The biological safety of condom material can be determined using an in vitro cell culture system

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Latex products have long been recognized as a cause of latex protein allergy. The increased usage of latex gloves, with the consequent increased occurrence of latex allergies appears to have escalated with increasing awareness of the transmission of HIV–AIDS and other infections. The use of condoms as a means to prevent the transmission of hepatitis B and sexually transmitted diseases such as HIV/AIDS has been widely promoted [2,11,12]. Latex is a natural sap extracted from the Brazilian rubber tree, Hevea brasiliensis [2,10,12]. It is used to produce natural rubber and is one of the important vegetable materials with extensive applications of over 40,000 products in the USA [2,9,10] including industrial and surgical products, chewing gums, balloons and toys [7,10,13]. The worldwide increase in reported reactions of latex is related particularly to the increased use of poorly manufactured rubber gloves and condoms used as a barrier against diseases [11,12]. Allergy to natural latex has become a major health problem and is frequent in certain occupational settings, especially among health care workers, among individuals producing articles containing natural latex and also in patients with a history of repeated operations [2,9–12].

Latex products contain two types of compounds that cause allergies, namely: added chemicals such as antioxidants that cause dermatitis, and natural proteins that cause systemic, potentially life threatening allergic reaction [5]. Reactions to an allergen are usually of immediate type and are elicited by an IgE response [7,10]. The predominant manifestation is contact urticaria, and the less frequent are rhinitis, conjunctivitis, asthma or anaphylactic shock [6,9,10,12,13].

The manufacturing process is similar for condoms and surgical gloves and includes the dipping of the mold in the liquid latex containing additives such as antioxidants, flavors and dyes. However, differences may exist in the stringency of the manufacturing process and the quantities of latex in different condoms may vary [4,13].

Like rubber gloves, most condoms contain both natural latex and chemical allergens. Contact dermatitis

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attributable to processing chemicals probably the most common problem, but anaphylactic reactions to condoms have also occurred [4]. In men, the symptoms include itching and edema of the distal shaft of the penis. Eczematous can occur and spread to the scrotum, inguinal area, and inner thighs as well as to more distal locations including the face and neck. Women may experience symptoms that range from mild vulval pruritus or vaginal burning to redness and edema of the vulva and a diffuse eczema or dermatitis of the adjacent skin [4].

Although extensive testing is done to evaluate the physical quality of condoms [13], no information is available regarding the biological safety of condoms. This study was undertaken to determine the effects of short-term exposure to physiological levels of condom surface material on cell viability and cell growth using cell culture experiments.

2. Materials and methods

2.1. Direct cell culture testing method

A modification of the direct cell culture testing method that is specified by the American Test Method, F813-83 of 1998 was followed [1]. This method of cell culture testing is useful for assessing cytotoxic potential as part of quality control for established medical materials and devices. It assumes that assessment of cytotoxicity potential provides a method for predicting the potential for cytotoxic or necrotic reactions to medical materials and devices during clinical applications to humans [1].

2.2. Cell cultures

Fibroblast cells (ATCC, CCL1 NCTC clone 929 strain designated L-929) were grown in minimum essential medium (MEM) with L-glutamine, 5% fetal bovine serum, 5% L-glutamine and 5% penicillin and streptomycin. Cells, medium and additives were from Highveld Biological Company, Johannesburg, South Africa. Cultures were maintained at 37°C and 5% CO2 and passaged by trypsinisation using 0.25% trypsin in Hanks buffered saline solution. Cells were plated at a cell concentration of 2 × 10^4 cells per ml in 24 well plates and were kept for 24 hours at 37°C and 5% CO2 before conducting each experiment.

2.3. Medium containing condom washings

Three different types of latex condoms were tested, namely condoms without spermicides, with spermicides and flavored condoms with spermicides.

The different mediums were prepared as follows: 1 g of each type of condoms was cut into thin strips and placed into 20 ml medium. Following incubation times of 0, 4 and 8 minutes at 37°C, the medium was removed. Cells were exposed to different dilutions of (0–66%) this medium. Following 20 or 48-hours exposures, cell viability and cell numbers (referred to in this paper as cell survival) were determined by the MTT [3] and Crystal Violet assay [8], respectively.

2.4. MTT assay

A 50 µl volume of 0.1 mg/ml MTT stock solution was added to each well. The cultures were maintained for a further 20 hours at 37°C and 5% CO2. The medium was removed, 200 µl of isopropanol: HCl solution (24:1 (1 M HCl)) was added to each cell well and the plates were shaken for 20 minutes. The solution was transferred into a 96-welled plate and absorbency at 545 nm was measured using a spectrophotometer (EL900) plate reader. All assays were done in triplicate.

2.5. Crystal Violet assay

A 100 µl volume of 11% glutaraldehyde was added to the medium, the plates were shaken for 30 minutes and then washed with water and dried over night at room temperature. A 300 µl volume of 0.1% methylene blue dye solution prepared in 200 mM of formic acid pH 3.5. The plates were shaken well, washed and dried. The bound dye was dissolved with shaking for 10 minutes in 300 µl of 10% acetic acid. Absorbency at 595 nm was determined as described for the MTT assay. All assays were done in triplicate.

2.6. Statistical analysis

The Crystal Violet assay and MTT Assay were analysed separately with an appropriate ANOVA for the split–split plot study design that was used to conduct the experiments. Testing was performed at the 0.05 level of significance and pairwise comparisons were done using Fisher’s LSD test.
3. Results

The effect of different exposure times on cell survival was determined using the Crystal Violet assay (Fig. 1(a)–(c)). Figure 1(a)–(c) displays the average percentage cell survival plotted against percentage condom medium of the control (exposure time $T_0$). A decrease in cell survival was observed for all condom types. For non-lubricated condoms (Fig. 1(a)) a 10–20% decrease in cell survival was observed for exposure times $T_4$ and $T_8$ where 8.3% to 50% of the medium was replaced with condom washings. A dramatic decrease in cell survival was seen at $T_8$ for non-lubricated condoms where 66% of the medium was replaced with condom washings.

Lubricated condoms (Fig. 1(b)) at exposure time $T_8$ where 66% of the medium was replaced with condom washings had a survival rate of only 22%. For flavored condoms a 10% decrease in mean cell survival was observed where 0–33% of medium was replaced with condom washings and a further decrease of 28% was observed at higher volumes of condom washings for both exposure times (Fig. 1(c)). At exposure time $T_4$ and $T_8$ and condom washing replacements of 66%, only 25% and 28% of the cells survived.

Cell viability of the cells using MTT assay was determined following 20 hours exposure to medium containing different volumes of condom washings. The decrease in cell survival is expressed as the percentage of the control (exposure time $T_0$). Results of different exposure times $T_0, T_4$ and $T_8$ are shown in Fig. 2(a)–(c). These figures represent the average percentage cell viability plotted against percentage condom washings for each condom type. Different volumes of condom washings appear to have only slight effect on cell viability for non-lubricated condoms (Fig. 2(a)) and lubricated condoms (Fig. 2(b)). Where medium was replaced with condom washing volumes above 40%, a rapid decrease of cell survival to 63% at $T_4$ and 28% at $T_8$ were observed (Fig. 2(c)).

Condom types are significantly different ($p = 0.0102$) and in particular non-lubricated condoms have significantly higher survival than lubricated and flavored condoms. However, there is also a significant interaction between type of condom and exposure time which is due to a much higher survival than expected for non-lubricated condoms at $T_2$ (Fig. 3). The significant differences between condom types are more likely as a result of this interaction. Furthermore, times are also significantly different ($p < 0.001$) and in partic-
Fig. 1. (Continued).
Fig. 2. Cell viability (MTT assay) following exposure of cells to washings derived from: (a) non-lubricated condoms, (b) lubricated condoms, and (c) flavored condoms.
Fig. 2. (Continued).

Fig. 3. LSH (T) means of survival by type versus time (Crystal Violet assay).
Fig. 4. LSH (T) of survival by type versus time (MTT assay).

Fig. 5. LSH (T) means of survival by condom washing volume added to media versus time (MTT assay).
Table 1
Analysis of variance table for cell survival (Crystal Violet assay)

| Source                                   | Degrees of freedom | p-value |
|------------------------------------------|--------------------|---------|
| Condom replicate                         | 2                  | 0.2561  |
| Condom type                              | 2                  | 0.0102  |
| Condom replicate v. condom type          | 4                  |         |
| Condom washing volume added to media     | 5                  | 0.0735  |
| Condom type v. condom washing volume added to media | 10          | 0.9249  |
| Condom replicate v. condom type v. condom washing volume added to media | 30          |         |
| Time                                     | 3                  | 0.0001  |
| Condom type v. time                      | 6                  | 0.0001  |
| Condom washing volume added to media v. time | 15          | 0.4468  |
| Condom type v. condom washing volume added to media v. time | 30          | 0.9885  |
| Condom replicate v. condom type v. condom washing volume added to media v. time | 108         |         |

Table 2
Analysis of variance table for cell viability (MTT assay)

| Source                                   | Degrees of freedom | p-value |
|------------------------------------------|--------------------|---------|
| Condom replicate                         | 2                  | 0.9858  |
| Condom type                              | 2                  | 0.1673  |
| Condom replicate v. condom type          | 4                  |         |
| Condom washing volume added to media     | 5                  |         |
| Condom type v. condom washing volume added to media | 10          |         |
| Condom replicate v. condom type v. condom washing volume added to media | 30          |         |
| Time                                     | 3                  | 0.0001  |
| Condom type v. time                      | 6                  | 0.0001  |
| Condom washing volume added to media v. time | 15          | 0.0001  |
| Condom type v. condom washing volume added to media v. time | 30          | 0.0015  |
| Condom replicate v. condom type v. condom washing volume added to media v. time | 108         |         |

The interactions between condom washing volumes and time and between condom type and time had a conservative influence on the main effect time. In the absence of these interactions the above differences will have been even more marked.

4. Discussion

The original FDA direct cell culture testing method is used to assess the cytotoxic potential for established medical materials and devices [1]. Since there is no specific documented cell culture testing method available for testing latex condoms, it was hypothesized that this test method may be suitable for use. Our re-

ular T0 higher survival than T2 which in turn is higher than T4 and T8 which are not different from each other. The increase of survival at T2 relative to T4 and T8 can also be attributed to the interaction between time and condom type resulting from the higher than expected survival for non-lubricated condoms at T2.

Exposure times are significantly different (p < 0.0001) and in particular T0 has significantly higher survival than T2 and T4 which in turn have higher survival than T8. Had there not been a significant interaction between time and percentage of condom washings added to medium, the better survival will have been even more marked (Fig. 4). The influence of the interaction between type and time is such that without interaction T8 will have had even lower survival (Fig. 5).
results indicate that the modified direct cell culture testing method is an effective, simple and reproducible method to determine the biological safety of condoms.

The cytotoxic effects of condom washings can be measured either by using cell survival (using the Crystal Violet assay) [8] or cell viability tests (using the MTT assay) [3]. However, we believe that measurement of cell viability may be the more sensitive method as it measures succinate dehydrogenase activity, an enzyme which is a component of the mitochondria complex II electron transport chain and is highly sensitive to changes in cell homeostasis [3].

For medium derived from non-lubricated condoms cell survival did not significantly decrease at exposure times of $T_4$. However, at $T_8$, where 66% of the medium was replaced with condom washings of media a decrease in cell survival was observed suggesting that exposure times may have an important role in cytotoxic effects. Cell viability was not significantly affected for all time intervals and percentage condom washings present in the media.

Cell survival for both time intervals, $T_4$ and $T_8$ decreased to 60% and 20% for lubricated condoms respectively, however, cell viability was not significantly affected for all time intervals and percentage condom washings added to media. These results indicate that condoms without spermicides (non-lubricated) do not decrease cell survival or cell viability, but condoms with spermicides (lubricated) decrease cell survival but do not affect cell viability of the remaining cells.

Flavoured condoms coated with spermicides on the other hand show a significant decrease in cell survival as well as cell viability. This is probably not necessarily due to spermicides, but due to the added chemicals used for flavouring.

Even though these tests were performed using the mouse fibroblast cell culture, the method is used by the FDA as a direct cell culture method. We therefore believe that our results reflect the cytotoxic effects of the added chemicals and latex proteins. Further research endeavors will focus on using human epithelial cells to confirm these results.

Promotion of condom use for contraception and the spread of HIV and STD’s is essential, however, we believe that the type of condom used is also important as little is known of the toxic and even carcinogenic effects of certain types of condoms. Furthermore, these results demonstrate that the biological safety of all commercially available condoms should be assessed to limit a possible health risk associated with prolonged use of certain types of condoms.

5. Conclusion

The modified, FDA test method F813-83 was found to be a sensitive test system for the evaluation of the biological safety of condoms. This study reveals the importance of evaluating the biological safety of all condoms that are commercially available, because of the potential health risk that may be associated with prolonged use of certain types of condoms.

References

[1] ASTM F813-83, Standard practice for direct contact cell culture evaluation of materials for medical devices, ASTM (1998), 229–231.
[2] A.B. Carey, K. Cornish, P. Schrank, B. Ward and R. Simon, Cross reactivity of alternate plant sources of latex in subjects with systemic IgE-mediated sensitivity to Hevea brasiliensis latex, Analyses of Allergy, Asthma and Immunology 74 (1995), 317–319.
[3] J.A. Cook and J.B. Mitchell, Viability measurements in mammalian cell systems, Anal. Biochem. 179 (1989), 1–7.
[4] P. Cullinan, J. Cannon, D. Sheril and A.N. Taylor, Histological evaluation of materials for medical devices, Anal. Biochem. 182 (1989), 16–19.
[5] D.A. Levy, S. Khouader and F. Leynadier, Allergy to latex condoms, Allergy 53(11) (1998), 1107–1108.
[6] C. Marcos, M. Lazaro, J. Fray, S. Quince, B. de la Hoz, M. Fernandez-Rivers and E. Losada, Occupational asthma due to latex surgical gloves, Analysis of Allergy 179(5) (1995), 319–323.
[7] P.C. Poter, Latex allergy – a major dilemma for health providers, Occupational Health 28(5) (1996), 12–15.
[8] B.B. Tan, J.T. Lear, J. Watts, P. Jones and J.S. English, Perioperative collapse: prevalence of latex allergy in patients sensitive to anaesthetic agents, Contact Dermatitis 26(1) (1997), 47–50.
[9] K. Turjaman and N. Reumala, Condoms as a source of latex allergen and cause of contact urticaria, Contact Dermatitis 20 (1989), 360–364.