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

N.A. Motsoane ^a, E. Pretorius ^{a,*}, M.J. Bester ^a and P.J. Becker ^b

^a Department of Anatomy, Medical Faculty, University of Pretoria, Pretoria, South Africa ^b Medical Research Council, Pretoria, South Africa

Received May 2001 Accepted 5 October 2001

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 STD's (sexually transmitted diseases) and HIV-AIDS has been widely promoted. Although extensive testing is done to evaluate the physical quality of condoms, 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 (MTT assay) and cell growth (crystal violet assay). A direct contact cell culture testing method (FDA test method F813-83 used to evaluate the cytotoxic potential of medical materials and devices) was used. The modified test method 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.

1. Introduction

Latex products have long been recognized as a cause of latex protein allergy [2,9,11,13]. The increased us-

Analytical Cellular Pathology 23 (2001) 51–59

ISSN 0921-8912 / \$8.00 © 2001, IOS Press. All rights reserved

age 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 40000 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

^{*}Corresponding author: E. Pretorius, Department of Anatomy, P.O. Box 2034, University of Pretoria, Pretoria 0001, South Africa. Tel.: +27 12 319 2533; Fax: +27 12 319 2240; E-mail: rpretori @medic.up.ac.za.

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 pruiritus 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 μ l 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 μ l 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 μ l 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 μ l 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 μ l 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 nonlubricated 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 (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. Cell survival determined from Crystal Violet assay following exposure of cells to washings derived from: (a) non-lubricated condoms, (b) lubricated condoms, and (c) flavored condoms.







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).

N.A. Motsoane et al. / Evaluation of the biological safety of condoms



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).

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	30	
volume added to media		
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 1 Analysis of variance table for cell survival (Crystal Violet assay)

Table	2
rabic	_

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	0.0001
Condom type v. condom washing volume added to media	10	0.2640
Condom replicate v. condom type v. condom washing	30	
volume added to media		
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	30	0.0015
media v. time		
Condom replicate v. condom type v. condom washing	108	
volume added to media v. time		

ular T_0 higher survival than T_2 which in turn is higher than T_4 and T_8 which are not different from each other. The increase of survival at T_2 relative to T_4 and T_8 can also be attributed to the interaction between time and condom type resulting from the higher than expected survival for non-lubricated condoms at T_2 .

Exposure times are significantly different (p < 0.0001) and in particular T_0 has significantly higher survival than T_2 and T_4 which in turn have higher survival than T_8 . 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 T_8 will have had even lower survival (Fig. 5).

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 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

- ASTM F813-83, Standard practice for direct contact cell culture evaluation of materials for medical devises, *ASTM* (1998), 229–231.
- [2] A.B. Carey, K. Cornish, P. Schrank, B. Ward and R. Simon, Cross reactivity of alternate plant sources of late in subjects with systemic IgE-mediated sensitivity to Hevea brasiliensis latex, *Analysis 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, Asthma following occupational exposure to *Lycopodium clavatum* in condom manufacturers, *Thorax* 48 (1993), 774–775.
- [5] A.A. Fisher, Management of "Consort dermatitis" due to combined allergy: seminal fluid and latex condoms, *Cutis* 54(2) (1994), 66–67.
- [6] C.P. Hamann and S.A. Kick, Immediate and delayed hypersensitivity to natural rubber latex, *Cutis* 52 (1993), 307–311.
- [7] R.G. Kaczmarek, B.G. Silverman and T.P. Gross, Prevalence of latex-specific IgE antibodies in hospital personnel, *Annals of Allergy, Asthma and Immunology* **76** (1996), 51–56.
- [8] W. Kueng, E. Silber and U. Eppenberger, Quantification of cells cultured on 96 well plates, *Anal. Biochem.* 182 (1989), 16–19.
- [9] D.A. Levy, S. Khouader and F. Leynadier, Allergy to latex condoms, *Allergy* 53(11) (1998), 1107–1108.
- [10] C. Marcos, M. Lazaro, J. Fraj, S. Quirce, B. de la Hoz, M. Fernandez-Rivers and E. Losada, Occupational asthma due to latex surgical gloves, *Analysis of Allergy* 67(3) (1991), 319– 323.
- [11] P.C. Poter, Latex allergy a major dilemma for health providers, Occupational Health SA 2(5) (1996), 12–15.
- [12] 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* 36(1) (1997), 47–50.
- [13] K. Turjanmaa and T. Reunala, Condoms as a source of latex allergen and cause of contact urticaria, *Contact Dermatitis* 20 (1989), 360–364.



The Scientific **World Journal**



Gastroenterology Research and Practice





Journal of Diabetes Research



Disease Markers



Immunology Research





Submit your manuscripts at http://www.hindawi.com





BioMed **Research International**



Journal of Ophthalmology

Computational and Mathematical Methods in Medicine













Research and Treatment





Oxidative Medicine and Cellular Longevity



Stem Cells International



Behavioural Neurology