# Toxicity of Medical Glove Materials: A Pilot Study

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*Cytotoxicity of 14 glove materials representing 4 natural rubber latex, 6 synthetic rubber and 4 synthetic polymeric materials was evaluated using dimethylthiazol diphenyltetrazolium (MTT), agar overlay and filter diffusion tests. Cell responses after contact with extracts of glove materials and contact with glove materials were assessed. One synthetic rubber glove (nitrile rubber) and 2 synthetic polymeric gloves (polyvinyl chloride) were non-toxic in all 3 tests, while 5 synthetic rubbers exhibited varying degrees of cytotoxicity, depending on the test. A severe cytotoxic response to both extracts of natural rubber latex materials and contact with natural rubber latex was verified in the 3 tests, indicating a need for consideration when selecting gloves, or other products, used in close skin contact.*

toxicity     natural rubber latex     synthetic rubbers     synthetic polymeric materials

## 1. INTRODUCTION

Few studies have focused on toxicity of glove materials, the majority addressing natural rubber latex (NRL) materials. NRL has been used as positive control in cytotoxicity testing [1]. Toxicity of NRL gloves has been identified in human in vitro fertilization programs [2, 3, 4, 5, 6]. Sperms exposed directly to latex gloves showed no survival, and the toxic substances in the latex material were readily transferred by touch [4]. In hospitals, the use of NRL catheters has caused side effects in patients [7, 8, 9, 10, 11]. Cytotoxicity testing using human cell lines revealed that direct contact with latex urinary catheters reduced cell viability, metabolic activity and cell proliferation indicating high toxicity of the catheters [12]. Some proteins in latex have been reported to cause a range from mild to severe allergic reactions, and health care personnel frequently using NRL are at risk [13, 14].

NRL gloves are usually manufactured in a conventional sulphur vulcanization process using sulphur and zinc oxide. Accelerators, either of dithiocarbamate-type (DTB-type) or 2-mercaptobenzothiazole-type (MBT-type), are added to create sulphur cross-linking, and antioxidants are added to prevent oxidative degradation [15]. DTB-type accelerators have been reported to show strong cytotoxicity [16, 17]. The cytotoxicity and tissue irritancies of NRL materials were correlated to the residual amount of zinc dithiocarbamate accelerators [15, 18]. MBT-type accelerators are known as contact allergens [19]. Cytotoxicity testing has shown that radiation vulcanized NRL materials are considerably less cytotoxic than sulphur-vulcanized materials [20, 21].

Other glove materials intended for use in the medical field are, e.g., synthetic rubber like butyl rubber, chloroprene, fluor rubber, nitrile rubber, styrene-butadiene and styrene-ethylene-butadiene, and several polymeric materials like ethylene-methyl acrylate (EMA), polyethylene, polyethene (PE) and polyvinyl chloride (PVC) [22].

Information on toxicity of butyl and nitrile rubber is scarce. Oshima and Nakamura [23] reported that extracts of butyl and nitrile rubbers yielded strong cytotoxicity on human gingival fibroblasts, while chloroprene and fluorine-contained rubbers showed little cytotoxicity.

PVC is the most extensively used polymeric material for single-use, pre-sterilized medical devices [24]. Rigid PVC materials contain modifiers, lubricants and stabilizers, while flexible PVC materials contain a range of plasticizers in order to produce materials with different properties. Toxicity testing of PVC has focused on plasticizers, particularly on the most widely used di(2-ethylhexy)phthalate (DEHP) and its metabolites [24]. Laboratory animals exposed to DEHP showed symptoms including cancer, but the health risk for humans could not be determined [25, 26, 27]. Cytotoxicity testing using human cell lines showed that the plasticizer DEHP was not toxic itself, but its presence in a polymer may permit the extraction of toxic additives from the matrix, or it may act synergistically with other polymer components [24].

Health care professionals frequently use protective gloves. Wransjö et al. [28] reported that 48% of dental personnel used gloves more than 6 hrs per day. NRL gloves were used by 40–60% of Swedish dental personnel, and more often by females according to another study. Moreover, frequent wearing of gloves was correlated with skin symptoms and hand dermatitis [29]. Latex sensitization is common among frequent wearers of NRL gloves [13, 14]. Due to the growing concern about latex allergies, alternative glove materials are used. The toxicity of the material is not well-known.

In vitro cytotoxicity tests can be used as the first step in biological evaluation of glove materials [30, 31]. These methods are designed to determine the biological response of cultured cells through exposure to extracts of the materials and/or in contact with the material [32, 33]. Cytotoxicity is determined qualitatively or quantitatively by measuring a variety of parameters such as assessment of cell damage by morphological means, measurements of cell damage, measure-ments of cell growth or measurements of specific aspects of cellular metabolism.

## 2. AIM

The purpose of the present study was to subject different types of commonly used medical glove materials to biological tests. Included in the tests were an extract test, a direct contact test and an indirect contact test.

## 3. MATERIALS AND METHODS

Fourteen different brands of medical gloves were selected for testing. The selected gloves were widely used in Sweden (according to the manufacturers), were non-powdered and were made from natural rubber, synthetic rubber and synthetic polymeric materials. Table 1 shows the gloves tested.

### 3.1. Cell Cultures

L 929 mouse fibroblasts (American Type Culture Collection CCL 1) were used in all three tests. Cells were maintained in continuous culture in MEM (minimum essential medium) supplemented with 100 units/ml of penicillin, 100 µg/ml of streptomycin, 2 mM L-glutamine, and 5% fetal bovine serum at 37 $^{\circ}$C, in air containing 5% $CO_2$. Cells were passaged when approximately 70% confluent by treating with 0.5g/L trypsin/0.2g/L ethylenediaminetetraecetic acid in Earl's balanced salt solution (all mediums and reagents were from GibcoBRL, Paisley, UK). Cell viability was measured using the trypan blue exclusion test [33]. Cells were plated in 96-well culture clusters (Costar, Corning, NY, USA), at a density of 15,000 cells/well in 100 µl, and incubated for 24 hrs to allow attachment. After incubation, the medium was replaced with 100 µl of a test or control medium, which had been equilibrated in air/5% $CO_2$ at 37 $^{\circ}$C for 30 min. After 24-hr incubation, cytotoxicity was assessed.

**TABLE 1. Gloves Tested**

| Glove Code | Name | Manufacturer | Type | Batch |
|---|---|---|---|---|
| | | Natural rubber latex materials (NRL) | | |
| NRL 1 | Amanita | Athena Nordic, Sweden | Latex | 3414 |
| NRL 2 | Biogel™ D | Regent Medical, UK | Latex | 97450 |
| NRL 3 | Sempermed | Semperit, Austria | Latex + silicone inner coating | 03971746x0007 |
| NRL 4 | P&G Latex | Procter & Gamble, USA | Latex | 804383 |
| | | Synthetic rubber materials (SRM) | | |
| SRM 1 | Nitril | Opti Pappers Gruppen AB, Sweden | Nitrile rubber | 90728003 |
| SRM 2 | Lirtin | SelefaTrade, Sweden | Nitrile rubber | 90928009 |
| SRM 3 | N-Dex Nitrile | Best Manufacturing, USA | Nitrile rubber | A98027B |
| SRM 4 | Nitra Touch | Ansell Medical, UK | Nitrile rubber | 8020311012 |
| SRM 5 | Tactylon® | Tactyl Techn., USA | Styrene-ethylene-butadiene | 7213-0002 |
| SRM 6 | Elastyren® | ECI Medical Tech., Canada | Styrene-butadiene | 96038 |
| | | Synthetic polymeric material, polyvinyl chloride (PVC)) | | |
| PVC 1 | Sensicare | Maxxim Medical, USA. | Polyvinyl chloride | 701M3F |
| PVC 2 | Metin | Medical Technology, Norway | Polyvinyl chloride | 0001/9741 |
| PVC 3 | Glads vinyl | Tena, Sweden | Polyvinyl chloride | 960926 |
| PVC 4 | Evercare | SelefaTrade, Sweden | Polyvinyl chloride | 2015 |

## 3.2. Preparation of Extracts

Pieces were cut from the glove materials and placed in glass vials with cell culture medium, $6\,cm^2$ of glove material/ml medium, according to an ISO standard procedure [34], for 24 hrs agitation in a water bath at 37 °C. Extracts were then filtered using a Millex-GS sterile filter (Millipore, France).

## 3.3. MTT Test

Cytotoxicity was assessed using the MTT (dimethylthiazol diphenyltetrazolium) assay [35, 36]. Twenty μl of a solution of 5mg/ml MTT (Sigma, MO, USA) in 37 °C phosphate-buffered saline (PBS) was added to each well and incubated at 37 °C, in air containing 5% $CO_2$ and 95% relative humidity for 4 hrs in the dark. After incubation, MTT was aspirated and 100 μl 0.04 M HCL in isopropanol was added. Plates were agitated until thorough formazan solubilization had occurred. Absorbance was read at 570 nm, using a Multiskan EX spectrophotometer (Labsystem, Helsinki, Finland).

## 3.4. Evaluation of Cytotoxicity Based on the MTT Test

Mean test absorptions were calculated and expressed as percentage of control cells. Each value represents the mean of 2 experiments, using at least 8 replicates of each extract per experiment. Cytotoxicity was rated based on cell viability relative to controls as severe, moderate, slight or not cytotoxic, where activity relative to controls was less than 30%, between 30 and 60%, between 60 and 90% or greater than 90% respectively (Table 2) [37, 38]. The materials were ranked consecutively based on their cytotoxicity from the highest to the lowest. They were then paired in a ranking order and the differences in the toxicity of each pair was compared using a two-tailed *t* test. The significance level was α = .05. Mean value was calculated together with the standard deviation (s) for each glove extract.

## 3.5. Agar Overlay

The cytotoxicity of contact with glove materials was assessed using the agar overlay test [39].

Culture dishes (Falcon, NJ, USA) were seeded with 10 ml of cell suspension ($3 \times 10^5$ cell/ml medium) and incubated at 37 °C, in air containing 5% $CO_2$ and 95% relative humidity for 24 hrs. After incubation, the medium was removed and the confluent cell monolayer was covered with 10 ml of agar medium. When agar had become solid, cells were stained using a 0.01% solution of neutral red vital dye (N-7005) (Sigma Aldrich Co., USA) and incubated for 20 min in the dark; excessive neutral red vital dye [34] was aspirated. The glove materials (circular, diameter 5 mm) were placed, inside surface down, on the agar layer, together with one positive control (REF 499/300/000/000, Portex, UK) and one negative control (REF 800/100/680/100, Portex, UK) and incubated at 37 °C, in air containing 5% $CO_2$ and 95% relative humidity for 24 hrs in the dark. Each test was carried out twice using at least three replicates for each experiment.

## 3.6. Evaluation of Cytotoxicity Based on the Agar Overlay Test

The cells were then examined under a microscope and the cytotoxic effect on the glove material was identified as lysis of the cells subjacent to the glove material and decolorization of the stained cells. The degree of cytotoxicity of the glove materials was based on the size of the decolorized zone (zone index 0–5), i.e., the diffusion ability of the toxic substance in the agar, and the percentage of the dead cells within the zone (lysis index 0–5), i.e., the toxicity of the test substance to cell membranes. The median values of lysis and zone indices were calculated, and the lysis indices were transformed to a relative degree of cytotoxicity (Table 2).

## 3.7. Filter Diffusion Test

The toxicity of contact with glove materials was assessed using the filter diffusion test [40]. Millipore filters (Millipore, USA), 47 mm in diameter, with a pore size of 0.45 µm, were placed on the bottom of culture dishes (Falcon, USA) and covered with 6 ml of cell suspension ($1.7 \times 10^5$ cell/ml medium). The cultures were incubated at 37 °C, in air containing 5% $CO_2$ and 95% relative humidity for 24 hrs to establish a cell monolayer on the filters. After incubation, the filters were placed on an agar medium (Eagles MEM × 2 and 1.5% agar) cell side down. Glove materials (circular, diameter 5 mm) were placed, inside surface down, on the filters, together with a positive control (4% phenol) and a negative control (Teflon) then incubated at 37 °C, in air containing 5% $CO_2$ and 95% relative humidity for 2 hrs. After incubation, test samples were removed and the filter gently loosened from the agar layer. The cells, still adherent to the filters, were incubated at 37 °C for 3 hrs to demonstrate the activity of succinate dehydrogenase [42]. After incubation, the filters were rinsed in distilled water and left to dry.

## 3.8. Evaluation of Cytotoxicity Based on the Filter Diffusion Test

The filters were examined macroscopically and the stain intensity of each test specimen contact area was compared with the background stain [40, 41]. Each test was carried out twice using at least three replicates for each experiment. Cytotoxicity of the glove materials was rated, based on a scoring system that takes into account the staining intensity of the zone and the diameter or extension of the affected area. The median values of indices were calculated and transformed to a relative degree of cytotoxicity (Table 2).

**TABLE 2. Cytoxicity Tests Expressed in Relative Values**

| Test Method | Relative Degree of Cytotoxicity | | | |
| --- | --- | --- | --- | --- |
| | None | Slight | Moderate | Strong |
| MTT (cell viability, %) | >90 | 60–90 | 30–60 | <30 |
| Agar overlay (lysis index) | 0–0.5 | 0.6–1.9 | 2.0–3.9 | 4.0–5.0 |
| Filter diffusion (score) | 0–0.4 | 0.5–1.4 | 1.5–2.4 | 2.5–3.0 |

*Notes.* M—MTT (dimethylthiazol diphenyltetrazolium).

# 4. RESULTS

The results of the three cytotoxicity tests are summarized in Table 3. The lysis indices were generally larger than the zone indices in the agar overlay test. The lysis index indicates the degree of toxicity to cell membranes, and was here used with MTT and filter diffusion tests as one method to assess the toxicity of glove materials. The zone index was used separately to evaluate the diffusion ability of the test substance.

The MTT assay showed that the most toxic extract reduced cell function by 98.4% and was from a PVC glove. Also extracts from NRL gloves and two synthetic rubber (SRM) ones exhibited strong cytotoxicity with over 95% reduced cell viability in the MTT assay. The seven strongly toxic extracts were ranked consecutively based on their cytotoxicity in the MTT assay from the highest to the lowest as PVC 1 > 1 > SRM 1 > SRM 2 > NRL 2 > NRL 3 > NRL 4. Paring them in ranking order and comparing the toxicity of each pair showed no significant difference in cytotoxicity. The six extracts determined as non-toxic in the MTT assay were ranked from the highest to the lowest as PVC 2 > SRM 3 > SRM 4 > SRM 6 > PVC 3 > PVC 4. Paring them in ranking order and comparing the toxicity of each pair showed no significant difference in cytotoxicity among non-toxic extracts. Interestingly, three extracts exhibited over 100% cell function in the MTT assay. The slightly toxic SRM 5 extract was significantly less toxic than strongly toxic extracts and significantly more toxic than non-toxic extracts.

All NRL materials released substances with the ability to diffuse through a filter and exhibit a strong toxic effect on the cell functions as shown in the filter diffusion test. The remaining materials were non- or slightly cytotoxic in the filter diffusion test (Table 3).

Substances that were released from the NRL materials diffused through agar and exhibited strong toxicity to the cell function for three out of four materials and moderate cytotoxicity for one material in the agar overlay test (Table 3). Four out of 6 SRM and 2 out of 4 synthetic polymeric gloves were moderately cytotoxic and the rest were not toxic in the agar overlay assay. The zone indices indicated that no glove material released substances with the ability to decolorize a zone larger than 1 cm from the test material (Table 3).

**TABLE 3. Results From the Three Cytotoxicity Tests**

| | Tests | | | |
| | MTT | Agar Overlay | | Filter Diffusion |
| | Cell Viability | Zone Index | | |
| Glove Code | % With *SD* (s) | (ZI) | Lysis Index (LI) | Score |
|---|---|---|---|---|
| | Natural rubber latex materials (NRL) | | | |
| NRL 1 | 2.2 (0.8) | 2.0 | 4.9 | 3.0 |
| NRL 2 | 3.1 (0.9) | 2.0 | 4.0 | 2.0 |
| NRL 3 | 3.5 (0.5) | 2.5 | 3.3 | 3.0 |
| NRL 4 | 4.8 (40.9) | 1.9 | 4.0 | 2.8 |
| | Synthetic rubber materials (SRM) | | | |
| SRM 1 | 2.2 (0.9) | 1.9 | 2.9 | 0.8 |
| SRM 2 | 3.0 (1.1) | 2.0 | 3.0 | 0.8 |
| SRM 3 | 91.9 (5.8) | 2.0 | 2.5 | 0.8 |
| SRM 4 | 97.1 (3.9) | 1.0 | 0.5 | 0.2 |
| SRM 5 | 65.5 (10.7) | 0.0 | 0.0 | 0.0 |
| SRM 6 | 101.1 (7.1) | 0.1 | 2.2 | 0.0 |
| | Synthetic polymeric materials (PVC) | | | |
| PVC 1 | 1.6 (1.1) | 0.5 | 3.9 | 0.9 |
| PVC 2 | 91.8 (4.5) | 0.0 | 0.0 | 0.0 |
| PVC 3 | 105.0 (6.3) | 1.0 | 0.0 | 0.0 |
| PVC 4 | 107.6 (5.5) | 0.0 | 0.0 | 0.0 |

*Notes.* M—MTT (dimethylthiazol diphenyltetrazolium).

**TABLE 4. Results From the Three Cytotoxicity Tests Expressed in Relative Values**

| Cytotoxicity | Natural Rubber Latex (NRL) | | | | Synthetic Rubbers (SRM) | | | | | | Synthetic Polymeric Materials (PVC) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 |
| Strong | MAF | MA | MF | MAF | M | M |  |  |  |  | M |  |  |  |
| Moderate |  | F | A |  | A | A | A |  |  | A | A |  | A |  |
| Slight |  |  |  |  | F | F | F |  | M | F | F |  |  |  |
| None |  |  |  |  |  |  | M | MAF | AF | M |  | MAF | MF | MAF |

*Notes.* M—MTT (dimethylthiazol diphenyltetrazolium), A—agar overlay, F—filter diffusion.

The result from the three tests was transformed and is expressed in relative values in Table 4. Five gloves exhibited identical results from the three tests, and 4 gloves exhibited the same result from 2 out of 3 tests. The five gloves with different degree of cytotoxicity in the three tests were slightly cytotoxic in the filter diffusion test and moderately cytotoxic in the agar overlay. In the MTT test, however, they were either strongly cytotoxic or non-cytotoxic (Table 4).

## 5. DISCUSSION

Severely cytotoxic reactions were observed after exposure to extracts of all NRL glove materials, and after contact with most NRL materials. One NRL glove with silicone inner coating was moderately toxic in the agar overlay test based on the lysis index (toxic effects on the cells). When evaluating the zone indices, however, the NRL glove showed the highest score of all materials, indicating high diffusion ability of the toxic substances released. One NRL glove exhibited moderate toxicity in the filter diffusion test, and thus higher toxic response than all the synthetic rubber and polymeric materials tested. The high cytotoxicity of NRL materials has been reported to correlate with the residual amount of DTB-type accelerators [15]. Also, leaching of chemicals has been identified as a causative factor to high cytotoxicity of sulphur-vulcanized NRL materials [21]. Proteins are acting as a stabilizer in NRL materials and some proteins, particularly 14kD, are considered to be latex allergens. Radiation vulcanization of NRL materials reduced cytotoxicity, but most proteins, including 14kD, were still present after irradiation [21].

SRM gloves exhibited lower toxic response than NRL ones in this study. The most biocompatible glove, an SRM glove (nitrile rubber), was non-toxic in all three tests, while the other three nitrile gloves were slightly toxic in the filter diffusion test, moderately toxic in the agar overlay and either strongly toxic or non-toxic, in the MTT assay. All nitrile gloves are manufactured of three monomers: acrylonitrile, butadiene and any one of many carboxyl acids. They are vulcanized similarly to NRL materials using zinc oxide, sulphur and accelerators, and they contain stabilizers [43]. The differences in toxicity between the four nitrile rubber gloves might be due to different composition, i.e., the type of stabilizer used. Interestingly, the toxic response was similar for two nitrile rubbers and one synthetic polymeric glove in all three tests, which might indicate occurrence of a similar toxic substance, probably a stabilizer. The SRM glove made of styrene-ethylene-butadiene was non-toxic in two out of three tests and slightly toxic in one, while the SRM glove of styrene-butadiene exhibited the same toxic response as one nitrile rubber (non-toxic in MTT, slightly toxic in filter diffusion, moderately in agar overlay). Three synthetic polymeric gloves (PVC) were non-toxic in all tests, except one PVC glove which exhibited moderate cell response in the agar overlay test.

The MTT test demonstrates the ability of cells to reduce the tetrazolium salt, MTT, to a formazan product indicating mitochondrial activity, which is seen only in living cells according to Edmondson, Armstrong and Martinez [33]. They reported that determination of formazan production in cells exposed to tests, compared to controls, enables the relative toxicity compared to controls to be assessed. The extraction procedure using cell culture media limits the extractants to water-soluble substances. Thus, a strong toxic response in the MTT test indicates toxic water-soluble substances released from the glove material. The

long extraction time (24 hrs) used in this study might not have influenced the response in the MTT assay. Studies have shown that the leaching time is important but the complete effect of leaching was reached within an hour and longer leaching time did not reduce cytotoxicity [21].

The filter diffusion test demonstrates the ability of cells to transform a yellow succinate solution (succinate + nitro blue tetrazolium + phenazine methosulfate) to a blue furate product (succinate dehydrogenase) indicating mitochondrial activity, which is seen only in living cells. Determination of succinate dehydrogenase in cells exposed to test substances compared to controls enables relative toxicity, compared to controls, to be assessed [40]. In the filter diffusion test, the cells were in contact with the glove materials and they were separated only by a filter. The close contact between the cells and the test material enhances the possibility that all leachables, not only water-soluble substances, will reach the cells [38]. Thus, the shorter exposure time (2 hrs), compared to 4 hrs for MTT, might explain the lower toxic response in the filter diffusion test compared to the MTT assay, for all glove materials except NRL. NRL materials exhibited strong cytotoxic responses in the filter diffusion test, indicating a readily release of strongly toxic substances.

The agar overlay assay demonstrates the ability of a test substance diffused through the agar layer to damage plasma or lysosomal membranes of the cells, resulting in a release of the preloaded neutral red dye. Viable cells take up and retain the dye compound [37, 42]. Determination of lysis subjacent to test material, and loss of colour of the stained cells, enables relative toxicity to be assessed. In the agar overlay, test material was placed on the agar layer for 24 hrs (indirect contact). The stronger toxic response in the agar overlay test compared to the filter test might be due to the longer exposure time (24 hrs vs. 2 hrs). The lysis index was always larger than the zone index in this study, which is in agreement with results from another study on toxicity of NRL materials [15]. They also compared the cell response in agar overlay with tissue response and found that oedema observed 7 days after implantation, residual muscle fibres in inflammatory layer, infiltration of small round cells in the intermuscular layer and elevated plasma cell counts correlated with cytotoxicity determined in the agar overlay test. However, haemorrhage, giant cells, pseudo-eosinophils and lymphocytes did not correlate with the result in the agar overlay test [15].

The present study used three different methods to assess the cytotoxicity of glove materials. The mode of exposure and exposure time differed. In the MTT assay, cells were exposed to extracts of glove materials for 4 hrs. In the filter test, cells were exposed for 2 hrs to substances released from the glove materials. In the agar overlay test, substances released during from the glove material for 24 hrs had to diffuse through an agar layer before reaching the cells. When the results from the three tests were compared, the three tests exhibited a similar degree of toxicity for all NRL gloves, the majority of synthetic polymeric gloves and for two SRM gloves.

The high sensitivity of cell culture tests is due to the isolation of the test cells in cultures, and the absence of the protective mechanisms in the body. It is difficult to predict the in vivo toxicity of such data, and no material can be considered biocompatible based only on cell culture tests. However, the strong toxic response to extracts of NRL materials and contact with NRL materials showed in this study, and the reported correlation between cytotoxicity indices and tissue responses [15], indicate a need for concern when selecting gloves or other products used in close skin contact. Other products often made of NRL materials are baby dummies and condoms.

This study also showed that gloves made of synthetic rubber and synthetic polymeric materials exhibited a varying degree of cytotoxicity and few gloves could be classified as non-toxic in all three tests. This indicates that further biological testing of medical glove materials is needed.

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