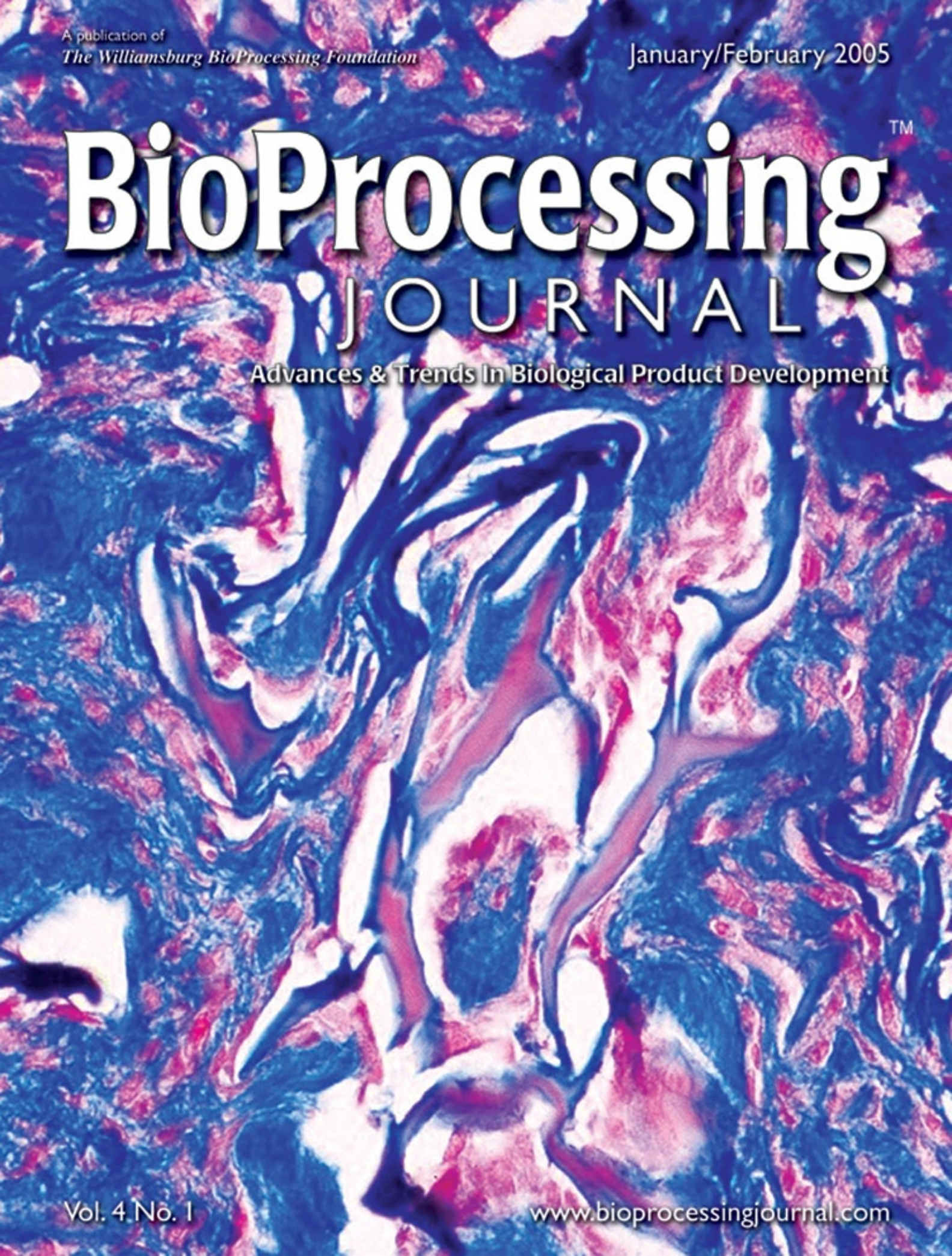
A publication of
The Williamsburg BioProcessing Foundation

January/February 2005

BioProcessing™ JOURNAL

Advances & Trends In Biological Product Development



Vol. 4 No. 1

www.bioprocessingjournal.com

Safety Assessment of a Renal Bio-Replacement Therapy System: *A Case Study*

By ZORINA PITKIN

Acute Renal Failure (ARF) is a severe inflammatory disease state often accompanied by multiple organ failure (MOF). ARF is precipitated by many factors such as blood loss, surgery, sepsis, toxins, trauma, and is most often linked to the loss of kidney tubule function.[1] Proximal tubule cells are specifically injured in acute renal failure.[2,3] Current therapies for ARF involve conventional kidney support with hemodialysis or hemofiltration. These therapies offer replacement of normal renal functions such as waste removal, fluid, and electrolyte balance, but they cannot provide vital endocrinological and metabolic functions of a healthy kidney.[4] Despite advances in synthetic materials and extracorporeal circuits for hemodialysis and hemofiltration, ARF is associated with a high mortality rate ranging between 55–70 percent.[5–8]

Nephros' Renal Bio-Replacement Therapy (RBRT) was designed to reconstitute these important biological functions and in so doing, treat ARF and facilitate natural recovery of a patient's own kidney function.[9]

This article describes the safety program for the RBRT system that was established at Nephros Therapeutics, Inc., and reports a case study pertaining to clinical use of the system, portraying how a methodical assessment of safety data collected from multiple data points allowed for the successful completion of an investigation.

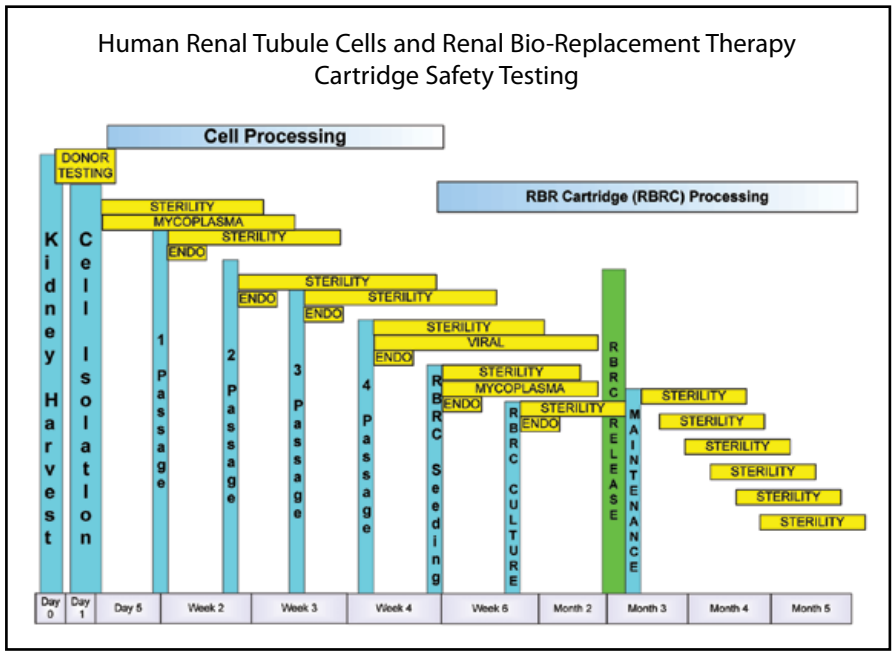


Figure 1. A schematic of safety testing using a time scale. Major procedural steps are shown as vertical bars; safety testing, which includes donor testing sterility, endotoxin, viral, and mycoplasma tests, are shown as horizontal bars in yellow. The schematic shows that all critical steps are associated with sterility and/or other safety tests.

Renal Bio-Replacement Therapy

The Renal Bio-Replacement Therapy (RBRT) is an extracorporeal treatment that employs renal tubule cells, and was designed to mimic the structure and function of the natural kidney. It is intended to replace the missing metabolic, endocrine, and immunological functions of the kidney, and allow time for the patient's own kidney to resume its normal functions.[9]

RBRT is a combination of a biological component (human kidney proximal tubule cells) and a device (a hollow fiber cartridge incorporating a biocompatible membrane). The RBRT is regulated as a biological product by FDA's Center for Biological Evaluation and Research (CBER). The cells are isolated from human kidneys that were obtained from eligible transplants and are expanded in a culture medium. The RBRT contains ~4,000 hollow fibers lined with proximal tubule cells that form artificial

Zorina Pitkin, Ph.D., RAC *(zpitkin@nephrostherapeutics.com) is vice president, regulatory affairs and quality systems, Nephros Therapeutics, Lincoln, RI.*

renal proximal tubules. This cartridge is intended for use as an extracorporeal system for up to 72 hours of continuous treatment. It is integrated in a conventional continuous veno-venous hemofiltration (CVVH) circuit. The patient's blood first enters a conventional hemofilter (dialysis cartridge), which acts like a normal glomerulus of the kidney, separating the blood into filtrate and cellular components. The filtrate is passed through the lumen of the fibers upon which these cells have been grown, while the blood cellular component is perfused around the outside of the hollow fibers and communicates with the tubular cells through the membrane.[9]

Each of the aforementioned steps is carefully controlled in order to avoid potential compromise of the system.

Clinical Development

A Phase I/II clinical trial to evaluate the RBRT in ten patients with ARF was completed in 2002. The predicted hospital mortality rate in studied patients averaged 86 percent. The primary purpose of the trial was to evaluate safety, tolerability, and 30-day mortality data. The study demonstrated that the RBRT was well tolerated and suggested improvement in patient mortality as compared to historical control.[10,11] Based on the results of this preliminary clinical study, Nephros Therapeutics initiated a randomized, controlled, multicenter Phase II clinical trial under an Investigational New Drug application (IND). This trial will further assess safety and efficacy of the RBRT for Intensive Care Unit (ICU) patients with ARF, as it may affect short-term mortality.

Quality Control Safety Testing

In-Process Controls

The human renal tubule cells (HRTCs) are obtained from explanted human kidneys available from qualified donors. Donors are screened for infectious diseases in accordance with draft FDA Guidances on Donor Eligibility for Human Tissues and Cells, and Screening and Testing of Donors of Human Tissue Intended for Transplantation.[12,13] The kidneys are aseptically processed in a classified cGMP environment, while the renal cells are isolated and expanded in a culture medium in such a way as to maintain their sterility, viability, and several of the metabolic functions of the kidney. The primary cell isolate from the donor kidney is tested to ensure that it is free from contamination by bacteria, fungi, and mycoplasma. In-process quality control (QC) testing includes sterility and endotoxin testing at each critical step of cell processing. The HRTCs are also tested for specific viral agents such as human immunodeficiency virus (HIV) HIV-1, HIV-2, hepatitis B virus (HBV), and hepatitis C virus (HCV) by a PCR method. In-process and final release quality controls measure the key production safety specifications of the cells at multiple points in the manufacturing process, as shown in Figure 1. The HRTCs are incorporated into a commercial hemofiltration cartridge, where the cells are grown to confluence along the inner surface of the hollow fibers.[14] Prior to the seeding of the RBRT cartridge (RBRTC), the confluence of the cells is assessed as well as the number and viability of the cells seeded into the cartridge. Safety of the product is determined (*initial* release), as measured by the endotoxin content, and by the absence of bacterial, fungal, and mycoplasma contaminants.[15] The RBRTC is maintained in a culture medium with weekly QC evaluations. Samples are taken to monitor cell functionality and sterility (*maintenance* release). The HRTCs that are incorporated into the RBRTC can be maintained under controlled conditions for up to five months.

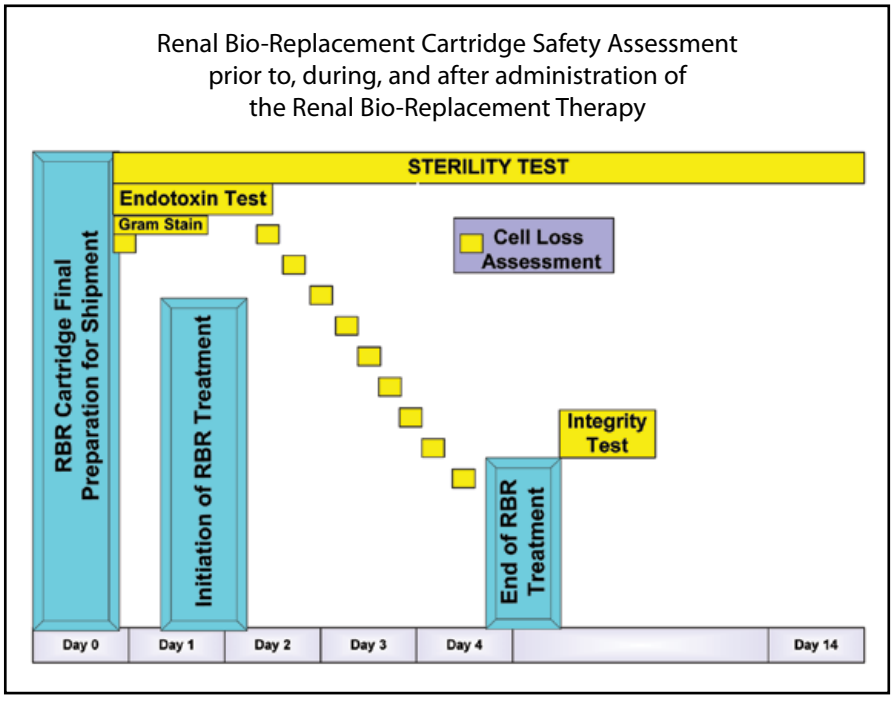


Figure 2. Procedural steps for RBRTC processing are shown in blocks with corresponding quality control testing. Each small yellow block ▭ represents cell loss assessment measured at the time the RBRTC is prepared for shipment, and every eight hours during patient treatment.

RBRTC Safety Assessment Prior to, during, and after RBRT

Once a patient is identified as being eligible for the RBRT (i.e., meeting all inclusion criteria of the clinical protocol) at one of the participating clinical sites, the RBRTC is prepared for shipment with a series of media flushes in a Class 10,000 cleanroom environment. The RBRTC is released for preparation for clinical use only when all in-process, *initial*, and *maintenance* release testing parameters have met the established criteria. Once the RBRTC is prepared for shipment, there is limited time within which the therapy must be initiated. The RBRTC is subjected to further QC evaluations such as Gram Stain, sterility, endotoxin, and cell loss (Fig. 2).

However, the final results of the sterility testing are not available for 14 days, which is far beyond the completion of the patient therapy. For the sake of product safety, and as dictated by appropriate federal regulations, it is imperative that a cell product delivered to the patients must be sterile, free of known adventitious agents, viable, and functional.[16,17] Accordingly, the RBRTC is conditionally released to the clinic based on the following:

- Review of the complete set of in-process, *initial* release, and *maintenance* release safety test results. On average, there are 20 sterility and functionality data points available for evaluation by the time the RBRTC is released for clinical use
- Visual observation of the culture medium for color and turbidity
- Results of the Gram Stain procedure
- Environmental and personnel monitoring
- Assessment of the cell loss during pre-shipment preparation

Once the RBRTC arrives at the clinical site, it is transferred to the patient's bedside, where it is incorporated into the RBRT–CVVH circuit. Cell loss is further evaluated during administration of the RBRT to the patient as a measure of the HRTCs' performance in the cartridge during therapy. This assessment is conducted every eight hours until the treatment is discontinued. Cell loss exceeding pre-determined criteria indicates that the RBRTC performance may be adversely affected. Normally, there is no detectable number of cells released from the cartridge into the waste at eight-hour intervals. Upon completion of each treatment, the RBRTC is subjected to integrity testing to make sure the membranes within the cartridge remain intact.

Case Summary

As described above, the RBRTC was shipped to the clinical site for treatment of a patient with ARF who met all inclusion criteria. The RBRTC met all quality control criteria prior to clinical use. However, during the RBRT, specifically during the cell loss assessment procedure at hour 40 after initiation of the treatment, clinical personnel noticed that the cells were sloughing off in detectable quantities (Fig. 3). The pH of the ultrafiltrate was also determined to be lower than anticipated. As a result, the therapy was discontinued. Although the cell number did not exceed the predetermined specification, this observation was unusual enough to initiate an investigation. The investigation concerned two areas: 1) the RBRTC, and 2) what was happening to the patient. The cell loss observation coupled with acidic ultrafiltrate and a strong odor suggested that the cells were affected by a contamination, either through the patient's blood or through another source.

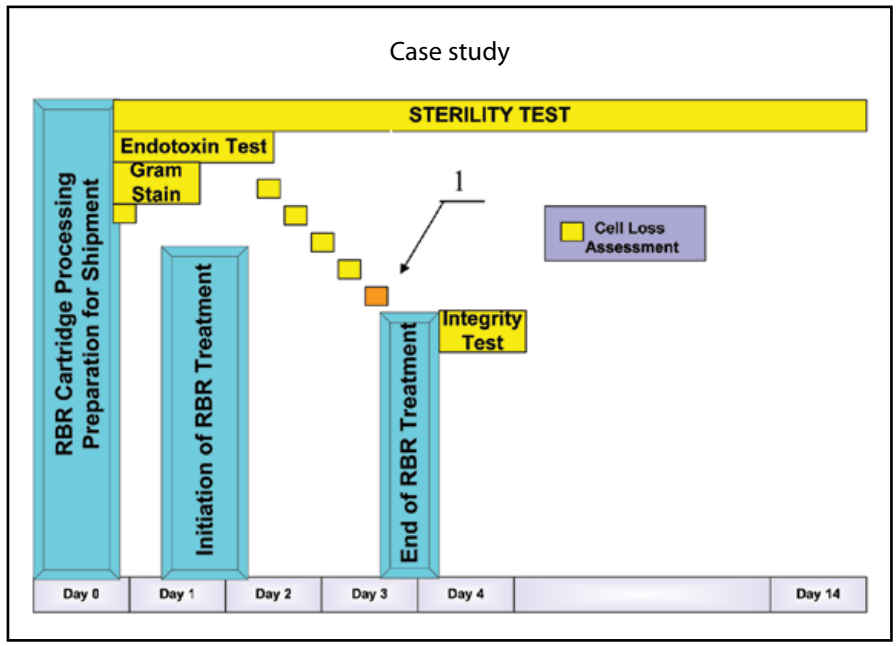


Figure 3. During patient treatment, an unusual observation (1) was made at the fifth measurement of the cell loss in the ultrafiltrate.

Investigation (RBRTC)

At the time of this investigation, the 14-day final product sterility data, obtained from the cartridge prior to shipment and necessary to fully confirm the sterility of the RBRTC, was not available. Nevertheless, a methodical assessment of all available data was performed to rule out the possibility of contamination.

The historical data on safety test results were once again reviewed — this was a re-evaluation of what was done prior to preparation for clinical shipment. The examination involved analyses of a) all steps of the RBRTC processing and historical data, b) preparation for and during shipment, c) receipt of the cartridge at the clinical site, d) clinical operations, and e) post-treatment assessment of the cartridge.

a) Historical data. The review demonstrated that all in-process safety test results were within specifications (i.e., no growth for 14 days) and that the sterility data for *maintenance* release were available from weekly sample collections during a time period of approximately five months. Sterility test results on a total of 18 data points were evaluated and confirmed to be acceptable. All environmental monitoring data were also acceptable.

b) Final preparations for shipment to the clinical site. The RBRTC processing before shipment to the clinical site was scrutinized and revealed that there was no visual observation of turbidity or change in the color of the culture medium. The Gram Stain results demonstrated no visible organisms. A three-day sterility test result post final manipulations was negative for bacterial and/or fungal growth.

c) Clinical operations. Records of the RBRTC receipt at the clinical site showed that the cartridge was intact upon receipt, and shipping temperature was within established specifications. There was no excessive cell loss for the first 32 hours of treatment obtained from four data points.

d) Post treatment assessment of the RBRTC. The cartridge passed the integrity test.

Investigation (Patient)

The course of the clinical treatment with the RBRT was analyzed to determine a) whether the patient experienced an adverse event, and b) whether there was an increased number of manipulations at the bedside that could have potentially led to the compromise of the RBRTC-CVVH system.

a) Adverse event. During the time of the investigation, the Principal Investigator (PI) determined that the patient, who was doing well for approximately 40 hours into the treatment, became septic. The patient's blood culture was tested and revealed *Staphylococcus epidermidis*, an inhabitant of the skin.

b) Bedside operations. There was no increase in the number of manipulations, such as change of a hemofilter and/or tubing sets, at the bedside. However, the PI noticed that the patient had a tear in the vascular catheter for the CVVH circuit and remained in the fetal position for more than 24 hours; therefore the patient's blood was exposed to open skin. This was a non-anticipated problem in the extracorporeal circuit.

This investigation showed that it was the compromised catheter that introduced bacterial contamination into the patient's blood stream, and hence adversely affected the HRTCs, consequently causing the beginning of the cell loss. A methodical assessment of safety data from multiple data points prior to and post RBRT allowed us to conclude that the RBRT used in patient treatment was not the source of contamination. The patient had a tear in the vascular catheter, which led to blood contamination by Gram-positive cocci. The catheter was used for conventional CVVH circuit and was determined to be the source of contamination. As all checkpoints of the Quality System showed no deviations from the established specifications, we were able to conclude that there were no sources of contamination other than the one identified by the PI. The patient recovered without sequelae.

Conclusions

The quality control safety program developed for the RBRT provides a high degree of assurance that a cellular therapy program can be carried out in a multisite mode, through strict adherence to cGMPs as set forth in existing regulations.

REFERENCES

1. Thadhani R, Pascual M, Bonventre JV. Acute renal failure N Engl J Med 1996;334:1448–1460.
2. Tang IY, Murray PT. Prevention of perioperative acute renal failure: what works? Best Pract Res Clin Anaesthesiol 2004;18:91–111.
3. Barretti P, Soares VA. Acute renal failure. Clinical picture and prognosis Rev Assoc Med Bras 1996;42: 67–72.
4. Abbate M, Remuzzi G. Acceleration of recovery in acute renal failure: from cellular mechanisms of tubular repair to innovative targeted therapies Ren Fail 1996;18:377–388.
5. Brivet FG et al. Acute renal failure in intensive care units--causes, outcome, and prognostic factors of hospital mortality; a prospective, multicenter study. French Study Group on Acute Renal Failure Crit Care Med 1996;24:192–198.
6. Liano F al. The spectrum of acute renal failure in the intensive care unit compared with that seen in other settings The Madrid Acute Renal Failure Study Group. Kidney Int Suppl 1998;66:S16–24.
7. Karsou SA, Jaber BL, Pereira BJ. Impact of intermittent hemodialysis variables on clinical outcomes in acute renal failure. Am J Kidney Dis 2000;35:980–991.
8. Mehta RL et al. A randomized clinical trial of continuous versus intermittent dialysis for acute renal failure. Kidney Int 2001;60:1154–1163.
9. Humes HD, Fissell WH, Weitzel WF. The bioartificial kidney in the treatment of acute renal failure Kidney Int Suppl 2002;80:121–125.
10. Humes HD et al. Renal cell therapy is associated with dynamic and individualized responses in patients with acute renal failure Blood Purif 2003;21:64–71.
11. Humes HD et al. Initial clinical results of the bioartificial kidney containing human cells in ICU patients with acute renal failure. Kidney Int 2004;66:1578–1588.
12. Food and Drug Administration [FDA]. Draft FDA Guidance on Screening and Testing of Donors of Human Tissue Intended for Transplantation. Rockville, MD. FDA; 1997 July.
13. Food and Drug Administration [FDA]. Draft FDA Guidance on Donor Eligibility for Human Tissues and Cells. FDA; 2004 May.
14. Humes HD et al. Metabolic replacement of kidney function in uremic animals with a bioartificial kidney containing human cells. Am J Kidney Dis 2002; 39:1078–1087.
15. Pitkin Z, McCarthy MC, Steel M. Quality Control Issues and Contract Manufacturing for Clinical Trials. Presented at the WCBP 2004 Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products; 2004. Washington, DC.
16. Barker LF. Food and Drug Administration regulations and licensure. Fed Proc 1975;34:1522–1524.
17. Food and Drug Administration [FDA]. FDA Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy. FDA; 1988 March 30.