Prophylactically Decontaminating Human Islet Product for Safe Clinical Application: Effective and Potent Method

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Background. Transplanting pancreatic islets into recipients must be safe and effective to treat type 1 diabetes. Islet quality and quantity are important; however, the final product must also be free from microbial contamination and low endotoxin levels.

Methods. This study explored a method to eliminate contamination in manufacturing islets for transplantation. A simple (single antibiotic n = 164) and refined (triple antimicrobial agents, n = 279) pancreas decontaminating methods were used to test their effects on reducing the contamination rates in the islet final product. A total of 443 pancreata were processed for islet isolations. Three samples for microbial tests (Gram stain, aerobic, and anaerobic culture) were taken at preprocess (pancreas preservation), postisolation, and postculture. Endotoxin levels were measured only for islets considered for transplantation.

Results. Of 443 pancreata used for islet isolation, 79 (17.8%) showed signs of contamination in preprocess samples; 10 (2.3%) were contaminated in both preprocess and in the final product (postisolation and postculture) samples. Contamination rates in which preprocess and final product samples were positive for contamination was significantly lower using the refined method (refined vs simple method: 5% vs 20.5%, P = 0.045). Identical microbial species were present in both preprocess and in the final product.

Conclusions. This study demonstrated that the refined method reduces the rate of contamination of the islet final product and is safe for clinical application. Moreover, it may be used as a standard method during human islet manufacturing facilitating the application of a biological license agreement from United States Food and Drug Administration.

Pancreatic islet transplantation is a promising approach in treating insulin-dependent diabetes.1-3 Currently, over 900 patients globally have been transplanted with islet allografts.4 Islet transplantation requires isolating islets from mass pancreatic acinar tissue, which involves extensive tissue manipulation and rigorous regulatory approvals to ensure a safe and high-quality final product for treating patients with type 1 diabetes (T1D).5-7 Studies in whole organ transplantation showed that graft failure was attributed to systemic infection due to the contamination in the organ preservation solution.8-10 The contamination issue is further exacerbated in islet isolation, which involves multiple steps, including preservation of the whole pancreas with a portion of the duodenum and/or spleen attached, trimming and decontamination of pancreas, perfusion of enzyme, digestion, collection, washing of pancreatic tissue, purification, culturing, and collection of islets.11 Although, the process of manufacturing islet final product is carried out in a current good manufacturing practice (cGMP) facility, the risk of introducing contamination at any stage of the procedure still remains.12-14 Aside from the media used in pancreas decontamination, all other solutions used throughout the pancreas preservation and islet isolation process are prepared within the cGMP facility without addition of antimicrobial agents.
to prevent a potential allergic reaction of the recipient. The T1D patients are more vulnerable to infection, especially after islet transplantation with the required immunosuppressive regimen to prevent allograft rejection. Thus, it is extremely important to take measures to prevent contamination during manufacturing the final islet product for transplantation.

With the advancement of islet transplantation as a standardized treatment strategy in a clinical setting, it eventually may require a biologic license application so that a safe and effective final product would be transplanted into patients. Therefore, sterility testing of the final product to be transplanted becomes one of the critical checkpoints. Hence, it is important to understand the route and rate of contamination in this whole process to implement an effective prevention plan to ensure the safety and potency of islet preparations.

A limited number of large-scale studies have been published regarding the contamination issues during the islet manufacturing process, and little is described regarding the effect of antiseptic agents on the outcome of the islet isolation process. In fact, the most recent article related to this topic was published 10 years ago, which evaluated the use of Cefazolin and Amphotericin B for decontamination in the islet isolation process. However, the current protocol from the Clinical Islet Transplantation Consortium centers only uses a single antibiotic (Cefazolin) dip to decontaminate the organ. We hypothesize that using multiple antimicrobial agents may further decrease or eliminate the contamination rate of the final product. Herein, we report a comprehensive review and analysis of contamination rates during islet isolation and the frequency of contamination by specific microbial species.

**MATERIALS AND METHODS**

**Pancreas Procurement and Transportation**

Human pancreata were donated and procured from donors for transplantation and/or research from organ procurement organizations. Unless otherwise stated, consent for research was obtained from next of kin. Pancreata, with a portion of the duodenum and the spleen attached, were transported in a multilayered sterile container with cold preservation solution without antimicrobial agents. Figure 1 outlines the entire procedure of the islet isolation. This study retrospectively summarized 443 pancreata received for islet isolation from 2004 to 2015.

**cGMP Facility for Human Islet Isolation**

The entire islet isolation facility is in a Class-10 000 (ISO 7) clean room environment with high-efficiency particulate air filters which remove 99.97% of 0.3 micrometers in diameter or larger particles. A CLiMET Particle Counter (Climate Instruments Company, Redlands, CA) is used for total particle and viable particle counts. Contact Plate (Biotest Laboratories, Inc., Brooklyn Park, MN) was used for detection any microorganisms on the surface. The facility and equipment used were decontaminated with Backdown Disinfecting Detergent (Thermo Fisher Scientific) and 70% isopropyl alcohol (EMD Millipore, Temecula, CA) at the end of the procedure.

**Pancreas Preparation and Decontamination**

Procured pancreas was immersed in cold preservation solution contained in a sterile plastic bag (inner bag), the bag was placed in a sterile Nalgene jar (Thermo Fisher Scientific), and the jar was placed in a second sterile plastic bag (outer bag). During transportation, the packaged pancreas was placed on ice in a Styrofoam box. Upon arrival to the cGMP isolation facility, the Nalgene jar was aseptically transferred into the biosafety cabinet. The first sterility sample (preprocess) was taken from the cold preservation solution before handling the pancreas and other tissues. The pancreas was removed from the inner bag and placed in a sterile stainless steel tray containing trimming solution (Mediatech, Inc, Manassas, VA) supplemented with Cefazolin (Hikma Pharmaceuticals, Sintra, Portugal). The duodenum and spleen were detached from the pancreas, then removed aseptically from the biological safety cabinets (BSC) and stored for biological waste disposal. The pancreas was carefully dissected, and the surrounding fat and connective tissue were removed using sterile surgical instruments. Figure 2 shows the process of pancreas cleaning and decontamination methods (simple and refined). The difference between the 2 methods was the additional step of dipping the pancreas in a solution containing 3 antimicrobial agents for 3 to 5 minutes to reinforce the antimicrobial prophylaxis, followed by rinsing 3 times in solution. The triple antimicrobial agents include: 1 g Cefazolin (7.69 mg/mL), 40 mg Gentamicin (0.3 mg/mL) (APP Pharmaceuticals, LLC, Schaumburg, IL), and 100 mg Amphotericin B (0.77 mg/mL) (X-GEN Pharmaceuticals, Inc., Big Flats, NY), that were reconstituted in solution. After decontamination, the pancreas was transferred to another sterile surgical pan containing 1 L trimming solution supplemented with 1 g Cefazolin for pancreas cannulation. In the event that a patient is allergic to Cefazolin, Vancomycin (Hospira, Inc., Lake Forest, IL) was used instead. The pancreas was then
Pancreas Digestion, Purification, and Culture

The cannulated pancreas was perfused with enzyme solution using an automatic perfusion apparatus (BioRep Technologies, Miami, FL). The pancreas was then cut into 6 to 9 pieces and placed into the Ricordi chamber for digestion in the presence of 7 marbles. Tissue digestion was conducted at 37°C using a previously described method. The digested pancreatic tissue was collected for purification of the islets using a refrigerated COBE 2991 Cell Processor (Terumo BCT, Inc., Lakewood, CO). The islets were washed and prepared for culture immediately after the isolation process. Islets were cultured in Connaught Medical Research Laboratories 1066 Supplemented media (pH 7.4) with 0.5% human serum albumin (Baxter Healthcare Corporation, Irvine, CA) and 0.1 μg/mL insulin-like growth factor-I (Cell Sciences, Canton, MA).

Before placing the islets in culture, islets were resuspended in 100 mL of Connaught Medical Research Laboratories culture media, and a second sterile sample (postisolation) was obtained for testing. Islets were cultured at 22°C/5% CO₂ for 24 to 72 hours before the final islet product was collected for quality control assessment. At this point, a third sample (postculture) was taken for sterility testing. The entire isolation process, islet culture, and collection of the final product were carried out in the cGMP facility.

Samples for Microbiology Testing

Figure 1 shows the flowchart of the islet isolation process indicating stages at which the sterility samples were taken according to standard operating procedure established at our institution. For the sterility test, the following samples were taken: Gram stain (5 mL in 15-mL sterile conical tube; Sarstedt, Inc, Sparks, NV), endotoxin (1 mL in endotoxin-free sterile Cryovial tube; Thermo Fisher Scientific), aerobic USP Tryptic Soy Broth culture (0.5 mL in TSB United States Pharmacopeia bottle; Hardy Diagnostics, Santa Maria, CA), anaerobic USP fluid thioglycollate medium (0.5 mL sample placed in USP THIO medium; Hardy Diagnostics), and fungus culture (0.5 mL in a flask containing SabDex agar; Hardy Diagnostics). Endotoxin samples were taken postisolation, postculture, and prior to islet transplantation. Gram staining and endotoxin results were reported within 2 hours of receiving the samples for islet transplantation. Endotoxin levels were measured at the Clinical Pathology Laboratory at the City of Hope using Endosafe Portable Test System (Charles River Laboratories, Charleston, SC) following the manufacturer’s instructions. Endotoxin levels 2 EU/mL or less were considered acceptable for transplantation. The samples in USP culture bottles were cultured at 35°C for 14 days to detect the growth of organisms. Fungus culture was incubated at 30°C for 28 days for identification of any microbial growth.

Statistical Analysis

GraphPad Prism (GraphPad Software 6.0, La Jolla, CA) was used to analyze the data and generate the figures. The χ² or Fisher exact tests were used for categorical variables. A P value less than 0.05 was considered statistically significant.

RESULTS

Overall Sterility Results

Figure 3 illustrates the overall sterility results of the samples tested. Contaminated samples represent positive testing either in Gram staining or in USP culture. Microbial contamination was positive with either bacteria and/or fungus in 79 of 443 pancreata (17.8%) for islet isolations (preprocess) (Figure 3). However, of the 443 preparations, only 10 (2.3%) were contaminated both in the preprocess sample and the final product (Figure 3). A final product is considered contaminated if either or both the postisolation or postculture samples were positive. In the total 443 preparations analyzed, only 9 cases (2%) were contaminated in the final product (postisolation or postculture) despite the preprocess samples being negative (Figure 3).

Antimicrobial Effect of the Simple and the Refined Method

Figure 4 illustrates the effect of antimicrobial prophylaxis between the simple and refined methods used to decontaminate the pancreata. One hundred sixty-four pancreata were decontaminated using the simple method, and the remaining 279 were treated with the refined method. The effects were
Comparison of microbial contamination between preprocess and final product samples. The rate of scenario 1 was significantly higher using the refined method compared to the simple method (refined vs simple: 95.0% vs 79.5%, P = 0.045). The rate of scenario 2 was significantly lower when the refined antimicrobial method was used (simple vs refined method: 20.5% vs 5.0%, P = 0.045). The results from scenarios 1 and 2 indicated that the refined decontamination method, carrying-over contamination was decreased significantly. There was no significant difference between the simple and the refined methods with regards to scenario 3 (P = 1.000).

Identification of Contaminated Microbial Species

Table 1 lists the microbes identified in preparations that were positive for both preprocess and final product. There were 10 bacterial species (Propionibacterium acnes, Peptoniphilus asaccharolyticus, Delftia (Camamonas) acidovorans, Staphylococcus species-coagulase negative, Escherichia coli, Staphylococcus aureus, and Enterococcus faecalis) and 4 fungi species (Candida strains) found in the preprocess and final product samples. Majority of cases in this double positive subset (scenario 2), identical microbial species were present in both preprocess and final product samples, indicating that the contaminations originated before receiving the organ, persisted through the isolation process, and remained in the final product. Interestingly, when the refined antimicrobial method was used, fungi were the only microbial agents found in 2 preparations. In contrast, when the simple antimicrobial method was used, both bacteria (5 of 8 cases, 62%) and fungal (3 of 8 cases, 38%) species were detected in the final product (Table 1).

We also analyzed the microbial species in preparations that were negative at preprocess but positive at the final product, using the refined or simple antimicrobial methods (Table 2).

In this category, a total of 9 cases were contaminated, of which 8 cases were bacterial contamination, and 1 case was contaminated with fungi. Contamination due to Gram-positive Staphylococcus species (7 of 9 cases) was the most frequent, with only 1 case of Ralstonia pickettii (Gram-negative bacteria) contamination and 1 case of Candida albicans (fungus) contamination.

**DISCUSSION**

Cellular therapy is entering a new phase for treating a number of diseases, such as diabetes, sickle cell disease, and beta thalassemia. In particular, the treatment of diabetes using insulin-producing β cells of islets of Langerhans isolated from native pancreata or derived from induced pluripotent stem cells has been reviewed extensively in the literature. Indeed, islet transplantation has become the standard method for curing hypoglycemia unawareness of T1D patients. Consequently, it is paramount to manufacture a product in a cGMP facility that is safe and functional to treat diseased organ/tissue or cells by following strict guidelines for cellular therapy. Accordingly, the food and drug administration (FDA) guidelines and regulations state that the final product to be used in treating patients must be safe and effective. This is especially critical for acquiring a biologic license application for clinical islet transplantation.

Although, whole organ transplantation is not overseen by the FDA, pancreatic islet transplantation is strictly regulated due to the complexity of the preparation of the final product. In compliance with FDA guidelines and regulations, the islet isolation process is completed without using antibiotics that may cause an allergic reaction in certain transplant recipients. Antimicrobial agents are only used in the initial step of pancreas decontamination upon receiving the organ to prevent carrying over the microbial contamination into the islet final product.

This is a retrospective study analyzing the overall frequency of microbial contamination and the effect of the refined antimicrobial prophylaxis method used during the process of manufacturing human islets. The results demonstrated that...
ferent time periods. It is suggested that the pancreas with the center, the frequency of contamination changed during dif-


dard operating procedure to decontaminate the pancreas and eliminate the possibility of having a contaminated final

were negative for any microbial contamination. However, two cases were positive for microbial contamination in the preproc-ess samples but the final products were free of any contamination. The data clearly showed that the refined method significantly reduced the contamination rate of the final product from 20.5% to 5%. Microbial species analysis showed that 4.9% of carried-over contaminations throughout the process were caused predominantly by Gram-positive staphylococcus species, and second, by Gram-negative bacteria and fungi, which is similar to previously reported results. Amphotericin B is an antifungal agent, Cefazolin is a broad-spectrum antibiotic to mostly Gram-positive bacteria, and Gentamicin is potent to mainly Gram-negative bacteria. Interestingly, the 2 cases of positive reports in the refined method group were both fungal contaminations (Candida albicans and Candida kefyr), whereas the bacterial contamination was negative.

The microbial contamination in the pancreas preservation solution has been quite common, with reported contamination rates from 25% to 84% by different centers in North America and Europe. Even within a single islet center, the frequency of contamination changed during different time periods. It is suggested that the pancreas with duodenum attached stored and shipped in the preservation solution may increase the likelihood of contamination. The microbial contamination positive may be attributed to not only the presence of microbes in the initial pancreas preservation solution (preprocess) but also in the methods of the pancreas procurement organization, cGMP facility setup, and sterility sampling.

In this study, 443 pancreata were procured for islet isolation at our center, 79 (17.8%) of them were positive for mi-
crobial contamination in the preservation/transport solution (preprocess), which is lower than the rates of previously published studies (25-84%). The exact reason behind this discrepancy is unknown; however, it may be related to the geographical location of organ origin and/or the surgical team involved in organ procurement. Additionally, a standardized microbial sampling may help to reduce the contamination rate.

It is acknowledged that the rate of initial contamination is reduced dramatically during the isolation process because the dilution effect of a large amount of solution is used to wash the pancreatic tissue during the process. To further reduce and eliminate the possibility of having a contaminated final product, antimicrobial prophylaxis has been used as a standard operating procedure to decontaminate the pancreas prior to islet isolation. Bucher et al reported that the initial decontamination of donor pancreata using the combination of Betadine, Cefazolin, and Amphotericin B reduced microbial contamination rate of the final islet product from 9.4% to 4.4%. In this study, we investigated the effect of a refined method using antimicrobial agents, Cefazolin, Gentamicin, and Amphotericin B, in addition to the first rinse in Betadine. The overall contamination rate from different stages of sampling was significantly lower than previously reported. Furthermore, the refined method using triple antimicrobial agents (Cefazolin, Gentamicin, and Amphotericin B) decreased, to a large extent, the frequency of microbial contamination in the final product.

The microbial contamination in the pancreas preservation solution is the most critical issue. In fact, a previous study reported that the incident rate of de novo contamination during islet isolation was 5%. Another study showed that de novo contamination occurred in 11% of the isolations performed in a standard laboratory; however, the contamination rate reduced to 2.2% when the islet isolations were conducted in a cGMP facility. Our retrospective analysis indicates de novo contamination occurred 2% of the time during islet isolation in the cGMP facility, using either the simple or refined method, which is consistent with a previously reported study.

Of the total 443 islet isolations performed in our center, 47 of them were transplanted into 20 T1D patients with single or multiple islet infusions. For all 47 cases, final products were negative for any microbial contamination. However, two cases were positive for microbial contamination in the preprocess samples but the final products were free of any contamination, and therefore were safe for transplantation (data not shown).

It was previously reported that although islet final product was found contaminated posttransplantation, successful islet

### TABLE 1.
Microbial species identified in preprocress, postisolation, and postculture sterility samples

| Isolation no | Decontamination method | Preprocess sample | Final product sample |
|--------------|------------------------|-------------------|---------------------|
| 555          | Refined                | Candida albicans  | Candida albicans    |
| 725          | Refined                | Candida kefyr     | Candida kefyr       |
| 850          | Simple                 | Propionobacterium acnes | Candida albicans |
| 851          | Simple                 | Peptostreptococcus asaccharolyticus | Gram-positive cocci |
| 883          | Simple                 | Delphina (Carnanomasa) | Cupriavidus paucilius, acidovorans | Gram-negative rods |
| 904          | Simple                 | Staphylococcus species-coagulase | Escherichia coli negative; Escherichia coli |
| 910          | Simple                 | Staphylococcus aureus | Staphylococcus aureus |
| 918          | Simple                 | Enterobacter aerogenes | Enterobacter aerogenes |
| 930          | Simple                 | Candida glabrata   | Candida glabrata    |
| 934          | Simple                 | Candida tropicalis | Candida tropicalis |

**TABLE 2.**
Microbial species identified in postisolation and postculture sterility samples

| Isolation no | Decontamination method | Preprocess sample | Final product sample |
|--------------|------------------------|-------------------|---------------------|
| 569          | Refined                | Gram-positive cocci |
| 581          | Refined                | Staphylococcus species-coagulase negative |
| 608          | Refined                | Gram-negative rods; Gram-positive cocci |
| 721          | Refined                | Enterococcus faecium, Lactobacillus species, Gram variable Cocccobacilli |
| 734          | Refined                | Gram-positive cocci |
| 757          | Refined                | Candida albicans |
| 778          | Simple                 | Raistonia (Burkholderia) pickettii |
| 786          | Simple                 | Gram-positive cocci |
| 845          | Simple                 | Staphylococcus species-coagulase negative |

The data clearly showed that the refined method significantly reduced the contamination rate of the final product from 20.5% to 5%. Microbial species analysis showed that 4.9% of carried-over contaminations throughout the process were caused predominantly by Gram-positive staphylococcus species, and second, by Gram-negative bacteria and fungi, which is similar to previously reported results. Amphotericin B is an antifungal agent, Cefazolin is a broad-spectrum antibiotic to mostly Gram-positive bacteria, and Gentamicin is potent to mainly Gram-negative bacteria. Interestingly, the 2 cases of positive reports in the refined method group were both fungal contaminations (Candida albicans and Candida kefyr), whereas the bacterial contamination was negative.
function was achieved with no risk of infection because the transplant recipients were prophylactically treated with antimicrobial agents.17 In fact, it has been reported that infection can result in enhanced alloreactivity and resistance to tolerance induction; therefore, the infections of transplanted recipients could have a detrimental effect on long-term allograft survival.3,5

It would be useful in identifying novel antibiotics that are sensitive to microbial contamination in the islet preparations. A defined concentration of antimicrobial agents used in preservation solution during the transportation and storage stages prevent preprep contamination upon arrive to CGMP islet isolation facility.16 The use of well-established standard operating protocols to manufacture islets is critical to achieve this goal. Furthermore, extensive cleaning of the fat and connective tissues from the pancreas and, in particular, the removal of the duodenum from the pancreas before antibiotic/antimitotic dip is paramount to the success and safety of the islet product preparation. Furthermore, it is important to clean the pancreas thoroughly, not only to reduce the likelihood of contaminated extrapancreatic tissue, but to also examine the pancreas for any abnormality or presence of tumors. Indeed, we have found two cases with abnormal nodules in the pancreas. The pathological examination showed a neuroendocrine tumor in 1 case and neoadiablastosis in the other case, information that was relevant to the healthcare of other organ recipients from the same donor (data not shown). In conclusion, this study demonstrates an effective and potent refined antimicrobial prophylaxis in reducing the contamination rate of the final islet product for clinical application. Moreover, it may be used as a standard method during human islet manufacturing facilitating the application of a biological license agreement from United States Food and Drug Administration.

ACKNOWLEDGMENTS

The authors are grateful to all members of the islet isolation team and the Department of Clinical Pathology at the City of Hope for their technical support. The authors also thank Randall Heyn-Lamb and Karen Ramos for providing and interpreting donor information, and Henry Lin and Leonard Chen for providing comments to the manuscript.

REFERENCES

1. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med. 2006;355:1318–1330.
2. Alejandro R, Barton FB, Hering BJ, et al. 2008 Update from the Collaborative Islet Transplant Registry. Transplantation. 2008;86:1783–1788.
3. Qi M, Kirner K, Danielson KK, et al. Five-year follow-up of patients with type 1 diabetes transplanted with allogeneic islets: the UIC experience. Acta Diabetol. 2014;51:833–843.
4. Collaborative Islet Transplant Registry Monthly Accrual Report. http://www.citreregistry.com
5. Ricordi C, Lacy PE, Finke EH, et al. Automated method for isolation of human pancreatic islets. Diabetes. 1988;37:413–420.
6. Weber DJ. FDA regulation of allogeneic islets as a biological product. Cell Biochem Biophys. 2004;40(3 Suppl):19–22.
7. Nanno R, Cisla B, Melzi R, et al. Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. Diabetologia. 2005;48:906–912.
8. Majerski JA, Alexander JW, First MR, et al. Transplantation of microbiially contaminated cadaver kidneys. Arch Surg. 1982;117:221–224.
9. Spees EK, Light JA, Oakes DD, et al. Experiences with cadaver renal allograft contamination before transplantation. Br J Surg. 1982;69:482–485.
10. Brayman KL, Stephanian E, Matas AJ, et al. Analysis of infectious complications occurring after solid-organ transplantation. Arch Surg. 1992;127:38–47.
11. Qi M, Luis V, Bilbao S, et al. Sodium levels of human pancreatic donors are sensitive to microbial contamination in the islet preparations. Transplant Proc. 1991;23:2655.
12. Rowinski W, Pacholczyk M, Chmura A, et al. Influence of positive cultures in donor and preservation medium on development of infection in cadaveric kidney transplant recipients: beneficial effects of antibiotic coverage at the time of nephrectomy. Transplant Proc. 1991;23:2655.
13. Wood PP, Melendez T, Langnas AN, et al. The viability of microorganisms in preservation solutions. Transplantation. 1991;51:239–242.
14. Meyers BR, Mendelsohn MH, Lansman S. Microbial contamination of solid-organ donor transplant fluids leading to systemic infection. Transplantation. 1992;53:1383.
15. Pouch SM. Infectious complications of pancreatic islet transplantation: clinical experience and unanswered questions. Curr Infect Dis Rep. 2015;17:482.
16. Yue OJ, McFarland RD, Iony J. Selected Food and Drug Administration review issues for regulation of allogeneic islets of Langerhans as somatic cell therapy. Transplantation. 2002;74:1816–1820.
17. Galya-Lopez B, Kin T, O’Gorman D, et al. Microbial contamination of clinical islet transplant preparations is associated with very low risk of infection. Diabetes Technol Ther. 2013;15:323–327.
18. Bucher P, Oberhofer J, Bosco D, et al. Microbial surveillance during human pancreatic islet isolation. Transplant Int. 2005;18:584–589.
19. Carroll PB, Ricordi C, Fontes P, et al. Microbiologic surveillance as part of human islet transplantation: results of the first 26 patients. Transplant Proc. 1992;24:2789–2799.
20. Clinical Islet Transplantation Consortium. http://www.isletstudy.org/.
21. Qi M, Valerie L, McFadden B, et al. The choice of enzyme for human pancreas digestion is a critical factor for increasing the success of islet isolation. Transplant Direct. 2015:1.
22. Qi M, McFadden B, Valerie L, et al. Human pancreatic islets isolated from donors with elevated Hba1c levels: islet yield and graft efficacy. Cell Transplant. 2015:24:1879–1886.
23. Murray L, McGowan N, Fleming J, et al. Use of the Bact/Alert system for rapid detection of microbial contamination in a pilot study using pancreatic islet cell products. J Clin Microbiol. 2014;52:3769–3771.
24. Johannesson B, Su L, Fryeys DO, et al. Toward beta cell replacement for diabetes. EMBO J. 2015;34:841–855.
25. Bhatia M, Sheft S. Hematopoietic stem cell transplantation in sickle cell disease: patient selection and special considerations. J Blood Med. 2015;6:229–238.
26. Mathews V, Srivastava A, Chandy M. Allogeneic stem cell transplantation for thalassemia major. Hematol Oncol Clin North Am. 2014;28:1187–1200.
27. Pagliuca FW, Milman JR, Gütler M, et al. Generation of functional human pancreatic beta cells in vitro. Cell. 2014;159:428–439.
28. Nostro MC, Keller G. Generation of beta cells from human pluripotent stem cells: potential for regenerative medicine. Semin Cell Dev Biol. 2012;23:701–710.
29. Linetisky E, Ricordi C. Regulatory challenges in manufacturing of pancreatic islets. Transplant Proc. 2008;40:424–426.
30. Wonnacott K. Update on regulatory issues in pancreatic islet transplantation. Am J Ther. 2005;12:600–604.
31. Scharp DW, Lacy PE, McLead M, et al. The bioburden of 590 consecutive human pancreata for islet transplant research. Transplant Proc. 1992;24:974–975.
32. Lakey JR, Rajotte RV, Warnock GL. Microbial surveillance of human islet isolation, in vitro culture, and cryopreservation. Cln Invest Med. 1995; 18:168–176.
33. Kin T, Roschuk S, Shapiro AM, et al. Detection of microbial contamination during human islet isolation. Cell Transplant. 2007;16:9–13.
34. Orr C, Stratton J, Riao O, et al. Quantifying insulin therapy requirements to preserve islet graft function following islet transplantation. Cell Transplant. 2015.
35. Chong AS, Alegre ML. Transplantation tolerance and its outcome during in vitro culture, in vitro culture, and cryopreservation. JClin Microbiol. 2015:34:841–855.
36. Lakshmaiah Narayana J, Chen JY. Antimicrobial peptides: possible anti-infective agents. Peptides. 2015;72:88–94.