Physicomechanical and cytotoxic properties of room temperature vulcanizing silicone prosthetic elastomers

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The physicomechanical and cytotoxic properties of two newly introduced room temperature vulcanizing (RTV) silicone prosthetic elastomers, Ideal and Silskin 2000, were investigated. Another RTV silicone, Elastosil M3500, was also investigated as a potential facial material. The in vitro cytotoxicity was assessed with the agar diffusion test and mouse fibroblast cells (L929). The properties investigated were tensile strength, percentage elongation, modulus, tear strength, hardness, and color changes (ΔE^*). The properties tested were selected because of their clinical significance in fabricating facial prostheses. The results indicate that Elastosil M3500 has a better combination of high tear strength, elongation at break, and low hardness than Ideal and Silskin 2000. All materials demonstrated a low cytotoxic profile. Elastosil M3500 warrants further attention with clinical trials. □ *Cytotoxicity; facial prostheses; physicomechanical properties; silicones*

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Extraoral maxillofacial prostheses (EMFP) consist of polymeric biomaterials used to restore missing and/or defective facial tissues. Ideally, maxillofacial prosthetic elastomers should not only be biocompatible but also have physicomechanical and esthetic properties similar to those of the natural tissues they replace (1). Today silicone elastomers, especially the room temperature vulcanizing (RTV) type, are the most popular materials for facial prostheses because they are less time-consuming, easy to process, flexible, and durable (2). Ideal (Orthomax, Bradford, UK) and Silskin 2000 (De Puy Healthcare, Leeds, UK) are two RTV silicone elastomers (addition reaction type) newly introduced for facial and body prostheses. The Elastosil M3500 (Wacker-Chemie GmbH, Munich, Germany) elastomer is an RTV silicone (condensation reaction type) whose potential application as a facial and body prosthetic material is being investigated. This study evaluated and compared these three silicone elastomers relative to tensile strength, modulus, percentage elongation, tear strength, hardness, color stability, and in vitro cytotoxicity.

Materials and methods

The silicone elastomers (non-pigmented) were mixed according to their manufacturers' instructions and processed in dental stone molds for 24 h at room temperature (23°C ± 2°C; relative humidity, 50% ± 5%). Perspex (ICI Ltd, Welwyn Garden City, UK) patterns machined to correspond to each method of testing (i.e. tensile, tear, hardness, color, and cell culture) were invested with dental stone (Tewestone, A. Kettenbach GmbH & Co. KG, Eschenburg, Germany) in conventional brass denture flasks to construct the molds. The mixing ratio of dental stone and water (100 g/23 mL) was according to the manufacturer's recommendation. After the stone had set, the flasks were opened and the patterns removed. Care was taken to ensure that samples were free of surface irregularities, tears or nicks at the edges, and internal defects. The same examiner visually inspected each sample. For the cell culture technique, samples were rinsed thoroughly with distilled water, dried with tissue paper, and stored in sealed plastic Petri dishes at room temperature before use. The samples were handled aseptically (i.e. with sterile water, forceps, and dishes) throughout these procedures. Ten acceptable samples were evaluated for each material and testing procedure.

Test methods

The test procedures conformed closely to specifications established by the American Society for Testing and Materials (ASTM) and the International Organization for Standardization (ISO). Testing and conditioning of samples were performed at 23°C ± 2°C and 50% ± 5% relative humidity. All of the samples were tested within 1 week after vulcanization.

Tensile test

Tensile tests were conducted according to the ISO 37:1977 procedure. Type II dumbbell-shaped specimens with a thickness of 1.8 mm were tested. Tensile strength, percentage elongation at break, and modulus at 100% elongation were measured with a Monsanto testing machine (Model T10, Monsanto Ltd., Swindon, UK)

Table 1. Definition of index values

Index	Description of zone
Zone index	
0	No detectable zone around or under sample
1	Zone limited to area under sample
2	Zone not greater than 0.5 cm in extension from sample
3	Zone greater than 1 cm in extension from sample
4	Zone greater than 1 cm in extension from sample but does not involve entire plate
5	Zone involves entire plate
Lysis index	
0	No observable lysis
1	Up to 20% of zone lysed
2	20% to 40% of zone lysed
3	40% to 60% of zone lysed
4	60% to 80% of zone lysed
5	Over 80% of zone lysed

Table 2. Cytotoxicity grading and interpretation

Index	Interpretation
0–0.5	Non-cytotoxic
0.6–1.9	Mildly cytotoxic
2.0–3.9	Moderately cytotoxic
4.0–5.0	Severely cytotoxic

equipped with a Monsanto TEO 44 automatic extensometer. The extensometer grips were set to a standard length of 20 mm using a setting gauge. The samples were stretched at a rate of 500 mm/min, and the cross-sectional area of each sample was entered into the program of the machine. The tensile strength and elongation at break were obtained automatically and displayed digitally by the machine, whereas the modulus was calculated from the stress–strain curves. The modulus at a given tensile strain (100%) is the stress in the test specimen when it is subjected to a given strain.

Tear test

Tear tests were conducted according to the ISO 34:1979 procedure with 90-degree-angle-shaped specimens with a thickness of 1.8 mm. Specimens were stretched at a rate of 500 mm/min in the Monsanto machine. Tear strength was calculated by the equation $T = F/D$, where T is tear strength, F is the force required to break the specimen, and D is the thickness of the specimen.

Hardness test

Indentation hardness was determined with a Shore A durometer (GS-706, Teclock Corp., Osaka, Japan) on specimens $25 \times 25 \times 10$ mm, according to the ASTM D2240:1988 method. Each specimen was placed on a hard horizontal surface, and 5 readings were made at least 6 mm apart. Readings were made 1 s after the pressor foot was in contact with the specimen.

Color test

Color changes (ΔE^*) were calculated with a Micro Color (Dr. Bruno Lange GmbH, Berlin, Germany) tristimulus colorimeter. This instrument was used to determine color characteristics in the CIE LAB color system. The system is an approximately uniform color space using three parameters (L*, a*, b*) to define color: L* is lightness, a* is redness–greenness, and b* is yellowness–blueness. Color change can be calculated from the following equation (3): $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$.

Color change was calculated from the data obtained before outdoor weathering (baseline) and after 6 months (March–August) of exposure to direct outdoor weathering in Athens, Greece. The samples were exposed according to ASTM G7-1983 on an untreated plywood-backed rack adjusted to a 5-degree angle from the horizontal to maximize the solar radiation on the samples. During the exposure period the average monthly solar radiation ranged from 1.04 to 1.70 $MJ.m^{-2}$ (personal communication, National Observatory of Athens, May 1998).

The specimens were disks 25 mm in diameter and 3 mm in thickness, and a positioning jig was used to ensure that readings were made at the same location on each specimen. Five readings were taken for each specimen, and the mean tristimulus values were automatically calculated and recorded by the instrument.

Agar diffusion test

This cell culture test was performed according to the method outlined in ISO 10993–5:1992. The silicone samples were disks 12 mm in diameter and 2 mm in thickness. Each 50×10-mm polystyrene culture dish (Costar, Cambridge, Mass., USA) was seeded with 3.5×10^5 mouse fibroblast cells (ATCC CCL1; NCTC clone 929, Flow Laboratories, Ayrshire, UK) in 5 mL of Eagle's minimum essential medium (E-MEM, Gibco Ltd, Paisley, UK). The nutrient medium had a bicarbonate buffer supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and 5% fetal bovine serum (FBS, Gibco Ltd). After 2 days' incubation (Biocenter 2001, Salvis S.A., Reussbühl, Switzerland) at 37°C, 98% relative humidity, and a mixture of 95% air and 5% carbon dioxide, a confluent cell layer had formed. The nutrient medium (E-MEM) was then aseptically aspirated from the culture dishes, and 3.5 mL of E-MEM containing 1.5% agarose (FMC Bio Products, Rockland, Me., USA) and supplemented with 5% FBS and antibiotics was added to each culture dish. The agarose solidified in approximately 10 min. The cells were then stained with 5 mL of 0.01% neutral red in 0.9% saline solution for 20 min in the incubator at 37°C. Subsequently, the excess dye was aspirated off. Silicone samples were placed on the surface of the agarose layer. Ten parallel cultures for each

Table 3. Means and standard deviations (*s*) of six tests on silicone elastomers*

Type of test	Silskin 2000		Elastosil M3500		Ideal	
	Mean	*s*	Mean	*s*	Mean	*s*
Tensile strength (MPa)	3.71^{A}	0.34	3.74^{A}	0.18	1.95^{B}	0.08
Modulus at 100% elongation (MPa)	0.65^{A}	0.06	0.67^{A}	0.06	0.42^{B}	0.03
Elongation (%)	304^{B}	35	365^{A}	21	283^{B}	18
Tear strength (KN/m)	14.58^{B}	1.80	21.35^{A}	1.90	12.34^{C}	1.39
Hardness (Shore A units)	22^{A}	0.88	19^{B}	0.63	17^{C}	0.57
Color change (ΔE^*)	4.09^{A}	1.47	2.94^{BA}	1.06	2.73^{BC}	0.90

* Mean values of each test designated with the same superscript letter ($^{A, B, C}$) are not significantly different ($P > 0.05$).

material were run. The dishes were incubated for 24 h at 37°C, 5% carbon dioxide, and 98% relative humidity. Ten negative (polyethylene) and ten positive (copper wire) control culture dishes were run. The copper wire specimens had been validated, as in-house positive controls, as being equivalent to using polyvinyl chloride containing dibutyl tin diacetate specimens. The cultures were examined under an inverted microscope (Leitz GmbH Wetzlar, Diavert, Germany) at ×125 magnification for cytolysis and cellular changes. Vital cells had a pink color, whereas affected cells were not stained. The results were evaluated according to the decolorization (zone) index and the lysis index, and are reported in terms of the median value (Table 1). Since the results are expressed as an index in a ranking scale, the median, instead of the mean, value was used to describe the central tendency of the scores. Then the cytotoxicity of the materials was graded and interpreted (Table 2). After the microscopic evaluation the samples were removed, and the cells were fixed in 10% formalin overnight and stained with 0.1% crystal violet in ethanol to maintain a permanent record of the experiments. The data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparisons test at the significance level $P = 0.05$.

Results

Table 3 presents means and standard deviations from six tests of the physicomechanical properties of the silicone elastomers and the outcome of Tukey's tests. In the agar diffusion test the negative control dishes revealed no change in the cell morphology. The results from control dishes with copper wire as in-house validated positive controls correspond to results from previous investigations (4) with polyvinyl chloride inducing severe cell lysis. All of the silicone elastomers showed decolorization zones limited to areas under the samples (zone index = 1), but cell lysis was mainly observed in zones 0 to 2 (lysis index) (Table 4). Accordingly, Elastosil M3500 and Silskin 2000 can be graded as mildly cytotoxic, whereas Ideal is a non-cytotoxic material.

Discussion

The six properties evaluated in this study are useful for screening and evaluating maxillofacial prosthetic elastomers. Although not inclusive of all service requirements, they are important physicomechanical properties and are useful for predicting how these materials will perform in practice. Materials used or proposed for facial prostheses should show high values of strength, toughness, and tear strength along with low values of hardness and modulus (5). Tensile strength, elongation, modulus, and tear strength together relate to the problem of elastomer tearing while in use and maintenance (i.e. at fine edges) and to compliance with facial movement. Hardness reflects the tactile response of lifelike perception. The limited service of facial prostheses is a result of the rapid degradation of the elastomer and its intrinsic and extrinsic color instability. Intrinsic color changes of non-pigmented elastomer is a contributing factor to the overall discoloration of facial prostheses observed clinically (6). It has been reported that a color change (ΔE^*) equal to 1 is considered visually detectable 50% of the time; a ΔE^* greater than 2, 100% of the time (3). For the purposes of this study a ΔE^* greater than 2 was used as the baseline. All of the silicone elastomers evaluated exhibited low values of modulus and hardness, and ΔE^* greater than 2, which is considered

Table 4. Cytotoxicity of silicone elastomers by agar diffusion test

Material	Zone index		Lysis index		Interpretation
	Median	Range	Median	Range	
Elastosil M3500	1	(1–1)	1.2	(0–2)	Mildly cytotoxic
Silskin 2000	1	(1–1)	1.7	(1–2)	Mildly cytotoxic
Ideal	1	(1–1)	0.25	(0–1)	Non-cytotoxic

visually detectable after 6 months of outdoor weathering. Elastosil M3500 had the highest tensile strength, tear strength, and percentage elongation; Ideal, the lowest. It has been reported that although the materials must possess reasonable tensile strength, high tear strength, primarily for edge integrity, is more important clinically than tensile strength and modulus (3, 7). In addition, Conroy et al. (8) concluded that a high-percentage elongation at break coupled with a high tear strength produces the most desirable combination. It seems from our results that Elastosil M3500 has the most favorable combination of mechanical properties—high tensile strength, tear strength, and percentage elongation, along with low modulus and hardness—among the three elastomers. The agar diffusion test revealed that the zone indexes were equal for all of the materials, indicating that diffusible substances were released. However, Ideal had a lower lysis index, indicating less cytotoxicity of the diffusible substances for this material.

Assessment of the cytotoxic potential of silicone elastomers cannot provide a definitive answer regarding whether the materials are acceptable for use in the construction of facial prostheses (4, 9). Extrapolation of cell culture tests to in vivo situations must be performed with due caution. In vitro cell culture techniques have been reported to be predictive for in vivo tests results (10). Ideal and Silskin 2000 are in clinical use today, and there are no reports of adverse effects in the relevant literature. The data obtained revealed that Elastosil M3500 has a favorable combination of physicomechanical properties that provide advantages in clinical applications, as well as a low cytotoxic profile that merits further attention in clinical investigation.

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References

1. Lontz JF. State-of-the-art materials used for maxillofacial prosthetic reconstruction. Dent Clin North Am 1990;34:307–25.
2. Andres CJ, Haug SP, Brown DT, Bernal G. Effects of environmental factors on maxillofacial elastomers. II. Report of survey. J Prosthet Dent 1992;68:519.
3. Seghi RR, Hewlett ER, Kim J. Visual and instrumental colorimetric assessment of small color differences on translucent dental porcelain. J Dent Res 1989;68:1760–4.
4. Polyzois GL, Hensten-Pettersen A, Kullmann A. Effects of RTC-silicone maxillo-facial prosthetic elastomers on cell cultures. J Prosthet Dent 1994;71:505–10.
5. Lewis DH, Castleberry DJ. An assessment of recent advances in external maxillofacial materials. J Prosthet Dent 1980;43:426–32.
6. Beatty MW, Mahanna GK, Dick K, Jia W. Color changes in dry-pigmented maxillofacial elastomer resulting from ultraviolet light exposure. J Prosthet Dent 1995;74:493–8.
7. Kouyoumdjian J, Chalian VA, Moore BK. A comparison of the physical properties of a room temperature vulcanizing silicone modified and unmodified. J Prosthet Dent 1985;53:388–91.
8. Conroy B, Haylock C, Hulterstrom A, Pratt G, Winter B. Report of a four year research and development programme involving the Inst. of Maxillo-Facial Technology and the Univ. of Wales Inst. of Science and Technology aimed at the production of a new facial prosthetic system. Proc Inst Maxillo-Facial Tech 1979. p. 218–45.
9. Grasso R, Gaydon J, Hendy RJ. The safety testing of medical plastics. II. An assessment of lysosomal changes as an index of toxicity in cell culture. Fd Cosmet Toxicol 1973;11:255–63.
10. Rice RM, Hegyeli AF, Gourlay SJ, Wade SW, Dillon JG, Jafe H, et al. Biocompatibility testing of polymers: in vitro studies with in vivo correlation. J Biomed Mater Res 1978;12:219–32.