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# Analysis of the Acute Cytotoxicity of the Erlanger Silver Catheter

**Summary: The Erlanger silver catheter consists of a new form of polyurethane, which contains finely dispersed metallic silver. The aim of this study was to establish the biocompatibility of this intravenous catheter by investigating the acute cytotoxicity of extracts from the Erlanger silver catheter on human fibroblasts and lymphocytes. Extracts of the Erlanger silver catheter were not cytotoxic for MRC-5 human fibroblasts nor for sensitized phytohemagglutinin (PHA)-stimulated human lymphocytes. The addition of silver powder of up to 2% by weight to the basic catheter polyurethane Tecothane™ led to no increase in acute cytotoxicity in comparison with untreated Tecothane™. The Erlanger silver catheter is a new intravenous catheter with good biocompatibility.**

## Introduction

Indwelling intravenous catheters play a very important role in modern medicine. With the use of intravenous catheters on the rise, however, catheter-associated complications are also increasing [1, 2]. Catheter-associated septicemia is the most frequent life-threatening complication of central venous access [3, 4]. The antimicrobial activity of silver has long been recognized (*Williams* et al.) [5]. The Erlanger silver catheter has been developed as a flexible intravenous catheter whose surface consists of finely dispersed metallic silver. The surface of a catheter has thus been provided with an effective, reliable and long-lasting antimicrobial substance. Newly developed medical biomaterials that may be used in patients must be subjected to standardized investigations before they are licensed for such use, in order to rule out possible harmful effects. Internationally accepted guidelines for the investigation of biocompatibility have been drawn up by the International Standard Organization (ISO) [6]. The Erlanger silver catheter has been tested for acute cytotoxicity on both human fibroblasts and sensitized, PHA-stimulated human lymphocytes.

## Materials and Methods

*Cell culture:* In accordance with the ISO 10993-5 [6], human embryonic fibroblasts of the MRC-5 cell line (American Type Culture Collection, ATCC, no. CCL 171, Rockville, MD, USA) were used to test cytotoxicity. MRC-5 cells were cultured in minimal essential medium (MEM, Gibco Life Technologies, Eggenstein) with Earle's salts, supplemented with L-glutamine (2 mmol, Biochrom, Berlin), HEPES (20 mmol, Biochrom), penicillin-streptomycin (100 U/ml and 100 µg/ml, respectively, Biochrom) and 10% fetal calf serum (FCS, Gibco) in culture flasks (75 $cm^2$, Falcon, Becton Dickinson Labware, UK) in a humidified incubator (37°C, 5% $CO_2$, 95% air). For subculture the adherent MRC-5 cells were treated with 0.25% (w/v) trypsin (Gibco) in phosphate buffered saline (PBS, Gibco) without $Ca^{2+}$ and $Mg^{2+}$ and washed with PBS (5 min, 200 g). The culture medium was changed weekly.

*Controls for cytotoxicity:* Catalyst-free polyethylene (PE, U.S. Pharmacopeia, Rockville, MD, USA) [7] in 1.5 $cm^2$ pieces served as a negative control. After sterilization with ethylene oxide (EO) the material was stored in sterile cell culture tubes. A film of polyvinyl chloride (PVC, Rehau Inc.) was used as a positive control. This film was composed of 60% PVC, 39% dioctylphthate (DOP) softener and 1% organostannic stabilizer. This stabilizer was based on a toxic stannic-dialkyl-dimercaptide.

*Neutral-red test with MRC-5 cells:* The test materials were cut into 1 cm pieces and extracted for 24 h at 37°C with culture medium (MEM-FCS). The ratio between the surface area of the test material and the volume of culture medium was 6 $cm^2$/ml. For the negative as well as the positive control a surface ratio of 0.6 $cm^2$/ml culture medium was used. The fresh extracts were then diluted with culture medium in a ratio of 2:3. To investigate the cytotoxicity of the extracts, 100 µl aliquots of a freshly prepared MRC-5 cell suspension (1.5 x $10^6$/ml) was pipetted into each well of a 24-well culture plate (Nunc, Wiesbaden). Four hundred cubic milliliters of extract dilution was added to each well (final concentration as described in Results). Experiments were performed in triplicates. Five cubic milliliters of neutral-red solution (0.4% in PBS) was added to each of these cultures. The cell cultures were kept in a humidified incubator (37°C, 5% $CO_2$/95% air). After 24 h the cell morphology, cell adherence, and the percentage of neutral-red negative MRC-5 cells were determined with an inverted microscope. The cytoplasm of living cells was stained red with neutral red, while dead cells remained unstained. The percentage of unstained cells was taken as a measure of the cytotoxicity of the extract tested. The extract was regarded as non-toxic, if the vitality of the cells and cell morphology remained unchanged in comparison with the negative controls [6].

*MTT test with MRC-5 cells:* The test materials were extracted with IMDM-FCS. The extracts of the test and control materials were diluted as described (see Results). One hundred and sixty cubic milliliters of the various dilution steps of the test and the control eluates was pipetted in triplicate into the culture wells of a 96-well culture plate. With the exception of the blank well, 40 µl of freshly prepared MRC-5 cell suspension (5 x $10^5$/ml) was added into each well. An MTT test was performed to determine the acute cytotoxicity of extracts of the test materials. The

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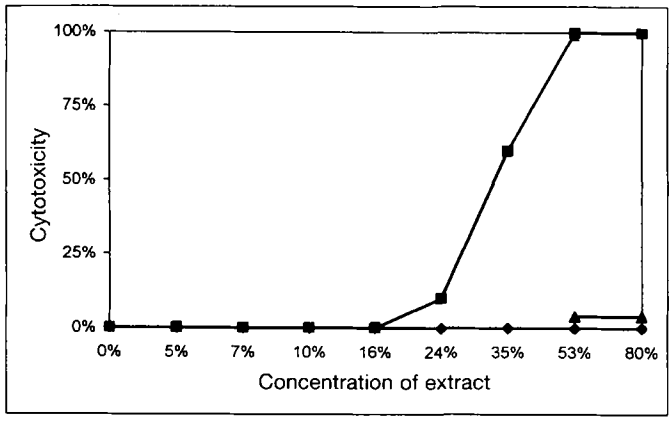


Figure 1: Acute cytotoxicity of the Erlanger silver catheter on MRC-5 cells in the neutral-red test. Triplicates of MRC-5 cell cultures (1.5 x $10^5$ cells/well) in 24-well plates were incubated in the presence of serial dilutions of extracts of the Erlanger silver catheter (◆) (final concentration in percentage volume). PE extract (▲) served as negative control. Extract of a cytotoxic PVC (■) film, stabilized with stannic-dialkyl-dimercaptide, was used as positive control. After 24-h incubation, the percentage of the neutral-red negative cells was determined with an inverted microscope. Mean values, expressed in percent, of the neutral-red negative MRC-5 cells of the respective cell cultures were used as a measure of cytotoxicity. A representative experiment is shown.

yellow, water-soluble color 3(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium-bromide (MTT, Sigma) is metabolized by mitochondrial enzymes of living cells into blue, water-insoluble formazan. The quantity of formazan formation by vital cells within a homogeneous cell population is directly proportional to the number of the vital cells in the culture [8]. Therefore, the MTT formazan production served as a measure of the viability and the growth of the test cells, and as a measure of the toxic effect of cytotoxic substances. After 72 h of incubation 100 µl of culture medium per well was discarded and 10 µl MTT solution (5 mg/ml in PBS) was added to each well. After 4 h 100 µl sodium dodecylsulfate (SDS, Serva, Heidelberg, 10% in 0.01 M HCl) was pipetted into each well to dissolve the blue, water-insoluble MTT formazan. The extinction was measured after 24 h with a test wavelength of 550 nm (optical density = $OD_{550}$) and a reference wavelength of 690 nm (Titertek Microplate Reader MK II, Lab-Systems, Turku, Finland).

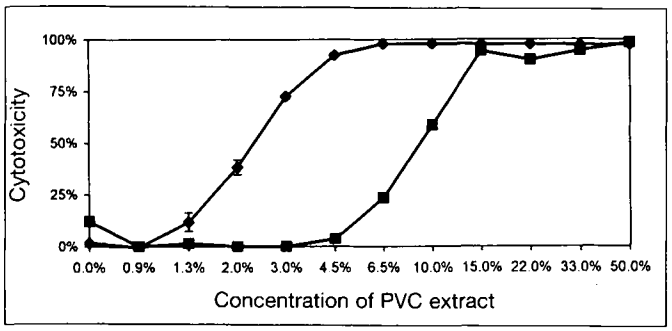


Figure 2: Sensitivity of MRC-5 cells and PHA-stimulated human lymphocytes for cytotoxic extracts. Triplicates of cell cultures with MRC-5 cells (■) (2 x $10^4$ cells/well) or PHA-stimulated lymphocytes (◆) (2 x $10^5$ cells/well) (see Materials and Methods) were incubated in the presence of extracts (final concentration in volume-percent) of cell-toxic PVC for 72 h. An MTT test was then performed. The mean and standard deviation of each set of triplicate $OD_{550}$ values were calculated. The calculated cytotoxicity ± standard deviation was expressed in percent. A representative experiment is shown.

Based on the $OD_{550}$-values of the triplicates, the mean value and standard deviation was calculated. The cytotoxicity was calculated according to the following formula and expressed as a percentage.

Cytotoxicity (%) = 100 - 100 x ($OD_{550}$ test extracts - $OD_{550}$ blank) / ($OD_{550}$ negative control) - ($OD_{550}$ blank).

By definition, a test material was regarded as having a cytotoxic effect if values exceeded 30%.

*MTT test with human lymphocytes:* In addition to experiments with MRC-5 cells, parallel experiments were performed with phytohemagglutinin (PHA) (Biochrom, 1% [v/v]) stimulated human lymphocytes.

Heparinized peripheral blood of healthy, adult volunteers was layered over Lymphoprep (Biochrom) in a sterile centrifugation tube. After 20 min centrifugation with 800 g the mononuclear cells were isolated from the interphase, resuspended with IMDM-FCS and washed once. Then, 100 µl of the cell suspension (2 x $10^6$/ml) in IMDM-FCS supplemented with PHA (2% v/v) was pipetted into the wells of a 96-well flat-bottom culture plate (Nunc). To each well 100 µl of the freshly prepared test extracts was added (final concentrations as shown in Results). Experiments were performed in triplicate. After a 72-h incubation, 100 µl medium per well was discarded and 10 µl MTT solution added. The evaluation of the test was performed as described above.

## Results

In the neutral-red test the vitality of the MRC-5 cell cultures was not inhibited by the addition of catheter extracts to the culture medium in comparison to the negative controls (Figure 1). The results showed that no substances were liberated in cytotoxic concentrations into the culture medium during a 24-h continuous contact of 6 $cm^2$ of test material surface from the catheter tube of the Erlanger silver catheter (type "jugular catheter, 4-way, lot no. C733105, EO-sterilized").

In accordance with ISO guidelines, an extract of a toxic PVC film was used as positive control. At a concentration of 35% or more this PVC extract showed a cytotoxic effect on MRC-5 cells. The PVC extract at a concentration of 24% was weakly cytotoxic to the MRC-5 cultures, because only 10% of the cells in the cultures were neutral-red negative (Figure 1).

In order to increase the sensitivity of the test system for cytotoxic substances, the neutral-red test system was changed in several ways: The vital dye neutral red was replaced by MTT. This modification also allowed the replacement of the time-consuming and subjective evaluation of the neutral-red uptake of vital cells with the objective photometric measurement of the MTT formazan produced by vital cells.

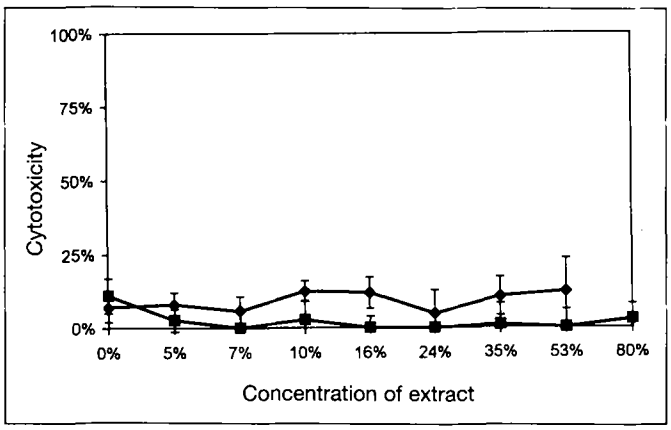


Figure 3: Acute cytotoxicity of Erlanger silver catheter extracts on MRC-5 cells or PHA-stimulated lymphocytes in the MTT test. Triplicates of cell cultures with MRC-5 cells (■) (2 x $10^4$ cells/well) or PHA-stimulated lymphocytes (◆) (2 x $10^5$ cells/well) (see Materials and Methods) were incubated in the presence of the extracts of the Erlanger silver catheter for 72 h. An MTT test was then performed. Based on the $OD_{550}$-values of the triplicates mean value and standard deviation were calculated. The calculated cytotoxicity ± standard deviation was expressed as a percentage. A representative experiment is shown.

In addition, the incubation period of the test cells with the extracts of the test materials was extended from 24 to 72 h. As an alternative to the MRC-5 cells, PHA-stimulated lymphocytes from healthy, volunteer donors were used in parallel experiments.

A PVC-extract concentration of 15% killed MRC-5 cells completely in the MTT test (Figure 2), while this extract concentration caused no decrease of the neutral-red uptake of the MRC-5 cells in the neutral-red test (Figure 1). PVC-extract concentrations of 4.5% or less showed no cytotoxicity on MRC-5 cells in the MTT test (Figure 2). These results indicated that the MTT test with MRC-5 cells was more sensitive for cytotoxic substances in the test extracts than the neutral-red test with MRC-5 cells.

A low PVC-extract concentration of 2% exhibited a cytotoxic effect on PHA-stimulated human lymphocytes. This result showed that PHA-stimulated lymphocytes were three dilution steps more sensitive to the toxic PVC-extract than MRC-5 cells (Figure 2). Therefore, the MTT test with PHA-stimulated lymphocytes was considerably more sensitive for cytotoxic substances in the test extracts than the MTT test with MRC-5 cells.

The analysis of the acute cytotoxicity of extracts of the Erlanger silver catheter on MRC-5 cells or PHA-stimulated lymphocytes with the MTT test showed less than 20% cytotoxicity (Figure 3).

In addition, the question of whether an increase of the silver content of the polyurethane Tecothane™ leads to an increase of the acute cytotoxicity was investigated. For this purpose Tecothane™ without silver, or with 0.5%, 0.6%, 1%, or 2% silver by weight was compared in the MTT test with PHA-stimulated lymphocytes. Extracts at a concentration of 50% from Tecothane™ with and without silver induced acute cytotoxicity on PHA-stimulated lymphocytes in a range of 20 to 25%. These results showed that the addition of increasing amounts of silver powder as high as 2% by weight did not increase the acute cytotoxicity of Tecothane™ (Figure 4).

## Discussion

The acute cytotoxicity of extracts of the Erlanger silver catheter was examined in accordance with the guidelines of the ISO. For this purpose, human embryonic fibroblasts of the cell line MRC-5 were tested. The acute cytotoxicity was measured with two different read-out systems, the neutral-red test and the MTT test. The Erlanger silver catheter showed no acute cytotoxicity in either of these test systems (Figures 1 and 3). To increase the sensitivity of the test system even further, PHA-stimulated human lymphocytes were used. Thus, the sensitivity of the test systems to cytotoxic substances could be increased by three dilution-steps in comparison with MRC-5 fibroblasts (Figure 2). Even at a concentration of 20 µM silver ions had a strong cytotoxic effect on cultured human lymphocytes [9]. The Erlanger silver catheter contained 0.3% to 0.6% silver by weight. Extracts of the Erlanger silver catheter had no acute cytotoxic effect on human PHA-stimulated lymphocytes (Figure 3). Therefore, on the basis of the absence of cytotoxicity of extracts of the Erlanger silver catheter, it can be concluded that *in vitro* the Erlanger silver catheter emits silver ions only in non-toxic concentrations, if at all.

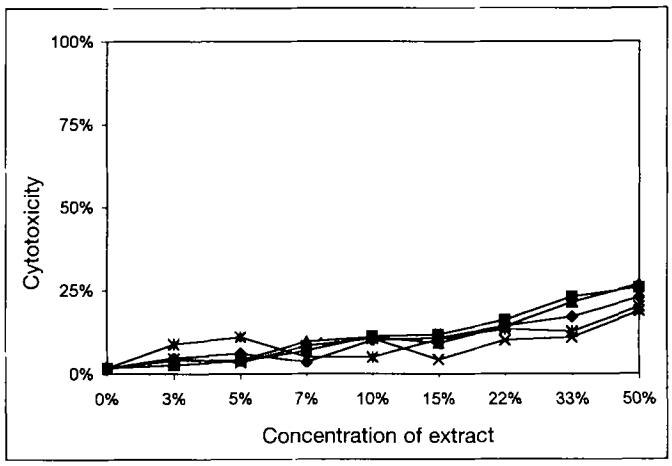


Figure 4: Acute cytotoxicity of Tecothane™ with various silver contents. Triplicates of cell cultures with PHA-stimulated lymphocytes (2 x $10^5$ cells/well) were incubated in presence of the extracts from Tecothane™ with a range of silver contents (0% ◆, 0.5% ■, 0.6% ▲, 1% ×, 2% ✳) for 72 h (final concentration in percentage volume). An MTT test was then performed. The means of each set of triplicate $OD_{550}$ values were calculated. The calculated cytotoxicity was expressed as a percentage. A representative experiment is shown.

The antimicrobial effect of silver-containing materials on *Escherichia coli* could be enhanced by increasing their silver content [10]. The addition of silver to Tecothane™ by as much as 2% by weight led to no increase in acute cytotoxicity on human PHA-stimulated lymphocytes (Figure 4). These results suggest that the antimicrobial effect of the Erlanger silver catheter can be improved even further by increasing its silver content, with no concomitant increase in cytotoxicity.

In conclusion, we were able to show that the Erlanger silver catheter is a new type of intravenous catheter with good biocompatibility.

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