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Cytotoxicity Assessment of Residual High-Level Disinfectants

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Some studies show the uptake of disinfectants on medical devices but no studies on their cytotoxicity have been reported. This study aimed to assess that cytotoxicity in a 3-dimensional culture system using HeLa cells grown in matrices composed of collagen. Plastic materials were soaked in the use solutions of the widely used high-level disinfectants, glutaraldehyde (GA), ortho-phthalaldehyde (OPA) and peracetic acid (PAA). After being rinsed, they were allowed to dry and were embedded into the cell medium to investigate the cytotoxicity of the residual disinfectants. Cytotoxicity was observed with the polyvinyl chloride, polyurethane and silicon tubes soaked in GA and OPA, indicating that both disinfectants were absorbed in the test pieces, whereas for PAA, none was observed. As for the polytetrafluoroethylene (PTFE) tubes, no disinfectant displayed cytotoxicity. GA and OPA are primary irritants, having a potential to cause anaphylaxis and other forms of allergic reactions. There should be consideration not only about the toxicity of the residual disinfectant from poor rinsing, but also about the toxicity that would result from the disinfectants that were absorbed and consequently released from the medical devices or materials.

Key words: High level disinfectants / Cytotoxicity assay / 3-Dimensional culture / Peracetic acid / Ortho-phthalaldehyde.

Glutaraldehyde (GA) has long been used as a high-level disinfectant; however, the health risks and danger to users from exposure to the disinfectant has become a problem. In Japan, it was recognized as a mutagenic substance that caused occupational health risks during the early 90’s. Due to this, safer alternatives were sought, leading to the investigation and eventually the approval of ortho-phthalaldehyde (OPA) and peracetic acid (PAA) as alternative high-level disinfectants.

As these disinfectants are frequently used during endoscopic procedures, there have been reports on the adverse effects of their residues, especially with OPA, since it causes discoloration of the skin and mucous membranes, irritation, anaphylaxis and other allergic reactions (Abdulla and Adams, 2007; Arrandale et al., 2012; Cooper et al., 2008; Miyajima et al., 2010; Suzukawa et al., 2007; Venticinque et al., 2003). These adverse effects are thought to be brought about by poor rinsing of medical devices. Nevertheless, in the case of allergic reactions, it cannot be disregarded that the mere existence of disinfectants themselves may cause allergic reactions regardless of their amount absorbed by medical devices or materials and released afterwards.

On the other hand, in evaluating the residual toxicity on surfaces of solid materials, since they do not dissolve in medium, evaluation is difficult. Although Lerones et al. (2004) and Miner et al. (2012) evaluated the residual toxicity of disinfectants with bacteria, because the structures of bacteria and cells differ, the results and method with bacteria are inapplicable. This present study used a 3-dimensional culture system with HeLa cells grown in matrices composed of collagen, enabling a study on solid materials. Each chosen plastic material was soaked in a disinfectant, rinsed and air-dried. Cell viability then served as an index to the toxicity of the disinfectant residues.

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HeLa cells (JCRB) were utilized with Eagles’s Minimum Essential Medium (EMEM, Dainippon Sumitomo Pharma Co. Ltd.) containing 10% fetal bovine serum (Life Technologies Corporation) as the medium.

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Sigma-Aldrich Co. LLC.

Phosphate buffer solution (PBS) was prepared with 8.0g NaCl, 0.2g KCl, 1.15g Na₂HPO₄, 0.2g KH₂PO₄ in 1L solution (All were purchased from Kanto Chemicals Co. Inc.).

The high-level disinfectants used for endoscopes, 0.3% PAA solution (Saraya Co. Ltd.), 2% GA solution (Maruishi Pharmaceutical Co. Ltd.) and 0.55% OPA solution (Johnson & Johnson services Inc.) were selected as test disinfectants.

For test materials, materials that constitute endoscope parts such as polyvinyl chloride (PVC) tubes, polyurethane tubes, silicone tubes and polytetrafluoroethylene (PTFE) test pieces were selected. After the tubes were cut to 5mm lengths and PTFE test pieces to 5x5mm squares, they were placed in a 10K bottle containing a solution of a disinfectant. Distilled water (DW) was used as the control, as well as for diluting the disinfectants to a solution according to the manufacturer’s instructions. The test pieces were allowed to soak in the disinfectants for 14 days. In order to maintain the use concentrations, the solutions were replaced with fresh ones on the 7th day. In the case of PAA, it was ensured that the concentration was still above the minimum recommended concentration (MRC, above 0.2%) before renewing the solution.

After the soaking period was over, the test pieces were brought into a laboratory bench and were handled aseptically. They were then rinsed thrice with 10mL DW, wiped with sterile gauze and allowed to dry.

The HeLa cells were cultured in a 10cm culture plate until they reached confluency followed by extraction with trypsin. Using CellMatrix I-A (Nitta Gelatin Inc.) and TransWell Permeable Support (Corning Inc.), the extracted cells were embedded into the collagen gel. The collagen gel was prepared according to the manufacturer’s directions. 10-fold of 10% EMEM, 10% of HEPES buffer solution (0.05M sodium hydroxide, 2.2% sodium bicarbonate, 200mM HEPES) and 80% CellMatrix I-A were mixed over ice. After the extracted cells were settled, the mixture was inoculated into the TransWell Inserts and incubated for 30 minutes to induce gelation. In order to displace the medium in the gel with FBS-containing EMEM medium, 2.5mL of EMEM medium was inoculated inside and outside the TransWell Inserts and allowed to stand in a 5%CO₂ incubator at 37°C.

While the medium was replaced daily, when the cells inside the gel reached confluency on the 3rd day, the cytotoxicity assay was performed using the test pieces immersed in disinfectants. The dried test pieces were gently embedded 2-3mm into the collagen gel. The assay was incubated in a 5%CO₂ incubator at 37°C for 3 days. After 3 days, the medium was removed and replaced with 0.5mg/mL MTT dye dissolved in medium followed by 2 hours incubation to dye the viable cells. The dye solution was removed and the gel was rinsed thrice with PBS.

First of all, with the PVC tubes, as shown in Fig.1, PAA and DW showed the same results: no cytotoxicity was observed as the area around the tubes was filled with living cells (black spots). With OPA, almost no living cells were observed around the tube. Based on these results, polyurethane tubes, silicone tubes and PTFE test pieces were used to evaluate the residual cytotoxicity of the 3 high-level disinfectants, PAA, GA and OPA. With living cells distributed around the polyurethane (Fig.2) and silicone (Fig.3) soaked in PAA, this disinfectant showed almost the same results as DW. However, the area surrounding the polyurethane and silicone soaked in OPA showed no living cells (Fig.4). As for the PTFE test pieces soaked in the three disinfectants, cytotoxicity was not observed (Fig.4).

Using a 3-dimensional culture system with HeLa cells grown in medium containing collagen enabled the study of cytotoxicity of solid materials. From the observed results, except for PTFE test pieces, it was clear that GA and OPA can be absorbed into plastic materials, eventually be released and are cytotoxic as well. PAA, on the other hand, did not display no cytotoxicity on all the test pieces.

This study only utilized tests pieces that were soaked

![Image](image_url)
in the disinfectants, rinsed, air-dried, and did not consider residual disinfectants due to poor rinsing as a factor of cytotoxicity. The cytotoxicity in terms of the amount of disinfectants absorbed cannot be discussed as they were not quantified but is assumed to be quite minimal due to rinsing. With this, it is thought that the presence of a substance is a problem regardless of its quantity. Furthermore, the surroundings of the tubes showed no living cells, suggesting that OPA and GA were absorbed into the tube materials and could be released later.

There have been several reports showing the presence of residual disinfectants on medical devices after soaking and rinsing, but almost no studies have
been done on the cytotoxicity of the disinfectants absorbed in medical devices. Studies on the uptake of GA to rubber and plastic materials have also been done, but none on its cytotoxicity (Power and Russell, 1989). Lerones et al. (2004) and Miner et al. (2012) did a unique study on the residual disinfectants and toxicity assay simultaneously. After soaking each test pieces in GA, OPA, etc., the toxicity was assessed in terms of the residual antibacterial activity. While there have already been studied on the residual disinfectants on medical devices after rinsing, none have reported the absorption and later release of residual disinfectants, as well as using the 3 selected high-level disinfectants used in this study. On porous materials (e.g. cotton and latex), the residual toxicity was reportedly high. On latex, OPA was reported to have a toxicity 300x higher than that of H2O2 (Lerones et al., 2004). Moreover, the antibacterial activity using Staphylococcus aureus showed that OPA effects lasted longest (Miner et al., 2012). This level of toxicity is risky for the patients undergoing an examination. While the number of rinsings can be increased as a countermeasure to residual disinfectants due to poor rinsing, their uptake by materials is thought to be cumulative through repetitions of the disinfection process. Hence, it is necessary to pay attention to what materials the medical devices are made of, especially those (e.g. PVC, polyurethane and silicone) that tend to absorb disinfectants. While reports on the adverse effects of residual disinfectants were thought to be due to poor rinsing, the absorption by materials and toxicity related to the later release of disinfectants should not be ignored, as anaphylaxis and other sorts of allergic reactions have been reported to be due to this phenomenon (Suzukawa et al., 2006; Suzukawa et al. 2007; Sokol, 2004; Zeiger et al., 2005).

The high-level disinfectants utilized in this study are sensitizing agents, especially in their concentrated form. GA is known to cause hereditary virulence and carcinogenicity including mutagenicity, and due to this, safer alternatives were sought. During the assessment of the risk alternative disinfectants would pose to the hospital staff, OPA was found to be a potential sensitizer towards the skin and respiratory system (Fujita, 2006), while no allergic reactions occurred with PAA and H2O2 (Rideout and Teschke, 2005; Zeiger et al., 2005). To protect the patients as well as the hospital staff, selection of safer disinfectants, improvement of the operating environment, modification of the rinsing process, use of materials less likely to absorb disinfectants, etc., are necessary.

From the evaluation of the present bioassay, it was clear that GA and OPA are absorbed in materials constituting medical devices such as PVC, polyurethane and silicone, and would eventually be released later. While it is widely known that poor rinsing leading to residual disinfectants and steam inhalation resulting in respiratory disorders pose risks to patients and hospital staff, the mere presence on materials of disinfectants, regardless of their amount, the cause adverse effects like allergic reactions, must not be neglected.

REFERENCES