Assessment of in vitro cytotoxicity of four RTV-silicone elastomers used for maxillo-facial prostheses

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The cytotoxic potential of four different and potentially interesting RTV-silicones was evaluated using an in vitro cell culture technique. Both human epithelial cells and mouse fibroblasts were used in this study and the results indicate that all materials tested were cytotoxic. Treating the surface of the discs with talc appeared to decrease the cytotoxic effects of the materials.

Key-words: Prosthetics; biocompatibility; cell culture studies

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The choice between rigid polymethylmethacrylate, PVC-plastisols, silicone or polyurethane elastomers in the manufacture of maxillo-facial prostheses depends to a great extent upon the technologist's personal preference. Most authors expressing their views on the choice of material give high priority to the requirement that the materials should be non-toxic. The assessment of toxicity profiles of many plastics used in medicine has been performed previously (1, 5, 8). However, little actual research appears to have been carried out to assess the biocompatibility of materials used for maxillo-facial prostheses (3, 4, 7, 9).

Before the manufacturing of Silastic 399 terminated in 1976, many maxillo-facial technologists looking for replacement materials found a large number of alternatives. Unfortunately, most of them were of so-called «industrial grade». Braley (2) warned against the use of industrial grade silicones for medical applications, although he mentioned that there might be exceptions.

The object of the present study was to assess the in vitro cytotoxicity of potentially interesting RTV (room temperature vulcanizing) silicones, using a cell culture technique originally developed for testing the biocompatibility of dental materials (6).

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Table 1. *Specification of materials and processing techniques used to manufacture 40 x 1 mm discs of 4 RTV silicones*

No.	Product	Batch No.	Processing technique	Remarks
1.	Silastic 399 Prosthetic Elastomer, Medical Grade, Dow Corning, USA	Base HH0012 Cat. 1 HH009 Cat. 2 HHU010	5 g base 15 drops crosslinking 3 drops catalyst Setting time – 2 hours	5 drops of catalyst No. 2 could not be used as the working time would be 30 seconds
2.	R&S330 T-RTV, Industrial Grade, Ringsted & Semler A/S, Denmark	Base – Cat. N.	5 g base 8 drops 24 hours setting time 24 hours airing	Amount of catalyst can be 1.5 to 5 percent. 8 drops = 2.5 percent.
3.	MDX 4–4210 Clean Grade Elastomer, Medical Grade, Dow Corning, USA	Base HHY059 Cat. HHJ079	5 g base 0.5 g catalyst Vacuum – 10mWs 30 min. 60 min. at 75°C	Open mould The curing time was extended to remove the surface stickiness.
4.	MDX 4–4210 Clean Grade Elastomer, Medical Grade, Dow Corning, USA	Base HHE076 Cat. HHE076	5 g base 0.5 g catalyst Over-night at 24°C Pre-curing 2 hours at 50°C and post cure 30 min. at 80°C.	Open mould No vacuum treatment Despite the extended curing time the surface was sticky, and therefore neutralized which talc.
5.	SK 43, Industrial Grade, Wacker Chemie, W. Germany	Base 1077	Directly from the tube 24 hours setting time 24 hours airing	Open mould

MATERIALS AND METHODS

Preparation of test specimens

The four materials tested are listed in Table 1, together with details of the processing technique.

Ten circular discs (40 x 1 mm) of each material were processed according to directions from the manufacturers, but with a slight modification for MDX 4–4210. This material did not vulcanize at room temperature, the discs were full of blisters when a closed mold was used, and the vulcanized material had a sticky surface. An aluminium ring was used to make a casting mold with sand-blasted glass slabs providing top and bottom covers. To facilitate easy removal of the vulcanized material, a sheet of mixing pad paper treated with detergent was used as separating medium. The number four discs were made by chief technician K.–G. Holmkvist, Malmö, Sweden. All specimens were stored in sealed plastic bags at room temperature until used.

Cells

Human epithelial cells (NCTC 2544), obtained from American Type Culture Col-

Table 2. *Relative cell growth (§) on the surface of silicone elastomers used for maxillo-facial prostheses*

No. Material	Test II NCTC 2544 cells		Test III NCTC 2544 cells	
	1d[a]	4d[a]	4d[a]	4d[b]
Control dish	45.7	100.0	100.0	100.0
1 Silastic 399	*	*	*	*
2 R&S330 T-RTV	14.3	25.7	41.3	46.5
3 MDX 4-4210	8.6	34.3	59.4	41.3
4 MDX 4-4210/TALC	25.7	62.9	64.5	61.9
5 SK 43	5.7	37.1	18.1	12.9

* Trypsin-treatment of Silastic 399 specimens to detach cells resulted in a cloudy, milky-white suspension prohibiting cell counting.
[a]) Specimens stored in sealed plastic bags until used.
[b]) Specimens stored in saline (25 ml/disc) for 7 days prior to use.
§) The cell growth is expressed as per cent of the total cell count in the control cultures at day 4.

lection, Rockville, Md., USA and mouse fibroblast cells (L 929), obtained from Microbiological Associates Inc., Bethesda, Md., USA, were grown in Eagle's minimum essential medium, supplemented with 10 % calf serum.

Test procedures

Test I: Both cell lines were used. The specimens were attached centrally to the bottom of plastic petri dishes (50 mm Ø, Falcon Plastic, USA) with autoclaved silicone grease (Midland Silicones, Barry Glamorgan, U.K.). To each dish was added $0.2 \cdot 10^5$ cells suspended in 10 ml growth medium. Incubation was performed in a humidified atmosphere of CO_2 in air at 37°C for 6 days.

Test II: Only NCTC-cells were used. To avoid shifting of the specimens in the culture chamber, something that happened to some of the specimens in Test I, glass rings with an inner diameter of 36 mm and a height of 15 mm were put on top of the specimens. The inner part served as incubation chamber for the cells.

To each dish was added $1.0 \cdot 10^5$ cells suspended in 5 ml medium. After 1 and 4 days incubation, the cells were detached with 2 ml 0.25 % trypsin and counted in an electronic counter (Celloscope 101, Ljungberg, Sweden).

Test III: Only NCTC-cells were used. One set of specimens were placed in saline (25 ml/disc) for 7 days. The other set of discs was taken directly from the polyethylene bags. The same experimental setup with glass rings as described in Test II was used, except that the sensitivity of the system was increased by using $1 \cdot 10^5$ cells in only 2 ml medium. After 4 days of incubation the cells were detached with 2 ml trypsin and counted as above.

RESULTS

Test I

R&S 330 T-RTV and SK43 were water-repellent. The silicone grease did not prevent some of the discs from coming loose during incubation, which necessitated the glass ring modification used in the two other tests. However, microscopy of the area *around* the specimens showed no living cells around Silastic 399, R&S330 T-RTV and SK43, whereas a few viable cells were seen around the two MDX 4-4210 samples. The epithelial cells

appeared to be more sensitive to these materials than the fibroblasts. Only the epithelial cells were therefore used in test II and III.

Test II

Trypsination of the cells on the Silastic 399 specimens caused the cell suspension to become cloudy and milky-white, making cell counting impossible. The other materials were not affected by the trypsination procedure and exhibited a varying degree of inhibition of cell growth. Surface treatment of MDX 4–4210 with talc gave a product that appeared less cytotoxic than the untreated version of the same material.

Test III

Silastic 399 was affected by the trypsination procedure as described above. Storage of the materials 7 days in saline prior to testing indicated that the untreated MDX 4–4210 (No. 3) became more cytotoxic. No obvious differences in cell growth were seen with the other materials. Again the talc-treated MDX 4–4210 appeared less cytotoxic than the untreated version.

DISCUSSION

All RTV-silicones included in this study showed cytotoxic effects on cells cultured in vitro, but to a varying extent. Except for the two varieties of MDX 4–4210 the materials are of different composition. It must therefore be emphasized that different mechanisms may be operative in determining the apparent cytotoxic effects observed. For instance, the waterrepellency observed for SK43 and R&S330 T-RTV may act by inhibiting the attachment of cells to the surface, causing difficulties in normal cell growth by their surface properties. The increased cell growth seen on the talc-treated specimens of MDX 4–4210 may be an effect of changes on the growth surfaces (making them more hydrophilic), more than a decrease in the release of toxic substances. Soaking the materials in saline for one week did not decrease this cytotoxicity.

Silastic 399 is a condensation polymer which, when the catalyst (stannous octoate) is stirred into the silicone base, yields propanol as a split-out product from the cross-linking reaction. Most silicones are believed to be chemically fairly stable though the trypsin treatment seemed to affect Silastic 399. The trypsin used in this investigation was a rough extract of proteolytic enzymes of the pancreas.

R&S330 T-RTV, a condensation polymer, has been used for the fabrication of maxillo-facial prostheses in Denmark since 1973, and has later been introduced in Sweden and England. About 200 prostheses have been manufactured from it in Europe. The residual split-out product is an alcohol. The apparent cytotoxicity can be attributed to the catalyst (unknown), the alcohol, the water-repellency, or a combination of these.

MDX 4–4210 is an addition polymer based on dimethylvinylsiloxy-polydimethylsiloxan with a hydromethylsiloxan polymer as crosslinking agent, where the reaction is catalysed with a platinum compound. If we look at the talc treatment of the silicone surface as a way to reduce the cytotoxicity, it is evident that the other materials may be treated similarly.

SK43 is used for extrinsic coloring of maxillo-facial prostheses and for permanent soft-lining of dentures. The residual split-out product of SK43 is acetic acid which can be neutralized by post-treatment in a sodium carbonate solution.

This assessment of the cytotoxic potential of materials cannot give a conclusive answer as to whether the materials are acceptable or not when used for maxillo-

facial prostheses. In view of the high sensitivity of cell culture tests, materials exhibiting cytotoxic effects should not be excluded on these grounds alone (5). However, the results indicate that a careful follow-up patients using prostheses made from these materials is warranted.

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