Cytotoxicity of cannulas for ophthalmic surgery after cleaning and sterilization: Evaluation of the use of enzymatic detergent to remove residual ophthalmic viscosurgical device material

Natacha S.M. Tamashiro, RN, Rafael Q. Souza, RN, MS, Cláudia R. Gonçalves, PhD, Tamiko I. Ikeda, BS, Reginaldo A. Luz, RN, BSN, Áurea S. Cruz, PhD, Maria C. Padoveze, RN, PhD, Kazuko U. Graziano, RN, PhD

PURPOSE: To evaluate the cytotoxicity of reusable cannulas for ophthalmic surgery after the cannulas were filled with an ophthalmic viscosurgical device (OVD) and cleaned with an enzymatic detergent.

SETTING: Microbiological Testing Laboratory, Department of Medical-Surgical Nursing, University of São Paulo School of Nursing, and Cell Culture Section, Adolfo Lutz Institute, São Paulo, Brazil.

DESIGN: Experimental study.

METHODS: The sample consisted of 30 reusable 25-gauge injection cannulas, 20.0 mm in length, whose lumens were filled with an OVD solution for 50 minutes. The following steps were used to process the cannulas: (1) presoaking, (2) washing the lumen using a high-pressure water jet, (3) backwashing with enzymatic detergent in ultrasonic cleaner, (4) preliminary rinsing with tap water, (5) final rinsing with sterile distilled water, (6) drying with compressed filtered air, (7) wrapping in surgical-grade paper, and (8) steam sterilization at 134°C for 4 minutes. The cannulas were then tested for cytotoxicity according to the United States Pharmacopeia 32.

RESULTS: The cleaning protocol used in this study removed residues of OVD solution and enzymatic detergent as shown by the lack of cytotoxicity of all sample extracts.

CONCLUSION: This cleaning protocol has the potential to minimize the occurrence of toxic anterior segment syndrome associated with residues of OVD solutions and enzymatic detergents.

Financial Disclosure: No author has a financial or proprietary interest in any material or method mentioned.

J Cataract Refract Surg 2013; 39:937–941 © 2013 ASCRS and ESCRS

Toxic anterior segment syndrome (TASS) is an acute and rare postoperative inflammatory reaction caused by noninfectious substances introduced into the anterior chamber of the eye during surgical procedures. Toxic anterior segment syndrome may damage intraocular structures, including the corneal endothelium and trabecular meshwork.[1] The major signs and symptoms of TASS are anterior chamber reaction, diffuse corneal edema, hypopyon, visual impairment, fibrin formation in the anterior chamber, pupil dilation, and increased intraocular pressure. The onset of symptoms usually occurs on the first postoperative day; however, in some cases symptoms are observed up to 2 weeks after surgery.[2,3] Possible causes of TASS include irrigating solutions with inappropriate pH and osmolarity; high levels of endotoxin[4]; wrong doses of ophthalmic drugs[5]; ointments that enter the eye[3]; and contamination of ophthalmic instruments with residual detergent, endotoxins, metal residues, or denatured ophthalmic viscosurgical device (OVD) substances.[6–12]

The Central Sterile Supply Department (CSSD) is responsible for ensuring the complete removal of residual enzymatic detergents, endotoxins, metal residues, and residual OVD substances from instruments. However, in practice, problems may occur during the processing of ophthalmic instruments at the CSSD, resulting in improperly cleaned instruments

contaminated with residues, which can be potential causative agents of TASS. To improve cleaning and sterilization practices at the CSSD and prevent TASS, the American Society of Cataract and Refractive Surgery (ASCRS) and the American Society of Ophthalmic Registered Nurses (ASORN) published guidelines in 2007 entitled "Recommended Practices for Cleaning and Sterilizing Intraocular Surgical Instruments."[13] However, it is necessary to determine whether these recommendations are effective in removing contaminants and residues that can cause TASS from ophthalmic instruments. The purpose of this study was to evaluate the cytotoxicity of reusable injection cannulas after the cannulas were filled with an OVD solution and cleaned with an enzymatic detergent according to the ASCRS–ASORN recommendations.

MATERIALS AND METHODS

This experimental laboratory study was performed at the Microbiological Testing Laboratory, Department of Medical-Surgical Nursing, University of São Paulo School of Nursing, and at the Cell Culture Section, Adolfo Lutz Institute, São Paulo, Brazil. The sample consisted of 30 reusable 25-gauge cannulas, 20.0 mm in length, for the injection of OVD substances in ophthalmic surgery. This type of cannula was selected for the study because of its narrow lumen and the difficulty cleaning it.

The lumen of the cannulas was completely filled with an OVD solution (methylcellulose 2%). The solution remained in the lumen for 50 minutes to simulate a worst-case scenario in health care delivery regarding the processing of instruments that had been in contact with OVD substances during presoaking. After this period, the cannulas were processed according to the ASCRS–ASORN recommendations.[13] The cleaning and sterilization of the cannulas consisted of the following steps: (1) presoaking in tap water for approximately 5 minutes, (2) washing the lumen using a high-pressure water jet for 5 seconds, (3) backwashing with an enzymatic detergent composed of 4 enzymes (Riozyme IV) in an ultrasonic cleaner (Medisafe SI Digital, Medisafe UK Ltd.) for 15 minutes, (4) preliminary rinsing of the external surfaces with tap water and washing of the lumen using a high-pressure water jet, (5) final rinsing of each cannula with 10 mL of sterile distilled water for the external surface and 20 mL for the lumen, (6) drying with compressed filtered air, (7) individual wrapping in surgical-grade paper, and (8) steam sterilization at 134°C for 4 minutes.

After sterilization, the cannulas were tested for cytotoxicity using the extraction method described in the United States Pharmacopeia 32.[14] For every 0.2 g of the sample, 1 mL of Eagle minimal essential medium (MEM) containing 5% fetal bovine serum (FBS) was used as extraction fluid. The lumen of each cannula was flushed with 2 mL of culture medium and remained in contact with extraction fluid at 37°C ± 1°C (SD) for 24 hours. To meet the required weight:volume ratio, an extract was prepared by processing 3 cannulas. The extracts were tested for cytotoxicity; National Collection of Type Cultures (NCTC) clone 929 cells were suspended in Eagle MEM containing 10% FBS at a concentration of approximately 1.5×10^5 cells/mL, seeded into 12–well plates, and incubated at 37 ± 1°C for 24 hours. After a cell monolayer was formed, the culture medium was shifted to the test medium containing extract at 100% concentration. The plates were incubated once more at 37 ± 1°C for 24 hours. Next, the plates were examined by inverted microscopy.[15]

The negative control consisted of 6 cannulas ready to use and as supplied by the manufacturer. The positive control consisted of 6 cannulas whose lumen was completely filled with an OVD solution (methylcellulose 2%) for 50 minutes. The cannulas were then immersed in enzymatic detergent, according to the manufacturer's directions regarding dilution and immersion time, but not rinsed. Next, they were transferred to a beaker using tweezers and dried in air for 12 hours.

The same wrapping and sterilization procedures and cytotoxicity testing were performed for the positive and negative controls and test group. Cytotoxicity was determined by qualitative evaluation of morphologic changes in cell monolayers, as described in the International Organization for Standardization 10993-5:2009 (Table 1).[16] Validation of the experimental model was performed using latex extract, a well-known cytotoxic substance, and culture medium as a noncytotoxic substance.[16] The latex extract exhibited severe reactivity (grade 4) and the culture medium exhibited no reactivity (grade 0), validating the model used in the study.

RESULTS

The cleaning protocol was followed completely; however, more time was necessary to remove residual OVD material. Most samples required 7 seconds of washing instead of the 5 seconds originally recommended in the step 2 of the cleaning protocol, and therefore a continuous high-pressure water jet was used for up to 7 seconds to flush the lumen of the cannulas. No reactivity (grade 0) was observed for all sample extracts and extracts from negative controls tested against NCTC clone 929 cells; therefore, the extracts were considered noncytotoxic (Figure 1).

In the positive control group, it was possible to see with the naked eye a white layer of OVD material coating the cannulas (Figure 2), even after immersion in

Submitted: August 21, 2012.
Final revision submitted: December 22, 2012.
Accepted: December 31, 2012.

From the School of Nursing (Tamashiro, Souza, Luz, Padoveze, Graziano), University of São Paulo, and the Cell Culture Section (Gonçalves, Ikeda, Cruz), Adolfo Lutz Institute, São Paulo, Brazil.

Support received from the Brazilian National Council for Scientific and Technological Development in the form of undergraduate and doctoral scholarships, respectively. This independent public foundation had no role in the design and conduct of the study, including collection, management, analysis, and interpretation of data, preparation, review, and approval of the article.

Corresponding author: Natacha S.M. Tamashiro, RN, NSM/EEUSP, Avenida Dr. Enéas de Carvalho Aguiar, 419; CEP 05403-000, São Paulo, SP, Brazil. E-mail: natacha.tamashiro@usp.br, natacha_tamashiro@hotmail.com.

Table 1. Cytotoxicity grading of extracts based on qualitative morphological changes in NCTC clone 929 cells.

Grade	Reactivity	Cell Culture Conditions	Cytotoxic Effects
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction in cell growth.	Negative
1	Slight	No more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells; slight growth inhibition.	Negative
2	Mild	No more than 50% of the cells are round, without intracytoplasmic granules; no extensive cell lysis; no more than 50% growth inhibition.	Negative
3	Moderate	No more than 70% of the cell layers contain rounded or lysed cells; cell layers not completely destroyed; more than 50% growth inhibition.	Positive
4	Severe	Nearly complete or complete destruction of the cell layers.	Positive

NCTC = National Collection of Type Cultures
Adapted from ISO 10993-5:2009[16]

enzymatic detergent. Slight to mild reactivity (grades 1 and 2) was detected for extracts from positive controls, indicating that the extracts were not cytotoxic; however, changes in cell morphology and a reduction in cell growth were observed.

DISCUSSION

Toxic anterior segment syndrome is a postoperative complication of multifactorial etiology; however, many of its causes are directly related to the processing of ophthalmic instruments at the CSSD. One hypothesis that has been proposed is that the enzymatic detergent used to clean instruments that had been in contact with OVD substances may trigger TASS. It is difficult to remove hardened residual OVD material from instruments, especially from the lumen. These residues may adsorb enzymatic detergent, making it difficult to completely clean the cannula.[1]

Our findings indicate that residues of OVD materials and enzymatic detergent can be completely removed provided the ASCRS–ASORN recommendations are followed.[13] The use of a recommended method to test the biocompatibility of medical products, reproducing the contact between sample extracts and living cells such as occurs during surgical procedures, showed the absence of cytotoxic residues in the cannulas tested. Thorough cleaning using presoaking, high-pressure water jets, ultrasonic cleaner, and enzymatic detergent was effective in removing residual OVD solution. The results show that the cytotoxicity of enzymatic detergents is a problem that can be solved by extensive rinsing and that this type of detergent is suitable for cleaning instruments used in ophthalmic surgery. The model used in this study is adequately sensitive to detect cell damage caused by the toxic effects of products used in health care. Despite the low level of reactivity found in positive controls, it was possible to identify changes in cell morphology and a reduction in cell growth.

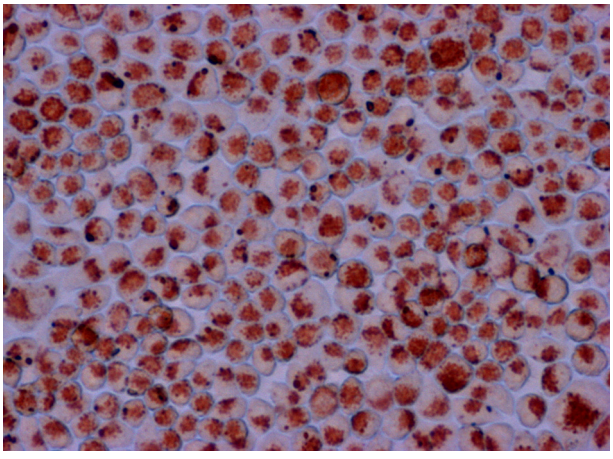


Figure 1. Micrograph of a confluent monolayer of NCTC clone 929 cells after 24 hours in contact with the extract from 3 reusable cannulas for injection of OVD substances (original magnification ×200).

The removal of residual OVD substances from cannulas with narrow lumens is a challenge for the CSSD. If the cleaning is inadequate, contaminants can be introduced into the surgical site during the procedure. In the preliminary cleaning procedures, additional flushing time was necessary to clear the lumen of a cannula from hardened OVD material. This suggests that measures should be implemented to prevent OVD substances from drying on the instruments during or after surgical procedures.

An important factor to be considered is the volume and quality of the rinsing water. Vague and subjective recommendations such as "rinse thoroughly" are not acceptable. The Association for the Advancement of Medical Instrumentation (AAMI) recommends that the volume of water used to rinse an instrument be 2 or 3 times the volume needed to completely immerse the instrument.[17] After the first rinse, the instrument should be rinsed again with high-purity water to minimize the deposition of substances present in the water, such as endotoxins and chemicals, which may be potential causative agents of TASS.[18]

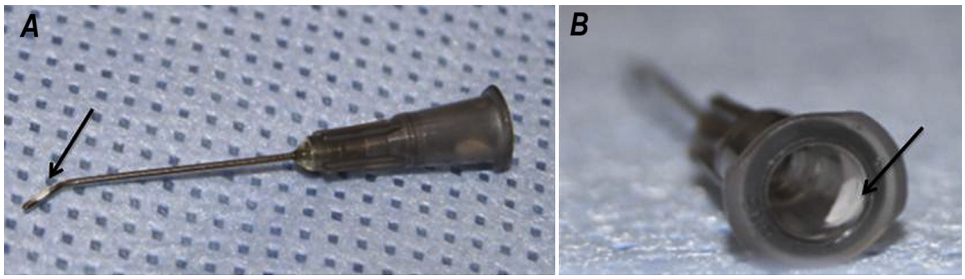


Figure 2. A 25-gauge cannula, 20.0 mm in length, for injection of OVD substances in ophthalmic surgery. White layer of OVD material coats the external surface of the cannula (*A*) and is seen on the internal wall (*B*).

In positive controls, dried OVD material adhered to the cannulas, forming a white layer, as described by Mathys et al.[10] Although the extracts from positive controls were classified as noncytotoxic (grades 1 and 2) based on the International Organization for Standardization 10993-5:2009,[16] reduction in cell growth and morphological changes were observed in cell monolayers, indicating that the extracts had potential cytotoxic effects. Changes induced in test cells may be associated with a precursor to or causative factor of TASS, as indicated in a study that compared eye and skin irritation tests in animals with in vitro cytotoxicity tests and reported on the predictive ability of cytotoxicity tests.[19]

The present study was designed to specifically evaluate in vitro the cytotoxicity of residual OVD materials in reusable cannulas for ophthalmic surgery after the cannulas were cleaned with enzymatic detergents. During ophthalmic surgery, cannulas also come into contact with blood and tissues, which represent other sources of contamination. This can potentially increase the amount of contaminants in cannulas, making removal of residues by the cleaning process more difficult. Further studies are being performed in the Microbiology Laboratory of the Department of Medical-Surgical Nursing and at the Cell Culture Section of the Adolfo Lutz Institute in Brazil to identify potential cytotoxic substances.

The ASCRS–ASORN recommendations regarding the cleaning and sterilization of instruments for ophthalmic surgery are in accordance with other guidelines, including those from the Association of Operative Registered Nurses,[20] AAMI,[21] and International Association of Healthcare Central Service Materiel Management,[A] which have stressed the extreme importance of cleanliness as the key factor in the reprocessing of surgical instruments and devices. In addition to these guidelines, cleaning instructions provided in the "Directions for Use" of many ophthalmic surgical instruments should be followed. These instructions may include, for example, the volume of sterile distilled water to be used to flush the instruments and the number of rinsing cycles. To achieve cleanliness standards, current technologies for cleaning and sterilizing surgical instruments must be used.

In conclusion, provided that cleaning and sterilization protocols used in CSSDs are in accordance with the ASCRS–ASORN recommendations, the use of enzymatic detergents to clean instruments that have been in contact with OVD substances does not result in cytotoxicity, which minimizes the occurrence of TASS.

WHAT WAS KNOWN

- ASCRS and ASORN published recommendations for cleaning and sterilization of intraocular surgical instruments.
- Empirical evidence and the peer-reviewed literature indicate that not following these recommendations may expose patients to residual cytotoxic substances and to the risk for TASS.

WHAT THIS PAPER ADDS

- Compliance with the ASCRS/ASORN recommendations for cleaning ophthalmic instruments ensured the removal of cytotoxic residues of OVD materials and enzymatic detergents, contributing to the prevention of TASS.

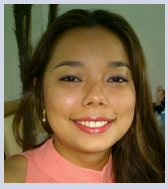
REFERENCES

1. Mamalis N, Edelhauser HF, Dawson DG, Chew J, LeBoyer RM, Werner L. Toxic anterior segment syndrome. J Cataract Refract Surg 2006; 32:324–333
2. Jehan FS, Mamalis N, Spencer TS, Fry LL, Kerstine RS, Olson RJ. Postoperative sterile endophthalmitis (TASS) associated with the MemoryLens. J Cataract Refract Surg 2000; 26:1773–1777
3. Werner L, Sher JH, Taylor JR, Mamalis N, Nash WA, Csordas JE, Green G, Maziarz EP, Liu XM. Toxic anterior segment syndrome and possible association with ointment in the anterior chamber following cataract surgery. J Cataract Refract Surg 2006; 32:227–235
4. Kutty PK, Forster TS, Wood-Koob C, Thayer N, Nelson RB, Berke SJ, Pontacolone L, Beardsley TL, Edelhauser HF, Arduino MJ, Mamalis N, Srinivasan A. Multistate outbreak of toxic anterior segment syndrome, 2005. J Cataract Refract Surg 2008; 34:585–590
5. Buzard K, Zhang J-R, Thumann G, Stripecke R, Sunalp M. Two cases of toxic anterior segment syndrome from generic trypan blue. J Cataract Refract Surg 2010; 36:2195–2199

6. Clouser S. Toxic anterior segment syndrome: how one surgery center recognized and solved its problem. Insight 2004; 29:4–7
7. Ünal M, Yücel I, Akar Y, Oner A, Altın M. Outbreak of toxic anterior segment syndrome associated with glutaraldehyde after cataract surgery. J Cataract Refract Surg 2006; 32: 1696–1701
8. Hellinger WC, Hasan SA, Bacalis LP, Thornblom DM, Beckmann SC, Blackmore C, Forster TS, Tirey JF, Ross MJ, Nilson CD, Mamalis N, Crook JE, Bendel RE, Shetty R, Stewart MW, Bolling JP, Edelhauser HF. Outbreak of toxic anterior segment syndrome following cataract surgery associated with impurities in autoclave steam moisture. Infect Control Hosp Epidemiol 2006; 27:294–298. Available at: http://www.jstor.org/stable/pdfplus/10.1086/501540.pdf. Accessed January 13, 2013
9. Centers for Disease Control and Prevention (CDC). Toxic anterior segment syndrome after cataract surgery—Maine, 2006. MMWR Morb Mortal Wkly Rep 2007; 56:629–630. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5625a2.htm. Accessed January 13, 2013
10. Mathys KC, Cohen KL, Bagnell CR. Identification of unknown intraocular material after cataract surgery: evaluation of a potential cause of toxic anterior segment syndrome. J Cataract Refract Surg 2008; 34:465–469
11. Cutler Peck CM, Brubaker J, Clouser S, Danford C, Edelhauser HE, Mamalis N. Toxic anterior segment syndrome: common causes. J Cataract Refract Surg 2010; 36: 1073–1080
12. Jun EJ, Chung SK. Toxic anterior segment syndrome after cataract surgery. J Cataract Refract Surg 2010; 36:344–346
13. American Society of Cataract and Refractive Surgery and the American Society of Ophthalmic Registered Nurses. Recommended practices for cleaning and sterilizing intraocular surgical instruments. J Cataract Refract Surg 2007; 33:1095–1100. Available at: http://www.eyeworld.org/ewsupplementarticle.php?id=200. Accessed January 13, 2013
14. The United States Pharmacopeia; the National Formulary, 32nd ed. Rockville, MD, United States Pharmacopeial Convention, Inc., 2009; 97–98
15. Cruz AS, Figueiredo CA, Ikeda TI, Vasconcelos ACE, Cardoso JB, de Salles-Gomes LF. Comparação de métodos para testar a citotoxicidade "in vitro" de materiais biocompatíveis [Comparison of methods to test the "in vitro" cytotoxicity of biocompatible materials]. Rev Saude Publica 1998; 32:153–159. Available at: http://www.scielo.br/scielo.php?script=sci_pdf&pid=S0034-89101998000200008&lng=en&nrm=iso&tlng=pt. Accessed January 13, 2013
16. International Organization for Standardization. Biological Evaluation of Medical Devices – Part 5: Tests for Cytotoxicity. Geneva, Switzerland, ISO, 2009; (ISO 10993–5)
17. Association for the Advancement of Medical Instrumentation. Water for the reprocessing of medical devices. (Technical information report n. 37). Arlington, VA, AAMI, 2003
18. Sarobe Carricas M, Segrelles Bellmunt G, Jiménez Lasanta L, Iruin Sanz A. Síndrome tóxico del segmento anterior: investigación de un brote [Toxic anterior segment syndrome (TASS): studying an outbreak]. Farm Hosp 2008; 32:339–343. Available at: http://apps.elsevier.es/watermark/ctl_servlet?_f=10&pident_articulo=13133027&pident_usuario=0&pcontactid=&pident_revista=121&ty=56&accion=L&origen=elsevier&web=www.elsevier.es&lan=es&fichero=121v32n06a13133027pdf001.pdf. Accessed January 13, 2013
19. Pinto TJA, Ikeda TI, Miyamaru LL, Santa MC, Santos BRP, Cruz AS. Cosmetic safety: proposal for the replacement of in vivo (Draize) by in vitro test. Open Toxicol J 2009; 3:1–7. Available at: http://www.scielo.br/pdf/rsp/v32n2/p153-159.pdf. Accessed January 13, 2013
20. AORN Recommended Practices Committee. Recommended practices for sterilization in the perioperative practice setting AORN J 2006; 83:700–722. Available at: http://download.journals.elsevierhealth.com/pdfs/journals/0001-2092/PIIS0001209206601981.pdf. Accessed January 13, 2013
21. Association for the Advancement of Medical Instrumentation. A compendium of processes, materials, test methods and acceptance criteria for cleaning reusable medical devices. (Technical information report n. 30). Arlington, VA, AAMI, 2003

OTHER CITED MATERIAL

A. Westermann G. Report on IAHCSMM in Wonderland; 45th annual conference of the International Association of Healthcare Central Service Materiel Management in San Diego on 1 – 5 May 2010. Zentralsterilisation 2010; 3:161–168. Available at "News Update": http://www.mhp-verlag.de/en/journals/zentralsterilisation/content/70/. Accessed January 13, 2013



First author:
Natacha S.M. Tamashiro, RN

School of Nursing, University of São Paulo, São Paulo, Brazil