Assessment of the biocompatibility of process chemicals used for decontamination of medical instruments

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To assess the biocompatibility of residues of process chemicals on the surfaces of reprocessed medical instruments, risk assessment data on the cytotoxic properties, systemic toxicity, irritation and sensitization potential as well as, if applicable, on the haemocompatibility products must be taken into account. In a stepwise test program the cytotoxic characteristics of four products containing detergent and disinfectant substances typically used for decontamination of medical instruments were investigated. The tests revealed that, as expected, disinfectant substances are cytotoxic even in diluted solutions, albeit less intensely so. Other constituents, such as non-ionic surfactants or corrosion inhibitors can also be cytotoxic. The adsorption behaviour evinced by process chemicals when interacting with process challenge devices (PCDs) is a determinant of the cytotoxicity. The stepwise test program performed with diluted solutions and PCDs is suitable for elucidating the cytotoxicity of process chemicals. In combination with the data already available in the majority of cases on the systemic toxicity as well as on the irritation and sensitization potential of the constituent substances, the biocompatibility of process chemicals can thus be evaluated within the framework of risk assessment.

KEY WORDS
- instrument decontamination
- biocompatibility
- cleaning
- disinfection

Introduction

Before medical devices are first used on patients, the safety-related characteristics of a device must be ascertained during a risk assessment in accordance with the European Medical Devices Directive (1, 2). For reprocessed medical devices the same safety standards apply as for the original devices. For that reason similar safety assessments must be performed for medical instruments decontaminated using a manual or automated method of cleaning and disinfection. Likewise, the potential risks posed by the process chemicals used for decontamination must be estimated.

For automated decontamination of medical instruments this safety assessment is carried out at the time of validation of the cleaning and disinfection processes. To that effect, the amount of residues in the rinse water is determined, either by measuring the conductivity of alkaline cleaning processes or using analytical methods for neutral detergents. Based on calculations made for dilution of the process chemicals while the process is being executed in the washer-disinfector (WD), a safety estimate is obtained (3, 4). Investigations into the cytotoxic and haemolytic properties of typical process chemicals for such decontamination processes demonstrate that in the majority of cases the calculation method provides adequate safety in terms of the biocompatibility of potentially adhering process chemicals (5).

While compiling the guidelines for «Standardized manual cleaning as well as manual chemical disinfection of medical devices» and for «Validation of automated cleaning and disinfection processes for decontamination of heat-sensitive endoscopes» it was necessary to devise methods to identify and evaluate chemical residues on medical devices after decontamination, in particular with regard to biocompatibility with human tissues.

To devise and specify a uniform methodology for assessment of the biocompatibility of such chemical residues as well as to verify the acceptance values at the time of performance qualification of the respective decontamination processes, a working group was set up comprising experts from member enterprises of the German Industrial Association for Hygiene and Surface Protection (IHO), Faculty of Healthcare. Moderated and coordinated by Assistant Professor Dr. Holger Biering, the following experts are offering their services to the working group: Dr. Richard Bloß (Bode Chemie), Dr. Kai Groh (Merz Hygiene), Dr. Thomas-Jörg Hennig (B. Braun Medical), Dr. Elmar Hjorth (Dr. Schumacher), Markus Kamer (Dr. Weigert), Dagmar Martini (Bode Chemie), Dr. Andreas Otte (Ecolab), Axel Schneider (orochemie), Michael Schreiner (Schülke & Mayr), Anna-Maria Sprünken (Merz Hygiene), Dr. Matthias Tschoerner (Dr. Weigert).

This paper presents the results of cytotoxicity tests of four products containing the detergent and disinfectant substances typically used for manual decontamination as well as for automated chemothermal decontamination. Cytotoxicity testing was conducted using a stepwise test program, where the concentration limits of the products were first determined in diluted solutions and, next, the cytotoxicity implications of interaction with the relevant materials of the decontaminated medical devices were ascertained. On the basis of the experimental cytotoxicity results, together with the data available for the constituent substances on systemic toxicity as well as on the irritation and sensitization potential, the various options for using one uniform method to assess the biocompatibility of such detergent and disinfectant products is discussed.

Material and Methods

Products tested

The following process chemicals used for cleaning and/or disinfection of medical instruments were tested:

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Table 1: Proliferation inhibition in [%] of diluted solutions of the frame formulations (values greater than 30 % proliferation inhibition – in red – are classified as being cytotoxic)

Concentration		Frame formulations			
Vol%	ppm	Product A	Product B	Product C	Product D
1	10000	100	100	100	100
0.1	1000	100	100	100	100
0.01	100	100	12	100	100
0.001	10	8	0	65	78
0.0001	1	0	2	22	23

- Product A: liquid disinfectant containing 10 – 25 % glutaral, 10 – 25 % ethanol and water.
- Product B: liquid two-component disinfectant, Component 1 containing 1 – 5 % peracetic acid, 8 – 35 % hydrogen peroxide, < 10 % acetic acid and water; Component 2 containing 2 – 5 % sodium hydroxide and water.
- Product C: liquid disinfectant and detergent, containing < 10 % quaternary ammonium compound (QAV), < 10 % diamine, non-ionic surfactants, solvents, complexing agents and water.
- Product D: liquid detergent containing 5 – 15 % fatty alcohol alkoxylate, solvent and water.

Process challenge devices (PCDs) tested

The PCD materials used for cytotoxicity testing were as follows:

- Test discs measuring 50 × 50 × 2 mm and made of stainless steel X20Cr13 (1.4021/AISI 420), brushed surface, representative of non-cutting medical instruments,
- Test discs measuring 30 × 50 × 1 mm and made of liquid silicone rubber (LSR) endowed with 30 Shore A hardness, representative of anaesthesia equipment.

Cytotoxicity test

To test for any cytotoxic effects, in vitro cytotoxicity tests were performed in accordance with DIN EN ISO 10993-5 (6).

The test organisms employed were L 929 cells (DSM ACC 2, mouse fibroblasts, strain L clone). The cell culture medium (Dulbecco's Modified Eagle Medium – DMEM) contained 10 Vol% foetal bovine serum (FBS), 100 U/ml penicillin and 100 µl/ml streptomycin.

The positive control used was 6.0 Vol% dimethyl sulfoxide solution (PK-DMSO). As an additional positive control, polyvinyl chloride (PK-PVC) was eluted with the cell culture medium, while protected against light, for 24 ± 2 h at 37 ± 1 °C. As a negative control, polyethylene was eluted with cell culture medium, while protected against light, for 24 ± 2 h at 37 ± 1 °C. Based on EN ISO 10993-5 (6), proliferation inhibition of more than 30 % compared with the reagent control was considered to be a cytotoxic effect.

Concentration limits in solutions

To identify the cytotoxic concentration limit, solutions with different concentrations of the test products were prepared and mixed with the cell culture medium. A physiological saline solution, which likewise was mixed with cell culture medium, was used as reagent control. Triplicate batches of aliquots of 100 µl were pipetted into the wells of a 96-well cell culture plate. Aliquots of 50 µl of a freshly prepared cell suspension ($7.0 \times 10^4 - 1.5 \times 10^4$ cells/ml) were seeded out to the batches. The final concentrations of the solutions in the test batches were 5.0, 1.0, 0.1, 0.01, 0.001 and 0.0001 Vol%). The cell culture plate was incubated for 72 ± 6 h at 37 ± 1 °C. Next, the protein content was measured using a colorimetric method (7) and the proliferation inhibition calculated.

Cytotoxicity to PCDs

PCDs made of instrument steel and silicone were used to identify the effect of process chemicals on the materials used to manufacture medical devices. On the test day, solutions of the products to be tested were prepared with deionized water in the concentrations 1.0, 0.1, 0.01 and 0.001 Vol %. One each of the two PCDs was immersed for 1 h in the respective test solution, then positioned vertically for 15 s on a paper support to dry and then left to dry for 1 h at room temperature.

The PCDs were eluted with 1.5 Vol% DMSO in cell culture medium, while protected against light, for 24 ± 2 h at 37 ± 1 °C. A surface-volume ratio of 4.5 cm^2/ml elution medium was used, which according to the specifications of EN ISO 10993-12 (8) corresponds to a ratio of 3 cm^2/ml in the cell culture batch. As reagent control 1.5 Vol% DMSO in cell culture medium was incubated for 24 ± 2 h at 37 ± 1 °C without test material.

The elution solutions and reagent control were pipetted in triplicate batches into the wells of a 96-well cell culture plate, as had been done for identification of the concentration limit, seeded out with cell suspension, incubated in an incubator, followed by determination of the protein content and calculation of proliferation inhibition.

Results

Determination of the concentration limits of products in solutions

The concentration limits below which no cytotoxic effects could be measured on further dilution varied greatly for the test products in accordance with their composition (Table 1). The highest concentration limit, and hence the relatively lowest cytotoxic properties, was ascertained for Product B, a disinfectant based on neutralized peracetic acid, in a 100 ppm dilution. Product A, the disinfectant based on glutaral, did not exhibit any cytotoxic effects as from a 10 ppm dilution, which was greater by one order of magnitude. Even more pronounced cytotoxic effects were observed for Product D, the neutral detergent based on non-ionic surfactants, and for Product C, the disinfectant based on QAV/amine. For both these two products no cytotoxic effects were observed only in the 1 ppm dilution.

Determination of the cytotoxicity of products to PCDs

To identify the cytotoxicity of product residues to PCDs made of silicone and instrument steel the same test concentrations, as used for the dilutioned solutions, were chosen but without the upper and lower dilution stages of 5 Vol% and 0.0001 Vol%. As expected in these tests, higher product concentration limits were observed in diluted solutions (Tables 2 and 3). The glutaral-based disinfectant (Product A) and neutralized peracetic acid (Product B) did not exhibit any cytotoxic effects up to the maximum test concentration of

Table 2: Proliferation inhibition in [%] on surfaces of instrument steel PCDs after one-hour interaction with diluted solutions of the frame formulations (values greater than 30 % proliferation inhibition – in red – are classified as being cytotoxic)

Concentration		Frame formulations			
Vol%	ppm	Product A	Product B	Product C	Product D
1	10000	0	15	100	71
0.1	1000	5	20	79	33
0.01	100	3	13	33	17
0.001	10	3	19	31	17

1 Vol% to either the instrument steel or silicone PCDs.

Weakly toxic effects to both materials were observed for the neutral detergent based on non-ionic surfactants (Product D) at a concentration of 0.1 Vol%, but these were not manifested when further diluted. The greatest cytotoxic potential was observed for the disinfectant based on QAV/amine (Product C). For this product cytotoxic effects were seen up to a concentration of 0.01 Vol% on silicone PCDs and 0.001 Vol% on PCDs made of instrument steel.

Discussion

Following decontamination of medical instruments, the medical devices must meet the same safety requirements in terms of functionality and biocompatibility as when first used. As such, the process chemicals used for cleaning and disinfection during decontamination, or any of their residues found on the surfaces, must not have any negative impact on the biocompatibility of the medical device. That can be assured by thoroughly rinsing medical instruments after each step of the decontamination process for which chemicals are used.

However, in medical device decontamination practices the question arises as to when a cleaning process is sufficiently intense to assure biocompatibility. For automated decontamination in washer-disinfectors (WDs), the cleaning processes are preset. Tests show that dilution of process chemicals with a calculated entrainment of up to 10 % cleaning solution from one process step into the next is adequate for the majority of detergents and neutralizing agents to assure biocompatibility. Special attention must be paid at the time of validation of automated decontamination processes when using neutral detergents based on non-ionic surfactants and for disinfectants. In such cases it is recommended to analyze the residual amount of process chemicals in the final rinse water during performance qualification and compare this with the specifications issued by the manufacturer of the respective process chemicals (5).

For manual cleaning and disinfection of medical instruments there are in most cases no defined specifications for rinsing instruments. While compiling the guideline on «Manual decontamination» it was necessary to devise methods and strategies for verification of the cleaning processes in standard operating procedures at the site of use. That task was assumed by a group of experts from the Industrial Association for Hygiene and Surface Protection, Faculty of Healthcare.

As a first step, the manufacturer/supplier of detergents and disinfectants for manual decontamination of medical instruments set about developing a uniform methodology to identify an acceptance value (limit value) for process chemicals on the surfaces of medical devices.

Discussion of the methodology for assessment of the biocompatibility

For new medical devices biocompatibility testing is performed in accordance with the standard ISO 10993 «Biological evaluation of medical devices». Depending on the expected contact time between the medical instrument and the human body and the type of body tissue, Part 1 of the standard «Evaluation and testing in the risk management process» (9) stipulates the parameters to be taken into account. It is obvious that this procedure should also be used for assessment of process chemicals. The majority of manually reprocessed medical devices have a contact time < 24 h with human tissue. From this, methods can be devised for evaluation and/or testing of systemic toxicity, cytotoxicity, irritation and the sensitization potential of process chemicals. Depending on how the instruments are used, it may be necessary in addition to take account of the haemocompatibility of the products.

To estimate the potential hazards posed by process chemicals, first of all, orientational tests were conducted on the cytotoxicity of four products containing the typical detergent and disinfectant substances used for manual instrument decontamination in aqueous solutions in different concentrations. It was revealed that, as expected, disinfectants are also cytotoxic in diluted solutions, albeit less intensely so depending on the microbicidal agent (Table 1). Product B based on neutralized peracetic acid was no longer cytotoxic as from a 100 ppm concentration level, whereas for Product A (based on glutaral) a dilution that was higher by one order of magnitude, and for Product C (active ingredient: QAV/amine) by two orders of magnitude were needed to reach that level. Other constituent substances, such as non-ionic surfactants, can also have a cytotoxic effect (10), as confirmed by the results obtained for Product D. Non-ionic surfactants are also used to achieve a detergent effect in disinfectants, in particular in those based on quaternary ammonium compounds and/or amines. In such cases the constituent substances generate a cumulative cytotoxic effect, something that explains the very low concentration limit in the case of Product C.

Following orientational cytotoxicity testing of aqueous solutions of the test products, cytotoxicity testing of product residues on surfaces after contact with the product solutions should permit insights of relevance to daily practice. As expected, the results obtained for the concentration limits in these series of tests were higher than those obtained for direct contact between cells and the solution. The adsorptive effects of the constituent substances of process chemicals when they interact with the silicone and stainless steel surfaces seem to be the chief determinant of the cytotoxicity potential of products.

It is well known that quaternary ammonium compounds are easily adsorbed to surfaces. That explains the results achieved for Product C, which reached, or undershot, the cytotoxicity limit value on both (PCD) materials only in the highest dilution stage of 10 ppm. Moreover it must be borne in mind that other potentially cytotoxic

Table 3: Proliferation inhibition in [%] on surfaces of silicone PCDs after one-hour interaction with diluted solutions of the frame formulations (values greater than 30 % proliferation inhibition – in red – are classified as being cytotoxic)

Concentration		Frame formulations			
Vol%	ppm	Product A	Product B	Product C	Product D
1	10000	25	5	100	59
0.1	1000	11	0	100	33
0.01	100	2	0	47	8
0.001	10	20	12	25	5

constituents of Product C, such as non-ionic surfactants and complexing agents, also adsorb to surfaces and can reinforce the product's cytotoxic properties on surfaces. The results for the detergent (Product D) confirm the effect mediated by the adsorptive properties of non-ionic surfactants on the cytotoxicity of the product.

Other constituents, such as the microbidical agents glutaral or peracetic acid, have a lower adsorption potential. Accordingly, for Product A (active ingredient: glutaral) and Product B (active ingredient: PAA) no cytotoxic effects can be identified on the surfaces of either materials up to the highest concentration of 1 % tested (Tables 2 and 3).

The tests demonstrate that it is not just the cytotoxic properties of the constituent substances of process chemicals that are of importance, but also their ability to interact with the surfaces of medical instruments. For that reason it is recommended that the cytotoxic characteristics of process chemicals be elucidated in experiments.

The systemic toxicity as well as the sensitization and irritation potential of process chemicals can in the majority of cases be assessed on the basis of the vast repository of data available for the constituent substances. Experimental testing should be performed for process chemicals only if no valid data are available for certain substances or if the substances in the respective mixture interact with each other and the toxicological potential of the reaction products is not known.

Proposal for assessment of biocompatibility

The Working Group recommends the following methodology for assessment of the biocompatibility of process chemical residues:

- Experimental elucidation of the cytotoxic properties of diluted solutions of process chemicals and of product residues on various surfaces.
- Assessment of the systemic toxicity as well as of the irritation and sensitization potential on the basis of the data already available for the respective substances.
- Evaluation of data within the framework of biocompatibility assessment.

It may be necessary to take account of other parameters for risk assessment in cooperation with the manufacturer of the medical instrument:

- The haemocompatibility of process chemicals depending on the intended use of the medical device
- Ageing processes in synthetic materials and associated changes in the surface properties of medical devices
- Penetration of constituent substances into medical devices.

Based on risk assessment, limit values are defined for acceptance of the respective process chemical on medical instruments in µg/cm² or µg/instrument. It may be beneficial to specify different acceptance values for a process chemical in accordance with the material composition of the instrument (e. g. synthetic material or stainless steel) or the area in which reprocessed instruments are used.

Methods are being currently developed for verification of these acceptance values, and possibly also for routine control, while reviewing the standard operating instructions for decontamination processes at the user's premises, and these will soon be published.

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References see p. 32

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