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Quality monitoring of microbial contamination of cryopreserved parathyroid tissue

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Abstract Cryopreservation of parathyroid tissue (PT) provides patients undergoing parathyroidectomy with an option for delayed autologous heterotopic parathyroid transplantation. A standard protocol for quality monitoring of PT has not been established. This article describes a method for detecting the presence of bacterial contamination in PT tissue intended for autologous transplantation. PT was received in the tissue bank, processed under aseptic conditions, and placed into cryopreservation medium. Sterility testing was performed at 2 time points prior to cryopreservation. From January 2005 to October 2008, 47 PT samples were cryopreserved. The following bacteria were isolated from 11 PT specimens: *Staphylococcus epidermidis*, *Staphylococcus capitis* subspecies *ureolyticus*, *Staphylococcus lugdunensis,* *Bacillus pumilus*, and corynebacteria (diphtheroids). 23% of PTs were contaminated at the time of collection, predominantly with indigenous bacteria. Quality monitoring using this protocol is a useful tool to identify tissues contaminated with bacteria.

Keywords Bacteria · Culture · Hypoparathyroidism · Autotransplant · Parathyroid

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Introduction

Implantation of fresh autologous parathyroid tissue has become the standard of care for patients undergoing subtotal or total parathryoidectomy as a method to prevent severe, post-operative hypocalcemia (Cohen et al. 2005). Greater than 80% of patients will achieve a euparathyroid state after fresh autologous heterotopic parathyroid transplantation (AHPT); however, for those who experience graft failure or hypofunction, treatment options are limited (McHenry et al. 1997). Cryopreservation of parathyroid tissue (PT) provides patients undergoing parathyroidectomy with an option for delayed autologous heterotopic parathyroid transplantation to treat post-surgical hypoparathyroidism. It has become an increasingly common practice for patients undergoing subtotal or total parathyroidectomy and in patients undergoing reoperative

parathyroidectomy should the first tissue fail to engraft or become hypofunctional (Neyer et al. 2002).

A standard protocol for the cryopreservation of PT does not exist and many institutions utilize their own in-house protocols. Furthermore, quality monitoring procedures for parathyroid tissue cryopreservation are not standardized. Hospital oversight of the collection, processing, storage and issuing of human tissue for transplant has traditionally been conducted by a variety of different departments depending on hospital policy. In 2005, the Joint Commission on the Accreditation of Healthcare Organizations (JCAHO) issued new regulatory standards regarding tissue services and has encouraged hospitals to centralize oversight of all tissue services (Parham 2006). Because of the similarities between blood products and tissue products, many hospital blood banks have taken on this responsibility. According to the 2007 National Blood Collection and Utilization Survey, blood banks are the second most common hospital department, with operating rooms the most common department, responsible for human tissue products (US Department of Health and Human Services 2007). At this institution, the stem cell laboratory, a section within the division of transfusion medicine, oversees parathyroid tissue procurement and transplantation.

Because no universal protocol exists for the cryopreservation of parathyroid tissue, we developed a quality monitoring protocol that incorporates the detection of microbial tissue contamination, both at the receipt of tissue and post processing. The prevention of tissue transmitted infectious disease is a goal for hospital departments offering tissue services. Furthermore, there has been increasing regulation by the Food and Drug Administration (FDA) and state health departments to improve the quality of Human Cells, Tissue and Cellular and Tissue Based Products (HCT/Ps) and the AABB has recently published hospital Tissue Bank guidelines (Eisenbre et al. 2008). Assessing for tissue contamination is one of the standards of practice for quality monitoring of cellular and tissue based products intended for future clinical use as this practice ensures quality assurance and increases patient safety. In this article, the quality monitoring protocol for sterility is discussed in the setting of a series of cases in which contaminated PT had been cryopreserved for future transplantation.

Materials and methods

Tissue procurement and cryopreservation

Parathyroid tissue was cryopreserved using methods similar to those previously reported (Saxe et al. 1990; Wagner et al. 1991). Briefly, PT tissue removed during surgery was placed in a sterile container containing 10 ml of sterile 0.9% sodium chloride solution and transported at room temperature to the tissue bank laboratory. Also at the time of surgery, 10 ml of whole blood was drawn via peripheral IV stick into two sterile, non-additive glass tubes and transported to the laboratory with the tissue. The tubes were then centrifuged at 2,000*g* for 10 min to separate the serum. The tissue was either processed on the same day or stored overnight at 4°C in a refrigerator either adjacent the operating room or in the tissue laboratory until processing the following day. Under aseptic conditions, the PT tissue was removed from the transport container and placed into a Petri dish with approximately 1 ml of the saline from the transport container. The PT was minced into 1–3 mm fragments and transferred into Nunc (Nalgene Nunc, Rochester, NY) cryotubes containing 1 ml freshly prepared cryopreservation medium. The cryopreservation medium consisted of 10% dimethyl sulfoxide (Edwards Life Science, Bedford, OH) and 10% autologous serum in Plasma-Lyte-A electrolyte solution (Baxter Healthcare, Deerfield, IL). The tissue was frozen at −80°C using either a controlled rate freezer or mechanical freezer with a rate of freezing of 1°/min and the vials were transferred for storage at −180°C over liquid nitrogen vapor phase.

Microbial culture, identification, and susceptibility testing

Bacterial cultures were performed on all PT tissues immediately before processing the tissue, at the end of processing before cryopreservation, and on the corresponding cryopreservation medium. Specifically, aerobic pediatric blood culture bottles (Peds Plus/F, BD Diagnostics, Sparks, MD) were inoculated with 1–2 ml of the fluid the tissue was received in (the 0.9% sodium chloride solution from the transport container), the remaining fluid from the Petri dish at the end of the mincing procedure, and the cryopreservation medium. The cryopreservation

medium was cultured immediately upon constitution. All bottles were incubated at 37°C for 5 days in an automated continuous-monitoring BD BACTEC™ 9240 blood culture system (BD Diagnostics, Sparks MD). If the growth index signaled as positive, culture bottles were Gram-stained and sub-cultured onto appropriate media, following routine protocol. Bacterial identification to the species level and antimicrobial susceptibility testing were performed according to routine clinical microbiology protocols. Isolates were classified as susceptible (S), intermediate (I), or resistant (R) according to the minimum inhibitory concentration (MIC) interpretive breakpoint values as recommended by the Clinical and Laboratory Standards Institute (CLSI/NCCLS-Clinical and Laboratory Standards Institute 2006). In-house validation studies of the BD BACTEC™ 9240 blood culture system were performed with seeded samples from both fluid types, the normal saline and the cryopreservation medium. The in-house studies determined that the lower limit of detection was 50 CFU/ml for the most commonly isolated organisms.

Molecular typing

Staphylococcus capitis isolated from PT tissue of three patients' were genotyped by pulsed-field gel electrophoresis (PFGE) using the CHEF Mapper system (Bio-Rad, Hercules, CA) as previously described with modifications detailed below (Goering and Winters 1992; Cimiotti et al. 2007). Briefly, *S. capitis* isolates were inoculated into 5 ml of nutrient broth and incubated for 3 h at 37°C with shaking to attain exponential growth. Agarose plugs were prepared by mixing the cultures with 1.5% low-melting-point agarose at 55°C, and solidified at 4°C. The bacterial cells were lysed in situ with 2 mg/ml lysostaphin and 30 U/μl of mutanolysin for 2 h at 37°C, followed by proteinase K treatment overnight at 55°C. The genomic DNA was digested with *Sma*I restriction endonuclease, and the DNA fragments generated were separated by PFGE. Running parameters were as follows: voltage, 200 (6 V/cm); initial switch time 1 min; final switch time 12 min; run time, 18.5 h. The gels were stained with ethidium bromide and the PFGE patterns were visualized and interpreted as indistinguishable, closely related, related, possibly related, and different according to established criteria (Tenover et al. 1995).

Statistical methods

The statistical significance of the difference between the two culture groups (cases with positive bacterial cultures compared to cases with negative bacterial cultures) and between the two processing groups (those processed the same day compared to those processed the next day) were calculated using the Wilcoxon Rank Sum test and the Fisher's Exact test with SAS software (SAS Institute, Cary, NC). Results were deemed significant with a P-value ≤ 0.05.

Results

Statistics and overview

From January 2005 to October 2008 a total of 47 PT samples from 47 patients, 31 females and 16 males ages 18–84, were cryopreserved at New York-Presbyterian Hospital/Columbia University Medical Center for potential implantation in the future. Of the 47 cases, 3 patients developed post-operative fresh autograft failure and severe hypocalcemia requiring re-implantation of cryopreserved PT. To date, two patients have successfully engrafted with resolution of symptoms and return to normocalcemic levels. The patients' tissue had been cryopreserved for approximately 11 and 12 months, respectively. The remaining patient engrafted after receiving a second transplant. The second transplant had the longest tissue cryopreservation time of approximately 14 months. No measures of post-thaw organ viability were conducted prior to re-implantation as accepted standards for such testing do not exist, are not readily available, and clinical significance has not been definitively established.

Bacterial contamination of the harvested parathyroid tissue for cryopreservation was 23% overall (11 of 47 cases, Table 1). Contamination was present before processing the tissue in 10 cases. In 3 of the 10 cases, contamination was present both before and after tissue processing. The discrepancy between pre-processing and post-processing cultures may reflect differences in the amount of fluid used for the culture inoculum, because on average slightly less fluid was used for the post-manipulation culture. Finally, one case had contamination only after processing the tissue. All cryopreservation reagent cultures were

Table 1 Number of positive bacterial cultures

Year	Total number cases	Total with positive cultures	Yearly percentage	Organisms
2005	5	1	20	*S. epidermidis*
2006	9	2	22	*S. epidermidis* *S. capitis*
2007	21	4	19	*S. epidermidis* (3) *S. lugdunensis* + *B. pumilus* (1)
2008 (Jan–October)	12	4	33	*S. epidermidis* (1) *S. capitis* (2) Diphtheroids (1)
Total	47	11	23	

negative. To date, no contaminated PT tissue has been re-implanted.

Of the 11 positive cases, 6 grew *Staphylococcus epidermidis*, 3 grew *S. capitis* subspecies *ureolyticus*, 1 grew *Staphylococcus lugdunensis* and *Bacillus pumilus*, and 1 grew corynebacteria (diphtheroids) (Table 2). The average time to cryopreservation for all cases was 10.1 h (range 0–27 h). 27 cases were cryopreserved the same day as surgery with an average time from harvest to cryopreservation of 1.7 h (range 0–4.5 h). 20 cases were cryopreserved the following day with an average time to cryopreservation of 21.3 h (range 15.5–27 h). The bacterial contamination rate between the cases processed the same day compared to those processed the following day was not statistically significant, 18.5 and 30%, respectively, *P*-value 0.48. Furthermore, the time to cryopreservation averaged 13.05 h for culture positive cases and 9.15 h for culture negative cases. The difference between the time to cryopreservation between culture positive and negative cases was not statistically significant, *P*-value 0.21.

Table 2 Bacterial contaminants identified pre- versus post-manipulation

Number of patients	Pre-manipulation	Post-manipulation
5	*S. epidermidis*	No growth
1	*S. epidermidis*	*S. epidermidis*
1	*S. capitis*	No growth
1	*S. capitis*	*S. capitis*
1	No growth	*S. capitis*
1	*S. lugdunensis*; *B. pumilus*	No growth
1	Diphtheroids	Diphtheroids

Molecular typing of *S. capitis* cultures

During 2008, two *S. capitis* cases occurred within 7 days. Due to the proximity of the events, we initiated an investigation to determine if the contaminants had a common source. The strains were typed using pulsed-field gel electrophoresis (PFGE), which is a highly discriminatory method that determines strain relatedness by comparing chromosomal DNA restriction fragment patterns, and is commonly used to identify the source of hospital-acquired contamination (Reich-Slotky et al. 2008). The PFGE results of the 3 *S. capitis* isolates from 2 patients revealed a greater than 6 band difference between patients. These data demonstrate that the strains were unrelated and did not come from the same source.

Discussion

Implementation of the Good Tissue Practices in May 2005 by the FDA requires HCT/Ps processing facilities to monitor the sterility of their products (Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments 2004). Autologous parathyroid tissue that is cryopreserved and reimplanted at the same hospital is considered the same surgical procedure and therefore FDA registration under 1271.15(b) is not required

(21 C.F.R. § 1271.15 2008). However, monitoring is nonetheless essential for quality assurance, patient safety and prevention of future contamination. Because many transfusion medicine departments process and issue blood and hematopoietic progenitor cell products, it is likely that future additional tissue banking activities will fall under their supervision (Brecher and Hay 2004). As such, product specific tissue banking protocols need to be developed to meet laboratory standards and AABB guidelines. This PT cryopreservation protocol involves culturing the tissue at 2 time points; upon arrival to the lab and at the end of processing prior to cryopreservation. For each specimen received the cryopreservation solution was tested as well. This protocol facilitates the identification of the pathogen source and thus guides the optimization of procurement, transport, and laboratory policies.

The microbial contamination rate for the 46 month study period was 23%. To our knowledge, this is the first report of positive bacterial cultures from harvested PT cryopreserved for potential future transplant. As such, the expected rate of contamination is unknown and the significance of these positive bacterial cultures has yet to be determined. These results are similar to the contamination rates reported from a study analyzing cadaveric tissue allografts where 17–21% of the harvested tissue was contaminated with bacteria (Eastlund 2006). Because parallel control cultures were not conducted, it is difficult to know if the positive cultures represent true organ infection or contamination from the surgical field, transport, or laboratory processing. No patients were known to have parathyroid infection at the time of surgery and the pattern of culture positivity suggests the majority of the PT was contaminated upon procurement or transport and not during laboratory processing, as evidenced by the negative cryopreservation medium cultures. Nonetheless, these findings raise safety concerns for patients and the need for standardized cryopreservation procedures that minimize the risk of true infection as several of the cultures have grown potentially pathogenic bacteria.

Staphylococcus epidermidis, *S. lugdunensis*, and *S. capitis* subspecies *urealyticus* are coagulase negative staphylococci considered indigenous cutaneous flora. However, all these species have also been implicated in a variety of human infections (Irlinger 2008). *S. epidermidis* and *S. lugdunensis* have been associated with acute cutaneous infections including soft tissue abscesses and infections of sebaceous glands (Tan et al. 2006). Coryneform bacteria (diphtheroids), a heterogeneous group of gram-positive bacilli, are also indigenous microflora and are usually considered contaminants in clinical specimens (Mandell et al. 2005). However, like coagulase-negative staphylococci, coryneforms can also cause skin and soft tissue infections (Tan et al. 2006). Bacillus species are gram-positive bacteria usually isolated from the soil and are not considered usual constituents of the normal skin flora (Aunpad and Na-Bangchang 2007). Interestingly, *B. pumilus* is used in commercially available probiotic products (Mandell et al. 2005). Although probiotics could be a possible source of this contamination, it is not known if the patient or medical staff involved with this case was using a probiotic product.

With the possible exception of the Bacillus species, the staphylococcal and coryneform bacteria pose a risk for potential post-transplant infections as PT autografts are surgically implanted in pre-sternal subcutaneous tissue or intramuscular forearm tissue (Echenique-Elizondo et al. 2007). Although bacterial survival post-cryopreservation is undetermined, evidence from hematopoietic stem cell cryopreservation suggests that bacteria will survive, as cases of positive pre- and post-cryopreservation bacterial cultures have been reported (Patah et al. 2007). Due to the irreplaceable nature of the cells needed for the patient's care, contaminated hematopoietic stem cell transplants have been infused with the concomitant use of antimicrobial therapy and generally did not result in adverse events (Kamble et al. 2005). PT transplants, like hematopoietic stem cells, may be medically necessary despite contamination and similar antimicrobial regimens may be necessary in patients receiving these PT transplants. Although the use of prophylactic antimicrobial agents is at the discretion of the surgeon, positive pre-cryopreservation cultures enables the laboratory to provide advance notification of potential contamination, often days before post-thaw cultures become positive.

This is the first reported series of bacterial contamination of PT tissue prior to cryopreservation, indicating a clinical need to optimize and standardize quality monitoring and culture methods and protocols. Suggestions for improving current methods used at our facility include using a larger inoculum for culture and adding anaerobic and fungal cultures to

the routine aerobic cultures. Parallel control cultures, obtained at the time of surgery, will be conducted. All tissue thawed for transplant will be cultured and recipients will be monitored for signs of infection. In cases with positive cultures, the pattern of culture positivity and the microorganism isolated will be analyzed. Completing a series of cultures on the organ, the cryopreservation medium, and the control sample will provide the necessary data to identify the specific contaminate source with subsequent remediating intervention.

The findings of this study prompted an open discussion with the surgeons to develop improved methods to reduce contaminations during surgery and transport and to review laboratory procedures. Improved coordination between the surgical team and laboratory has resulted in same day processing for all specimens and contamination rates are tracked and reviewed in quarterly inter-departmental quality assurance meetings. As more surgical procedures are completed we hope to gain a better understanding of the nature of the contamination and possible methods to reduce it. Additional studies are being planned to determine the rate of contamination of resected PT that is not cryopreserved. Our sterility quality monitoring approach for procurement and processing of resected PT tissue for cryopreservation and potential future transplant proved successful at capturing bacterial contamination and is recommended to increase laboratory quality assurance and patient safety.

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