Biocompatibility of medical devices after automated reprocessing in washer-disinfectors

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The cytotoxicity and haemolysis potential (indicators of biocompatibility) of five typical process chemicals used for automated reprocessing of medical devices were experimentally investigated in a graduated test program. The tests revealed that certain constituents of the process chemicals, such as microbicidal substances or non-ionic surfactants, had an important effect on the cytotoxic and haemolytic properties of the products. When validating process chemicals containing such substances it is recommended that the final rinse water be tested for entrainment of such products and that the rinse water test intervals be based on the results obtained.

KEY WORDS
- instrument reprocessing
- biocompatibility
- cleaning
- disinfection

Introduction

EN ISO 15883 decrees that for the automated reprocessing of medical devices the cleaning process must guarantee that a residual concentration of process chemicals is below a potentially dangerous limit (1). These limit values should be specified by the manufacturer or supplier of the process chemicals, but the standard does not stipulate the exact methods to be used to determine this.

On the other hand, the European directive on medical devices (2) specifies, inter alia, that medical devices must meet the safety requirements set out in the pertinent standards before being placed on the market. Consequently, every reprocessed medical device must be endowed with the same safety characteristics as the original device.

The mutual tolerance (biocompatibility) between the materials used and human tissue and body fluids can be verified and evaluated using the EN ISO 10993 series of standards «Biological evaluation of medical devices». The test parameters will depend on the nature of the intended contact between the medical device and human body as well as on the contact time. For the majority of medical devices reprocessed in washer-disinfectors (WDs) this in turn highlights the need for biocompatibility tests, taking account of cell toxicity, sensitisation potential, irritant effect and, if applicable, the haemocompatibility as well as the systemic toxicity, as dictated by Part 1 of the standard «Evaluation and testing within a risk management system» (3). Whereas some of the parameters call for corresponding tests, other parameters can be evaluated on the basis of the existing data.

For validation of automated processes in a WD for reprocessing medical devices, the residual quantity of process chemicals in the rinse water is determined on the basis of the conductivity in the case of alkaline cleaning processes or by means of analytical methods when using neutral detergents. This procedure involves calculations for dilution of process chemicals during the course of the process (4, 5) in the WD as well as confirmation of the biocompatibility of the calculated residual quantities by the process chemicals' manufacturer. This paper presents the results of cytotoxicity and haemolysis tests (as parameter of haemocompatibility) carried out for selected process chemicals that can be used in thermal or chemothermal reprocessing processes. First, the cytotoxicity and haemolysis limit concentrations of the process chemicals were determined in diluted solutions for both test models. Then the influence of the process chemical/medical device interaction on cytotoxicity was determined under laboratory conditions and in an actual reprocessing process. The results obtained are compared to the calculated residual quantities of process chemicals entrained in the rinse water during the cleaning cycles.

Materials and Methods

Products tested

The following process chemicals used for automated thermal and chemothermal reprocessing of medical devices were tested:

- Product A: liquid mildly alkaline detergent, contains phosphates, silicates, alkaline constituents and corrosion inhibitors, use concentration: 5 ml/l.
- Product B: liquid neutral detergent, contains non-ionic surfactants, enzymes, glycols and solubilizers, use concentration: 5 ml/l.

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Table 1: Concentration of process chemicals calculated in final rinse water in washer-disinfector with 5 % and 10 % entrainment as per (5)

	Entrainment into cleaning solution	Process steps				
		Pre-wash	Cleaning	Neutralisation or chemo-thermal disinfection	Intermediate rinse	Disinfection or final rinse
Detergent 5 ml/l	5 %	0 ppm	5000 ppm	250 ppm	12,5 ppm	0.6 ppm
	10 %	0 ppm	5000 ppm	500 ppm	50 ppm	5 ppm
Neutralising agent 3 ml/l	5 %	0 ppm	0 ppm	3,000 ppm	150 ppm	7.5 ppm
	10 %	0 ppm	0 ppm	3,000 ppm	300 ppm	30 ppm
Desinfectant 12 ml/l	5 %	0 ppm	0 ppm	12,000 ppm	600 ppm	30 ppm
	10 %	0 ppm	0 ppm	12,000 ppm	1,200 ppm	120 ppm

– Product C: liquid neutralisation agent, contains aqueous citric acid solution, use concentration: 3 ml/l.
– Product D: liquid neutralisation and detergent agent, contains phosphoric acid solution, use concentration: 3 ml/l.
– Product E: liquid disinfectant, contains 20 % glutaral, use concentration: 12 ml/l

Calculation of the theoretical concentration in final rinse water

While reprocessing medical devices in washer-disinfectors, process chemicals can be entrained into the rinse water via cavities in the medical devices and in the WD as well as in residues of the rinse water adhering to the medical devices. In general, this is thought to amount to 5 %, in rare cases to 10 %, entrainment in the various process steps (4, 5). The theoretical residual quantities reported in (5) for detergents at a use concentration of 5 ml/l and neutralisation agent for a use concentration of 3 ml/l were supplemented with similar calculations for the disinfectant at a use concentration of 12 ml/l (Table 1), where the disinfectant step was calculated instead of neutralisation. The residual quantities of process chemicals calculated with 10 % entrainment can be viewed as maximum quantities (5). Other process steps, such as intermediate rinses or neutralisation before disinfection, result in lower quantities of process chemicals being calculated in the rinse water. It must also be borne in mind that in the case of alkaline cleaning followed by neutralisation, the residual quantities are also reduced because of neutralisation reactions.

Cytotoxicity test

To investigate potential cytotoxic effects, in vitro cytotoxicity tests were conducted pursuant to DIN EN ISO 10993-5 (6).

In the cell culture medium (Dulbecco's modified eagle's medium with 10 % calf serum, 2% penicillin/streptomycin), Balb / 3T3 A31 mouse fibroblasts were cultured and transferred to microtiter plates in a density of around 2000 cells/well and incubated for 48 h (6). Used as controls were a cell culture medium (negative control) as well as a 0.01 % solution of sodium lauryl sulphate (positive control). A reduction in cell viability of 30 % or more is deemed to be cytotoxic as per DIN EN ISO 10993-5 (6).

Limit concentrations in solutions

To determine the cytotoxic limit concentration, solutions of the test products were prepared using graduated concentrations in the cell culture medium. The cell culture medium in the microtiter plates was replaced with the test product solutions. The viability of the cell cultures was determined by means of the MTT test (7) 24 h after substance application. A series of six such tests were carried out.

Cytotoxicity on process challenge devices (PCDs)

To determine the influence exerted by process chemicals on the materials used to produce medical devices, silicone tube segments measuring 6 cm in length and with an internal diameter of 10 mm and wall thickness of 2 mm were used as PCDs (supplier: VWR International, Order Number 228-0730).

Under laboratory conditions these PCDs were rinsed in each case in 18 ml diluted solutions of the test products for 15 minutes at room temperature. The test product solutions were produced using demineralised sterile water. After removal of the PCDs from the solution, they were left, half standing, to dry in the air for 30 minutes. The PCDs were extracted as per the specifications of DIN EN ISO 10993-12 with cell culture medium (8). All test steps were conducted in duplicate. An untreated

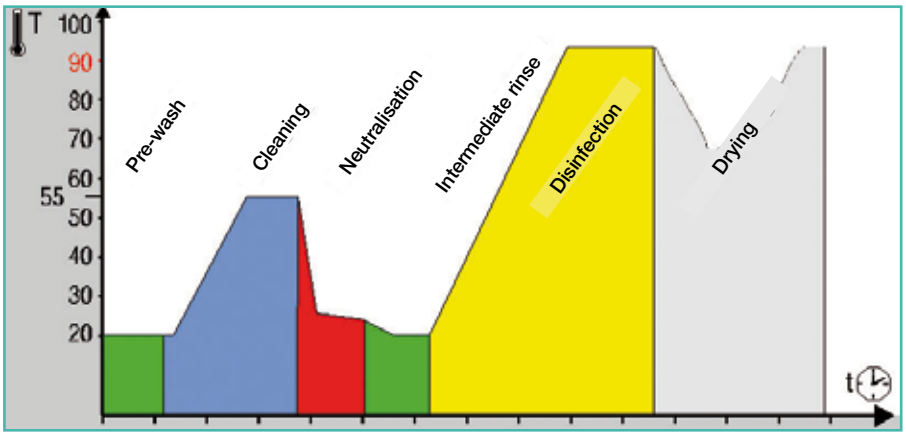

Fig. 1: Temperature and time course of process steps for thermal reprocessing in washer-disinfector

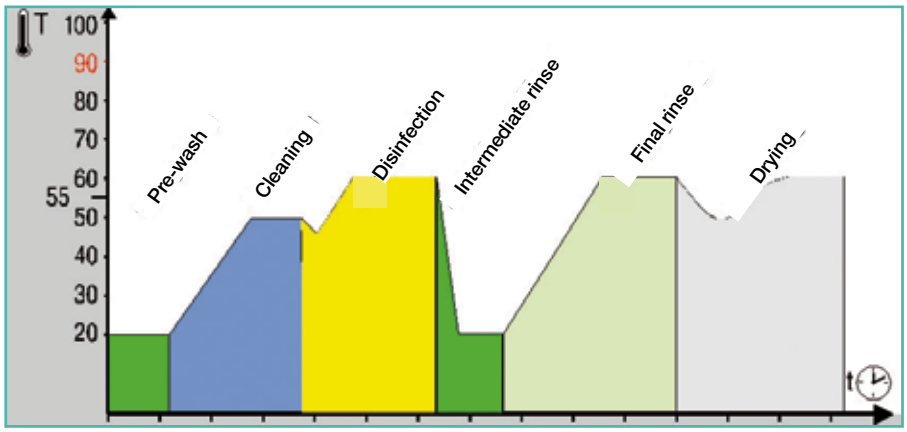

Fig. 2: Temperature and time course of process steps for chemothermal reprocessing in washer-disinfector

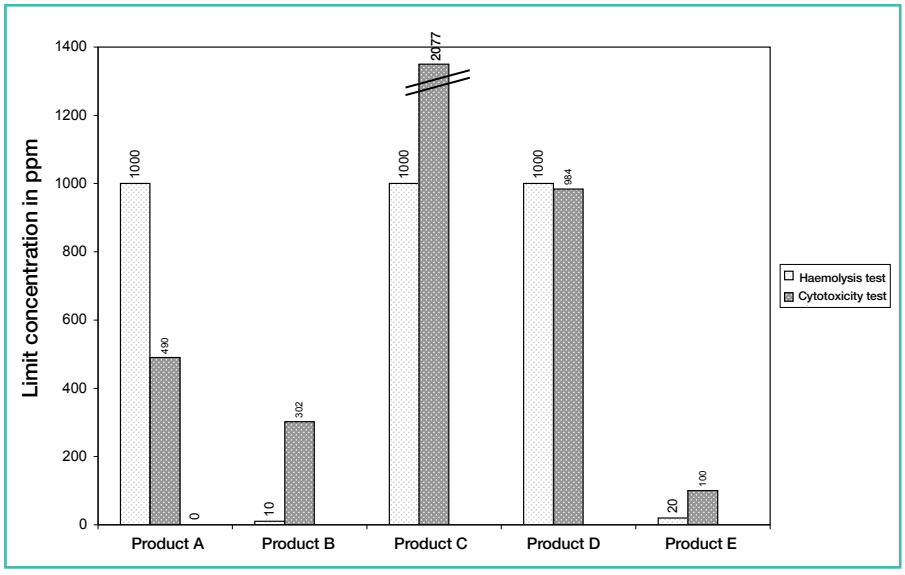

Fig. 3: Limit concentrations of test products in diluted solutions in the cytotoxicity and haemolysis tests

PCD was used as negative control for the further tests.

As in the test procedure used to determine the limit concentration, the cell culture medium was replaced with the extraction solution. Viability of the cell cultures was measured by means of the MTT test (7) 24 h after substance application. A series of six such tests were carried out.

Using a reprocessing process reflecting everyday use conditions, the PCDs were placed in a washer-disinfector G7836 manufactured by Miele and exposed to a thermal reprocessing process using the process steps featured in Figure 1 and to a chemothermal reprocessing process with the process steps illustrated in Figure 2 excluding the drying step. For the thermal reprocessing process, the detergents Product A and Product B were used in combination with the neutralisation agents Product C and Product D. For the chemothermal reprocessing process, product B was used in the cleaning step and Product E in the disinfection step. At the end of the reprocessing process, the PCDs were removed from the WD, left to dry in the air, half standing, for 30 minutes and then extracted in the same way as for the tests conducted under laboratory conditions. Next the viability of the cell cultures was determined. An untreated PCD was used as negative control for the further tests.

Haemolysis test

The haemolysis test was used as a parameter to investigate the haemocompatibility. To ascertain the haemolytic potential, solutions of the test products were prepared in graduated concentrations using demineralised sterile water.

The haemolysis test was performed pursuant to ASTM Standard F 756-00 (9). The suspension of red blood cells was sourced from human blood from healthy volunteers after removal of the plasma and washing in Ca- and Mg- free phosphate-buffered saline solution (PBS). The suspension was set to $5x10^7$ erythrocytes/ml PBS. The test product solutions were transferred to microtiter plates in graduated concentrations, then the erythrocyte suspension was added and incubated at 37 °C for 15 minutes. Following a centrifugation step at 700 g for 15 minutes the supernatant was transferred to another microtiter plate and the adsorption was photometrically measured at 540/690 nm. The quantitative lysis

of erythrocytes after addition of distilled water was defined as total haemolysis. The haemolytic potential was determined from the percentage of haemoglobin released in respect of the total haemoglobin quantity present at the start of the test (free haemoglobin concentration/total haemoglobin concentration x 100 %). 2% haemolysis above the negative control was evaluated as haemolytic.

Results

Determination of limit concentrations of the process chemicals in solutions

Pronounced variations were noted in the test product limit concentrations for both the cytotoxicity test and haemolysis test in accordance with their composition (Figure 3). The neutralisation agents, Products C and D, yielded comparatively high limit concentrations with values of around 1000 ppm or higher in both the cytotoxicity test and haemolysis tests. The values for the two detergents were of somewhat the same magnitude, measuring 302 ppm in the cytotoxicity test and 490 ppm in the haemolysis test. However, in the haemolysis test these differed essentially in their tolerance to red blood cells. Whereas for the alkaline detergent (Product A) a comparatively high threshold value of 1000 ppm was determined, at 10 ppm this was lower by two orders of magnitude for the neutral detergent. As expected, lower limit concentrations of 100 ppm in the cytotoxicity test and 20 ppm in the haemolysis test were measured for the disinfectant (Product E) in both tests.

Determination of the cytotoxicity of process chemicals on process challenge devices (PCDs)

To determine the cytotoxicity of residues of the process chemicals on silicone PCDs in the laboratory test, test product concentrations corresponding to a calculated ten percent entrainment of the product into the last WD rinse water plus a safety margin were calculated (Figure 4). The cell viability obtained for all test products at the concentrations used in the fictitious final rinse water was of the order of that of the untreated PCD and above the acceptance value of 70 % surviving cells specified in standard DIN EN ISO 10993-5 (6). That means that under these conditions all test products were non-cytotoxic.

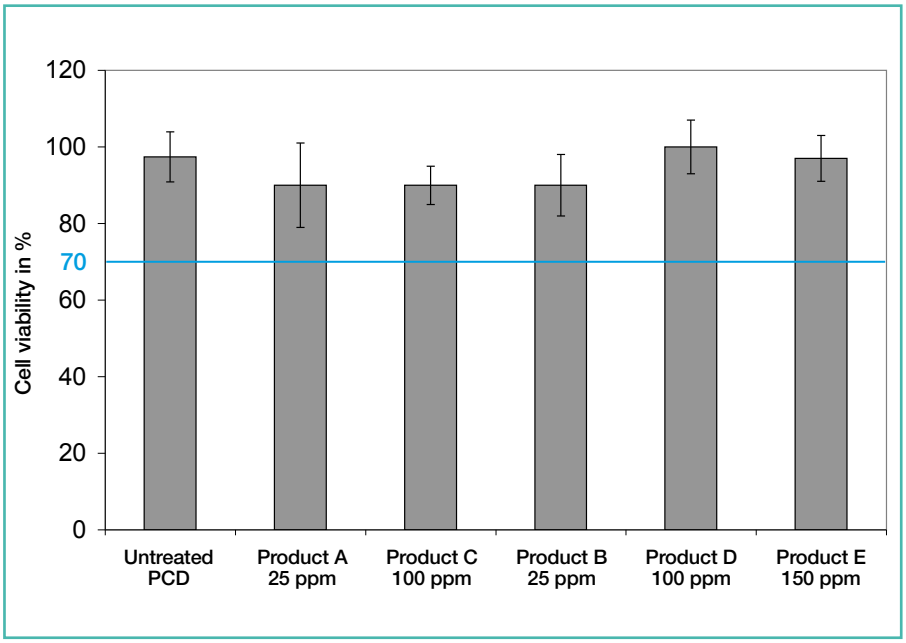

Fig. 4: Cytotoxicity tests on silicone PCDs after exposure to diluted solutions of the test product

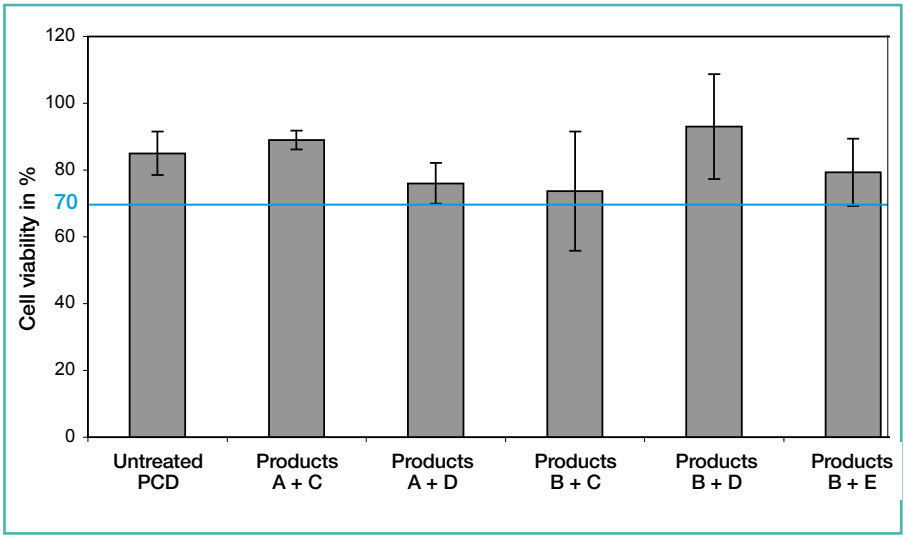

Fig. 5: Cytotoxicity tests on silicone PCDs after reprocessing once in washer-disinfector

Tests conducted with silicone PCDs in a WD revealed that in the thermal reprocessing process both the combination of alkaline detergent (Product A) and the enzymatic neutral detergent (Product B) with the neutralisation agents based on citric acid (Product C) as well as phosphoric acid (Product D) showed no major cytotoxic effects compared with the untreated PCDs and the cell viability values obtained were above the 70 % target value of surviving cells (Figure 5). Silicone PCDs reprocessed in a chemothermal reprocessing process using an enzymatic neutral detergent (Product B) and a disinfectant based on glutaral (Product E) also yielded cell viability above the 70 % mark.

As such, the residues of the test products and of any reaction products formed, for example because of neutralisation, are deemed to be non-cytotoxic under the test conditions selected.

Discussion

The reprocessing process must not result in changes to the characteristics of reusable medical devices which could adversely affect their functionality and/or tolerance of human tissue when they are put to use. Any residues of process chemicals found on a medical device after the reprocessing process must be below a potentially dangerous limit, which is determined inter alia by means of their biocompatibility. To assess the biocompatibility of process chemicals, pursuant to DIN EN ISO 10993-1 for the majority of reusable medical devices, the following must be considered: the intended contact with human tissue and the contact time, the irritant effect of process chemicals, their sensitisation potential, their cell toxicity, their haemocompatibility, and, if applicable, systemic toxicity (3).

For most constituents of process chemicals, extensive data are available on their toxicological properties and these can be consulted in public databases, the pertinent literature or obtained from the manufacturer of the chemicals. Based on the toxicological data available on constituent substances, experts in this field are able to conduct risk analysis to calculate and assess to what extent such constituent substances can be mixed in respect of irritant effects, sensitisation and systemic toxicity of the process chemicals.

Conversely, the cytotoxic properties and the haemolysis potential of process chemicals have to be determined by means of experiments.

The results obtained in our tests suggest that certain constituent substances determine to a large extent the cytotoxic properties and the haemolysis potential of the selected process chemicals used for automated reprocessing of medical devices. Whereas the acids, citric acid and phosphoric acid, used in neutralisation agents as well as the constituents of the alkaline detergent in the concentrations used in the diluted solutions produced medium to high values for the limit concentrations in the cytotoxicity test and haemolysis test and, as such, caused no, or only low, cytotoxic or haemolytic effects, some of the constituents of the neutral detergents and disinfectants are deemed to be critical. In terms of the cytotoxicity and the haemolysis potential this derives in particular from disinfectant constituents, such as glutaral, and from non-ionic surfactants for which limit value concentrations of less than 30 ppm were measured for the cytotoxicity and less than 5 ppm for haemolysis in respect of the relevant constituents.

Using a test procedure that differed from ours, M. Zottmann and B. Becker (10) obtained comparable values for the cytotoxic potentials of non-ionic surfactants. Evidence of effects against cell viability by a rinse aid based on non-ionic surfactants was obtained in these tests at a a concentration of 5 ppm.

On comparing the limit concentration values for the cytotoxicity and the haemolysis of process chemicals with the values calculated for the corresponding concentrations in the final rinse water with up to 10 % entrainment of the cleaning solution according to table 1, it emerged that for the alkaline detergent (Product A) and the neutralisation agents (Products C and D) the expected values in the rinse water were between two and three orders of magnitude lower than the cytotoxic and haemolytic limit concentrations. This was also confirmed in the cytotoxicity tests using silicone PCDs both in the laboratory and the WD, where as expected no effects could be found. Hence when using these products to reprocess medical devices a sufficiently large safety margin is assured, which means that following successful validation of the process longer intervals can be used for routine checks of the WD rinse water.

In the case of the enzymatic detergent based on non-ionic surfactants (Product B) the concentrations calculated for the final rinse water with 10 % entrainment according to table 1 were also below the cytotoxic and haemolytic limit concentrations, but with a value of 1.5 orders of magnitude for the cytotoxicity and less than one order of magnitude for the haemolysis potential the safety margin is considerably less. In the tests with the silicone PCDs in the laboratory when using the neutral detergent alone and in the WD when using the neutral detergent in combination with the neutralisation agents it was confirmed that the process chemical residues found on the PCDs were not cytotoxic under the test conditions. In view of the small safety margin with regard to the haemolysis potential, entrainment should be measured when validating the WD in situ, and the routine check intervals (for the WD final rinse water) should be set in line with the results obtained and the nature of the medical devices reprocessed.

The mechanism of action of highly effective microbicidal substances, such as glutaral, is based on chemical interaction with the constituents of cells. The values ascertained for the cytotoxic and haemolytic limit concentration for the glutaral-based disinfectant (Product E) were thus as expected low, and were even below the concentration calculated according to table 1 for the final rinse water with 5 % entrainment. In the tests with silicone PCDs in the laboratory using a disinfectant alone, and in the WD on using the neutral detergent (Product B) in combination with the disinfectant, it was demonstrated that process chemical residues found on the PCDs were not cytotoxic under the test conditions used.

Chemothermal processes are used predominantly in everyday practice to reprocess heat-sensitive medical devices, such as flexible endoscopes or anaesthesia accessories. These medical devices are used on intact skin or mucous membranes, hence the haemolytic properties are not of paramount importance in terms of their evaluation. However, if medical devices coming into contact with blood are reprocessed with such processes, in our opinion the disinfectant manufacturer should be consulted as regards the suitability to this effect, bearing in mind the haemolytic properties of the products.

In everyday practice chemothermal reprocessing predominantly involves the use of non-ionic surfactant detergents in combination with a disinfectant. In view of the safety margins in terms of the cytotoxic potential of both the detergent and the disinfectant, entrainment should be measured when validating the WD in situ, and the routine check intervals set in line with the results obtained.

The graduated method employed in our tests to determine limit value concentrations for the cytotoxic and haemolytic properties of process chemicals in solutions, together with laboratory tests on PCDs, using a calculated concentration in the rinse water with 10 % entrainment plus a safety margin, as well as tests carried out in the WD with PCDs under eve-

ryday use conditions, represents a model that permits realistic insights into the biocompatibility of products, while evaluating their constituents for sensitisation and irritant effects. Further tests are to be conducted to establish whether the model using PCDs to determine the cytotoxic potential can be extrapolated to the haemolysis test. Moreover, the range of PCDs is to be expanded to include other materials used to manufacture medical devices, such as metals or other synthetic materials.

However, it must be pointed out that the PCD-based model in the laboratory setting only simulates the effect of one-time use of process chemicals on the medical device. Ageing effects on synthetic materials as well as the penetration of constituents of process chemicals into the medical device, which can occur during the cleaning and/or disinfection step at a high temperature and concentration of process chemicals and have a potential effect on the biocompatibility, are to be investigated in further studies. ■

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