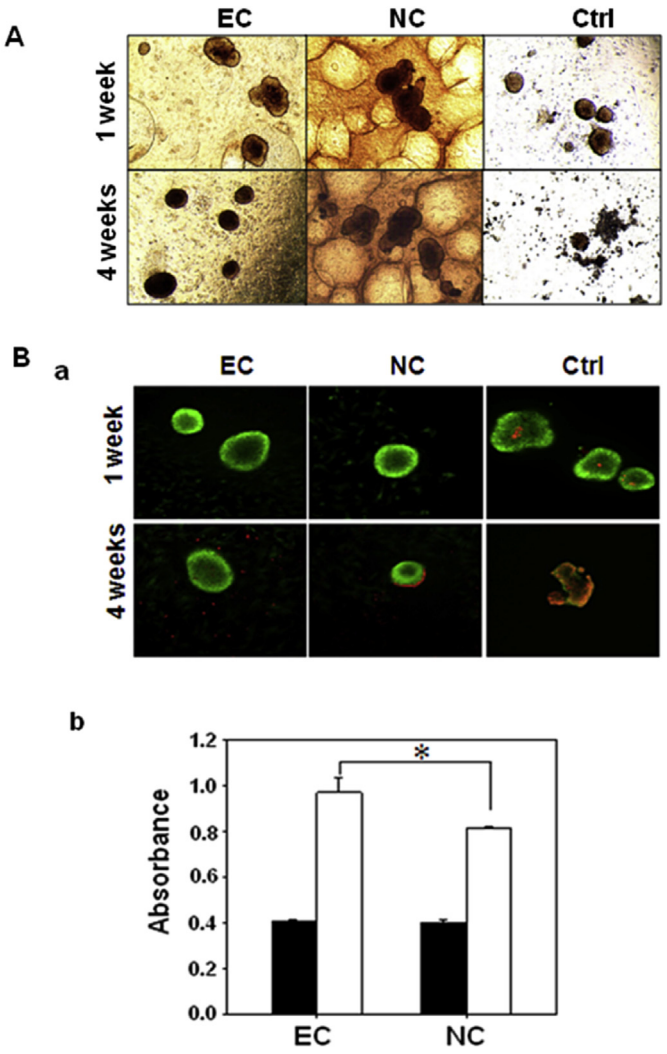

Fig. 2. Morphology (A) of rat islets cultured for 1 week and 4 weeks on an EC, NC, and non-treated (Ctrl) plate. (B) Confocal microscopy of islets cultured on EC or NC. The viability of the intact islet was investigated by FDA/PI staining after 1 week and 4 weeks of culture (a) and the viability of dissociated islet cells (1×10^5) was tested at 3 days and 7 days by an MTT assay (b) (*$P < 0.05$).

3.4. Morphological studies and viability of islets cultured on EC

The morphology and viability of islets cultured on EC, NC, or non-coated plates for 4 weeks were examined by FDA/PI staining. Fluorescent images demonstrated that rat islets cultured in EC maintained their intact shape and viability over 4 weeks of culture (Fig. 2A and Ba). However, in the control (non-coated) group, an increasing number of dead nuclei were detected within the islets after 1 week and 4 weeks. In addition, confocal microscopy of islets loaded onto EC sponges after 14 days of 3D culture showed green fluorescence (viable islets), which was intensively emitted along the edge and surface of islet clusters, whereas few dead islets were detected. In contrast, incomplete and damaged islets were observed in the NC sponge cultures (Fig. 2Bb). Thus, islet morphology and viability appears to be maintained during long-term culture on EC.

3.5. Functionality and related gene expression of islets cultured on EC

Although rat islets showed high viability at 1 week in both the EC and NC groups, the function of these islets was significantly different between the groups. The average SI values were 2.88 ± 0.5 and 4.05 ± 0.6 for islets in the EC after 1 week and 4 weeks, respectively. However, the average SI values over the same time points were 1.69 ± 0.2 and 2.03 ± 0.3, respectively, for the NC group and 2.1 ± 0.6 and 1.8 ± 0.2, respectively, for the control group (Fig. 3A). Thus, the average SI values after 4 weeks were approximately two-fold more in the EC group than in the NC and control groups in 2D culture.

We also analyzed the insulin release capabilities of 3D cultured islets at 1 week and 4 weeks of culture. At all time points tested, islets cultured on EC sponges exhibited enhanced insulin release compared to islets cultured on NC sponges. At 1 week, the stimulation indices for glucose-stimulated insulin secretion from islets cultured on EC sponges were significantly higher than those for islets cultured on NC sponges ($p < 0.05$). After 4 weeks in culture, the stimulation indices for islets cultured on EC sponges were 3-fold greater than those for islets cultured on NC sponges. In EC, a more significant difference in insulin functionality was seen for islets cultured under 3D conditions for 4 weeks (Fig. 3B).

To identify the genes that contributed to the functionality of islets cultured on collagen, we performed RT-PCR analysis for representative genes that play a role in islet functionality (insulin and glucagon), as well as insulin production and release (GCK, SUR1, GLUT2, and PDX-1) genes. As shown in Fig. 3C, GCK, insulin,

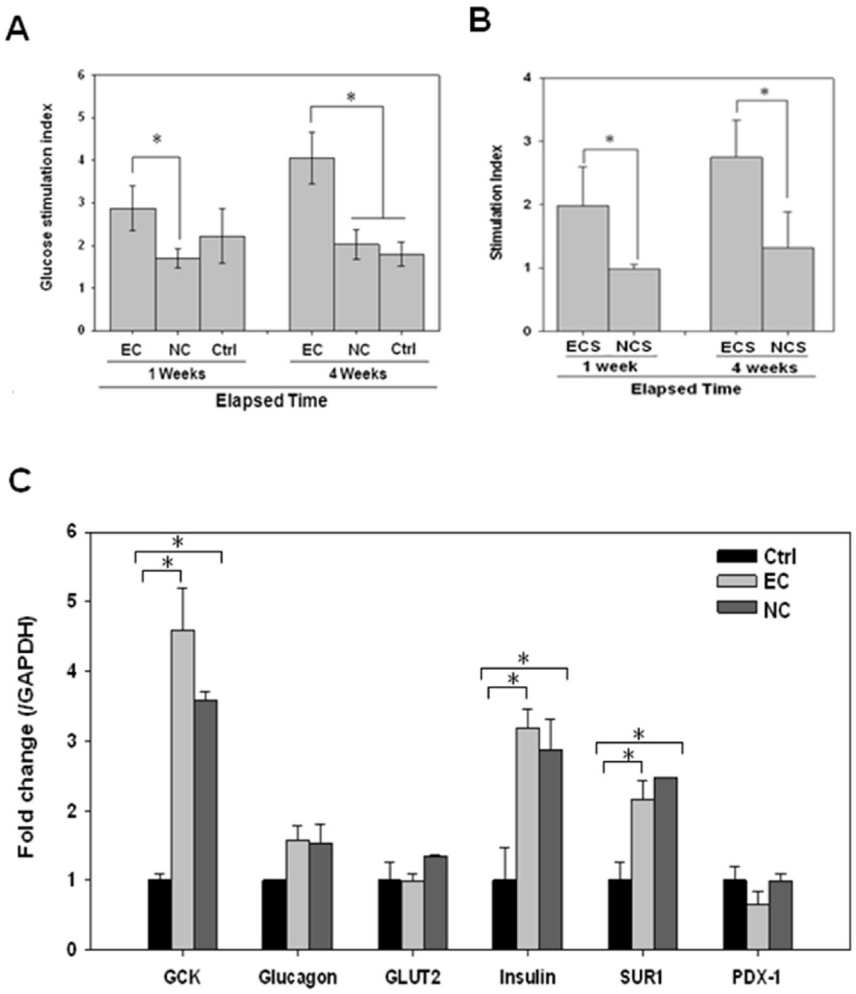

Fig. 3. Functionality and related gene expression of islets cultured on EC. In EC group, a more significant difference in insulin functionality was seen for islets cultured under 2D (A) and 3D (B) conditions for 4 weeks. A stimulation index was calculated as the ratio of stimulated to basal insulin secretion. (C) Relative gene expression levels of islets cultured on EC or NC after 1 week of culture. The gene expression levels of insulin, glucagon, PDX-1, GLUT2, GCK, and SUR1 were evaluated relative to the GAPDH gene expression levels and normalized (*$P < 0.05$).

and SUR1 gene expression levels were significantly elevated on NC and EC-coated plates compared to the control. Among these genes, the mRNA expression level of GCK, which is associated with glucose metabolism and insulin secretion, was higher on EC than on NC. GCK is a major glucose sensor in β-cells for glucose-stimulated insulin secretion [18] and regulates insulin release by maintaining glucose homeostasis [19], which may explain the high insulin release in islets cultured on EC.

3.6. SEM observation of islets loaded onto EC sponges

To evaluate the effects of 3D support provided by the EC sponges, the morphology of islets cultured on sponges were analyzed using SEM. As shown in Fig. 4, morphological analysis under SEM at day 14 demonstrated that most islets on EC sponges had relatively smoother surfaces with well-defined microvilli (black arrows) than those on NC sponges. On NC sponges, partially destroyed islet cells (white arrows) with rough surfaces and no trace of microvilli were observed.

4. Discussion

Pancreatic islet transplantation has gained attention for the treatment of type I diabetes. However, most transplanted islet grafts fail during the peritransplant period [20]. One of the main reasons for this failure is the disruption of the islet-ECM microenvironment during islet isolation, which leads to reduced islet function and loss of islet viability [21]. Thus, efforts to improve islet survival and function with the ECM have been made, leading to enhanced viability and glucose-stimulated insulin secretion [5,12,22–25]. Lucas-Clerc et al. reported that collagen increases viability and glucose-stimulated insulin secretion of human pancreatic islets over the long term [5].

Several studies have now reported that charged surfaces can affect cell adhesion and proliferation depending on the cell type [26–28]. In our current study, we chemically modified carboxyl groups and made the net charge of collagen more positive, confirming these modifications by zeta potential analysis. EC showed a higher mV value than NC, indicating that esterification increases the positive net charge of collagen molecules. To determine the effect of these positively charged collagens on cell proliferation, we used L929, rat MSCs, and K562 cells and measured proliferation by MTT assay. Our results indicated that L929 cells and rat MSCs, which are anchorage-dependent cells, were more proliferative on EC than on NC. In contrast, EC had no effect on the proliferation of K562 cells, which are anchorage-independent cells.

We next investigated the effect of positively charged collagen on the adhesion of islets compared to NC. NC promoted islet adhesion more strongly than EC. Strong adhesion may cause a loss of 3D islet morphology, eventually leading to the expansion of islets into a monolayer of cells that express little or no insulin [29]. Indeed, islets cultured on EC maintained their intact shape and viability in 4 weeks of culture. However, in our control (non-coated) group, an increasing number of dead nuclei were detected within the islet after 1 week and 4 weeks. In addition, confocal microscopy of islets loaded onto EC sponges after 14 days of 3D culture showed evidence of live islets as shown by green fluorescence, which was intensively emitted along the edge and surface of islet clusters, whereas few dead islets were detected. In contrast, incomplete and

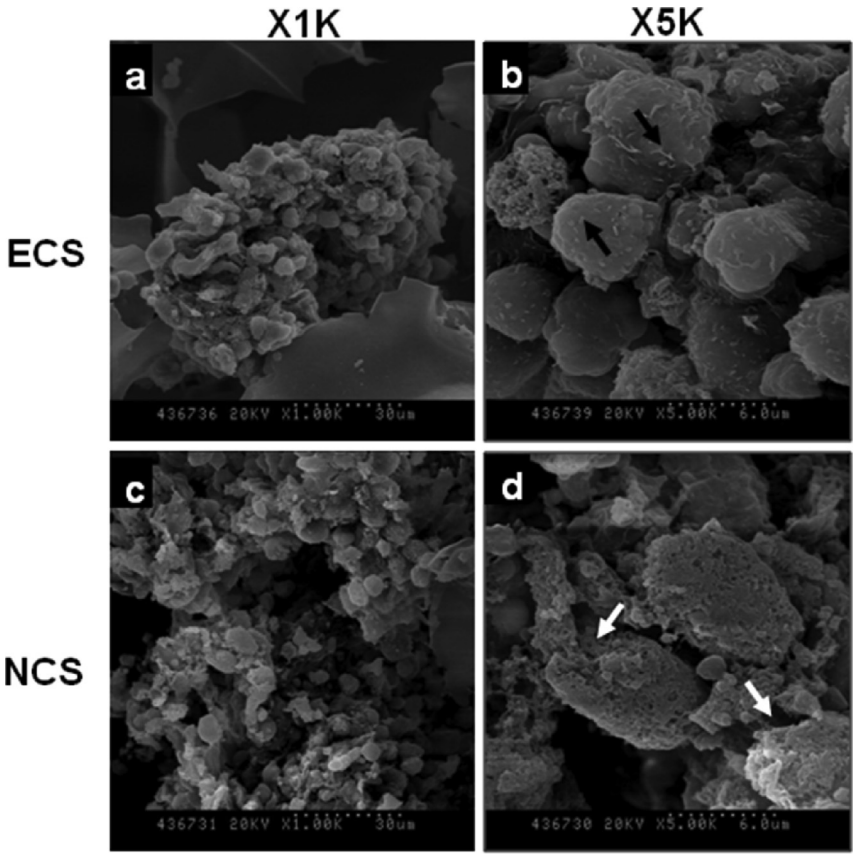

Fig. 4. SEM images of islets loaded on EC (ECS; a-b) and NC (NCS; c-d) sponges. Islets were loaded on EC or NC sponges and cultured for 2 weeks. The ultrastructure of islets was observed by scanning electron microscope (S-800, HITACHI).

damaged islets were observed in the NC sponge cultures, which suggested that both 2D and 3D cultures are capable of maintaining islet viability on EC. Krol et al. have revealed that encapsulated islets with a positive net charge exhibited glucose-stimulated insulin secretion, while islets encapsulated with a negative net charge showed glucose-independent insulin secretion. Our current results demonstrated that cells grown on positively charged collagen showed prolonged survival and enhanced function of islets, which is consistent with previous findings [30]. Released insulin concentrations under high glucose stimulation were similar for both the EC and NC groups, with no statistically significant difference between them. However, the SI of the NC group was approximately 1 for all assays. Islets cultured on NC sponges are assumed to maintain insulin secretion, but it was not a manner of glucose dependent way. By contrast, in the EC group, the SI was >1 in all assays. More importantly, glucose-stimulated insulin secretion in the EC group was maintained for up to 4 weeks, whereas much lower levels of insulin secretion were noted in the NC and control groups, suggesting that EC is an appropriate environment for islet viability and function.

Daoud et al. reported previously that type I collagen-cultured islets showed significantly higher insulin gene expression levels, and that the levels of GLUT2 and GCK, which are related to glucose metabolism, were highest on collagen I-coated surfaces [25]. Our present data also indicated that GCK, insulin, and SUR1 gene expression levels were significantly elevated on NC and EC compared to the control. Among these genes, the mRNA expression level of GCK, which is associated with glucose metabolism and insulin secretion, was higher on EC than on NC. GCK is a major glucose sensor in β-cells for glucose-stimulated insulin secretion [18] and regulates insulin release by maintaining glucose homeostasis [19], which may explain the high level of insulin release in islets cultured on EC.

Finally, our SEM images of islets grown on EC or NC sponge revealed the ultrastructure of the islets and islet endocrine cells. Islets on the sponges were examined as clusters of endocrine cells without an external surface, so that individual endocrine cells were identifiable. Interestingly, the majority of endocrine cells on EC sponges had relatively smoother surfaces than cells on NC sponges.

In conclusion, both EC and NC surfaces are capable of enhancing islet survival, but the addition of NC had no significant effect on glucose-stimulated insulin release over a 4-week culture. In contrast, islets cultured on EC maintained glucose-stimulated insulin secretion as well as improved islet survival in both 2D and 3D culture. These data indicate that positively charged collagen modification enhances the function and viability of 2D and 3D culture systems for pancreatic islet cells.

Financial support and competing interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by grants from the Korea Health Technology R&D Project (HI12C0433).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.062.

References

[1] A.N. Balamurugan, R. Bottino, N. Giannoukakis, C. Smetanka, Prospective and challenges of islet transplantation for the therapy of autoimmune diabetes, Pancreas 32 (2006) 231–243.
[2] R.P. Robertson, C. Davis, J. Larsen, R. Stratta, D.E. Sutherland, Pancreas and islet transplantation for patients with diabetes, Diabetes Care 23 (2000) 112–116.
[3] R.N. Wang, S. Paraskevas, L. Rosenberg, Characterization of integrin expression in islets isolated from hamster, canine, porcine, and human pancreas, J. Histochem Cytochem 47 (1999) 499–506.
[4] J.C. Stendahl, D.B. Kaufman, S.I. Stupp, Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation, Cell. Transpl. 18 (2009) 1–12.
[5] C. Lucas-Clerc, C. Massart, J.P. Campion, B. Launois, M. Nicol, Long-term culture of human pancreatic islets in an extracellular matrix: morphological and metabolic effects, Mol. Cell. Endocrinol. 94 (1993) 9–20.
[6] T. Meyer, S. Czub, I. Chodnewska, U. Beutner, W. Hamelmann, G. Klock, U. Zimmermann, A. Thiede, K. Ulrichs, expression pattern of extracellular matrix proteins in the pancreas of various domestic pig breeds, the Goettingen Minipig and the Wild Boar, Ann. Transpl. 2 (1997) 17–26.
[7] R. Montesano, P. Mouron, M. Amherdt, L. Orci, Collagen matrix promotes reorganization of pancreatic endocrine cell monolayers into islet-like organoids, J. Cell. Biol. 97 (1983) 935–939.
[8] R. Perfetti, T.E. Henderson, Y. Wang, C. Montrose-Rafizadeh, J.M. Egan, Insulin release and insulin mRNA levels in rat islets of Langerhans cultured on extracellular matrix, Pancreas 13 (1996) 47–54.
[9] C.H. Thivolet, P. Chatelain, H. Nicoloso, A. Durand, J. Bertrand, Morphological and functional effects of extracellular matrix on pancreatic islet cell cultures, Exp. Cell. Res. 159 (1985) 313–322.
[10] P. Metrakos, S. Yuan, S.J. Qi, W.P. Duguid, L. Rosenberg, Collagen gel matrix promotes islet cell proliferation, Transpl. Proc. 26 (1994) 3349–3350.
[11] 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. Transpl. 10 (2001) 447–451.
[12] N.A. Nagata, K. Inoue, Y. Tabata, Co-culture of extracellular matrix suppresses the cell death of rat pancreatic islets, J. Biomater. Sci. Polym. Ed. 13 (2002) 579–590.
[13] S.H. Chao, M.V. Peshwa, D.E. Sutherland, W.S. Hu, Entrapment of cultured pancreas islets in three-dimensional collagen matrices, Cell. Transpl. 1 (1992) 51–60.
[14] G. Reid, S. Gorham, J. Lackie, The attachment, spreading and growth of baby hamster kidney cells on collagen, chemically modified collagen and collagen-composite substrata, J. Mater. Sci. Mater. Med. 4 (1993) 201–209.
[15] J. Zhang, H.P. Wei, C.H. Quek, S.M. Chia, H. Yu, Quantitative measurement of collagen methylation by capillary electrophoresis, Electrophoresis 25 (2004) 3416–3421.
[16] C.D. Reyes, A.J. Garcia, A centrifugation cell adhesion assay for high-throughput screening of biomaterial surfaces, J. Biomed. Mater. Res. A 67 (2003) 328–333.
[17] Y. Zhu, C. Gao, X. Liu, J. Shen, Surface modification of polycaprolactone membrane via aminolysis and biomacromolecule immobilization for promoting cytocompatibility of human endothelial cells, Biomacromolecules 3 (2002) 1312–1319.
[18] F.M. Matschinsky, Regulation of pancreatic beta-cell glucokinase: from basics to therapeutics, Diabetes 51 (2002) S394–S404.
[19] A. Grupe, B. Hultgren, A. Ryan, Y.H. Ma, M. Bauer, T.A. Stewart, Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis, Cell. 83 (1995) 69–78.
[20] O. Korsgren, T. Lundgren, M. Felldin, A. Foss, B. Isaksson, J. Permert, N.H. Persson, E. Rafael, M. Ryden, K. Salmela, A. Tibell, G. Tufveson, B. Nilsson, Optimising islet engraftment is critical for successful clinical islet transplantation, Diabetologia 51 (2008) 227–232.
[21] R.N. Wang, L. Rosenberg, Maintenance of beta-cell function and survival following islet isolation requires re-establishment of the islet-matrix relationship, J. Endocrinol. 163 (1999) 181–190.
[22] R.B. Jalili, A. Moeen Rezakhanlou, A. Hosseini-Tabatabaei, Z. Ao, G.L. Warnock, A. Ghahary, Fibroblast populated collagen matrix promotes islet survival and reduces the number of islets required for diabetes reversal, J. Cell. Physiol. 226 (2011) 1813–1819.
[23] T. Kaido, M. Yebra, V. Cirulli, C. Rhodes, G. Diaferia, A.M. Montgomery, Impact of defined matrix interactions on insulin production by cultured human beta-cells: effect on insulin content, secretion, and gene transcription, Diabetes 55 (2006) 2723–2729.
[24] L.M. Weber, K.N. Hayda, K.S. Anseth, Cell-matrix interactions improve beta-cell survival and insulin secretion in three-dimensional culture, Tissue Eng. Part A 14 (2008) 1959–1968.
[25] J. Daoud, M. Petropavlovskaia, L. Rosenberg, M. Tabrizian, The effect of extracellular matrix components on the preservation of human islet function in vitro, Biomaterials 31 (2010) 1676–1682.
[26] S. Srivastava, S.D. Gorham, J.M. Courtney, The attachment and growth of an established cell line on collagen, chemically modified collagen, and collagen composite surfaces, Biomaterials 11 (1990) 162–168.

[27] R. Kumar, R. Sripriya, S. Balaji, M.S. Kumar, Physical characterization of succinylated type I collagen by Raman spectra and MALDI-TOF/MS and in vitro evaluation for biomedical applications, J. Mol. Struct. 994 (2011) 117–124.
[28] J. Zhang, H.P. Wei, C.H. Quek, S.M. Chia, H. Yu, Quantitative measurement of collagen methylation by capillary electrophoresis, Electrophoresis 25 (2004) 3416–3421.
[29] A. Lechner, A.L. Nolan, R.A. Blacken, J.F. Habener, Redifferentiation of insulin-secreting cells after in vitro expansion of adult human pancreatic islet tissue, Biochem. Biophys. Res. Commun. 327 (2005) 581–588.
[30] S. Krol, S. del Guerra, M. Grupillo, A. Diaspro, A. Gliozzi, P. Marchetti, Multilayer nanoencapsulation. New approach for immune protection of human pancreatic islets, Nano Lett. 6 (2006) 1933–1939.