Cellular Response to Pigmented Silicon Maxillofacial Prosthetic Material

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Abstract: Introduction: This study intended to assess *in vitro* the cytotoxicity of pigmented maxillofacial silicon M511 after immersion of the specimens in dissimilar simulated storage condition for six month period at 37^0 C. **Material and Methods:** Sixty disc shaped pigmented specimens were prepared according to the manufacturer's directions. The specimens divided into four groups. Group I served as control and the other three test groups were immersed in different storage solution. Cytotoxicity test was done using WST-1assay and hMSCs at 24 and 72hrs incubation. Results: Our result revealed that the test groups showed a significant increased of the survival cell rates at 24 and 72hrs.Comparison between the 24hrs and 72hrs in group IV showed a significant decreased of the survival cell rates. Conclusions: hMSCs is recommended for better screening of the cytotoxic effect of cosmesil elastomer. The pigmented cosmesil M511 cross linking elastomer had no cytotoxic effect and more compatible when immersed in alkaline and acidic solution while sebum showed minimal biocompatibility.

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Key words: silicon elaster, simulated solution, cytotoxicity assay

1. Introduction:

Acquired and congenital defects of the face require facial rehabilitation since they create an unfavorable condition and discomfort for the patients. Although the new materials have exhibited some excellent properties, still they have also exhibited some frustrating deficiencies. Thus reassessment of materials used in the field of maxillofacial prosthesis seems desirable [1].

The facial skin in human is believed to be a protective barrier against the variety of microorganisms or harmful conditions and serves as the interface with the external world throughout life [2].

Maxillofacial prosthesis silicone base material is colored using various pigments according to the patient's skin color. Primary color pigments are used in the coloring maxillofacial silicones, silicone base, water base, oil base and as dry pigments commercially available. Pigments play the important role of imparting color to prostheses. Evaluation of color stability using combinations of pigments, opacifiers and an understanding of the effects and interactions of each component or the ingredients is necessary to produce the most color stable prosthesis. The material should be compatible with human tissues and should cause no irritation or inflammation and noncarcinogenic [3,4].

Silicone is biocompatible and biodurable. It is easy to manipulate with adequate working time and good color stability [5,6]. Its physical properties of relevance include hardness, high tear resistance and reliable bonding to acrylic substructures which are frequently used along with them [7,8]. Finished facial prostheses rest on living human skin for extended periods and may absorb perspiration and sebum. These absorbed species may cause degradative changes in the elastomer structure, resulting in the ultimate deterioration of the prosthesis [9,6] within two years and reduced the clinical longevity of the prosthesis.

Due to the cytotoxicity or biocomptability, testing is necessary. The phenomenon of cytotoxicity depends on some factors as the potency of the material, time of exposure and concentration.These factors are antagonstic on the growth and function of the cells. Although biological safety of maxillofacial materials and pigments from various manufacturers has been approved separately prior to their applications, there are still concerns regarding hazards and toxicity potentially caused by chemical reactions and contaminations. Very few studies have been reported regarding effects of pigments on cytotoxic properties of maxillofacial silicone elastomers although the base materials and pigments seem to be almost not dangerous respectively, [10]. Since this issue is very important for pigmented maxillofacial materials, therefore the rationale of the study was to evaluate in vitro the cytotoxicity of pigmented maxillofacial silicon M511 after immersion in different simulated storage condition for six month period at 37^0 C.

2.Materials and Methods:

Sample preparation:

Sixty disc shape specimens of pigmented maxillofacial Silicone (Cosmesil Series maxillofacial

rubber M511, Medical grade Technovent Co, U.K) were prepared with 25mm in diameter and 3mm thickness [11].

The specimens were fabricated and polymerized as follow:

A ratio of 10 gm (part A) of silicon elastomer to 1gm catalyst (part B) (10:1 =11gm totally) were prepared and mixed with 0.2% by weight pigments (Intrinsic Colorants pigments agents, Product code: P409-P420, Technovent, U.K) until a homogenous color is obtained [9, 12]. Then the mix is poured into the molds premade to the specific dimensions required by International Standardization Specification. The molds closed, and polymerized in a dry heat oven at 100^0 C for 1hr.

The prepared specimens after polymerization were divided into four groups, each of fifteen samples.

Group I: fifteen pigmented specimens served as control and were not immersed in any storage solution.

Group II: fifteen pigmented specimens were immersed in Simulated acidic solution (solution a).

Group III: fifteen pigmented specimens were immersed in simulated alkaline solution (solution b).

Group IV: fifteen pigmented specimens were immersed in simulated sebum solution (solution c). All the pigmented specimens immersed in the simulated storage solution were placed in incubator at 37ºC for six months.

Composition of the different simulated storage solutions:

a) Simulated acidic:

Acidic perspiration (pH5.5) enclosing per liter of distilled water: 0.5g L-histidine monohydrochloride monohydrate, 5g sodium chloride, and 2.2g sodium dihydrogen orthophosphate dehydrate.

b) Simulated alkaline:

Alkaline perspiration (pH 8) containing the following per liter of distilled water: 0.5g L-histidine mono-hydrochloride monohydrate, 5g sodium chloride, and 5g disodium hydrogen orthophosphate dodececahydrate

The solution (a and b) was prepared according to International Organization for Standardization (ISO) specification [13].

c) Simulated sebum:

It was prepared using 10% palmitic acid and 2% tripalmitin dissolved in 88% linoleic acid [14].

Sterilization of specimens:

All the pigmented specimens groups (group I, II, III) were sterilized by immersion in 70% Ethanol for 10 min in 50 ml falcon tubes.

Washing of the specimens in 1× PBS (Phosphate Buffer Saline) and leaving under the hood to dry.

Cell culture media:

The immersed discs in simulated acid, alkaline and sebum solutions were placed in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Germany) with 15% fetal bovine serum (FBS, Sigma, Germany,) and 1% antibiotic(PAA, Germany) incubated at 37°C of 5% CO2 for 24hrs and 72 hrs. After the incubation periods, the extracts were filtered through 0.22µm filters and then used to evaluate cytotoxicity.

The experiment was divided into two groups as follow:

- Control group (groupI): hMSCs (human mesenchymal stem cells) cultured in complete culture media for 24hrs and 72hrs.
- Test groups (II, III, IV): hMSCs cultured in extracted media of acid, alkaline and sebum for 24 hrs and 72 hrs.

Cytotoxicity Assay:

Cytotoxicity was evaluated by WST1 assay (Roche applied science,Germany) and was performed according to the manufacturer's specifications. It is a colorimetric assay based on the cleavage of WST-1 tetrazolium salt by mitochondrial dehydrogenases [15].

Exponentially hMSCs at passage 8 were harvested in DMEM with 15% FBS and 1% antibiotic and plated into 96-well microplates at seeding density of 1.7 x 10^3 cells in a volume of 100 µl per well and incubated in complete medium at 37°C,5% CO_2 for 24 hrs. Removal of culture medium from the wells and 100 µL of the extracts were added in each well and incubated for 24 h and 72 hrs at 37°C and 5% CO_2.

The wells containing cells without tested substances served as control,100 µL of the complete culture media was added and incubated for 24 h and 72 hrs at 37°C and 5% CO_2.

After the exposure period, extract medium was changed, 100 µL of fresh medium were added per well. 10 µl of WST1 solution was added to all wells. The medium also was changed in the control wells. Culture plates were covered with aluminum foil to protect from light and cells were incubated in a dark for 4 h at 37°C and 5% CO_2. Shaking of the well plates thoroughly for 1 minute on a shaker.

Optical density was measured on a spectrophotometer plate reader (Multiscan MCC340, Labsystems Germany) at 450 nm.

The empty wells acted as blanks. WST-1 assay were repeated in three separate experiments to ensure reproducibility. The survival rates of the controls were set to represent 100% proliferation.

Statistical Analysis:

All statistical analyses were performed using Statistical Package for the Social Science software (SPSS, version 17, Chicago, IL, USA). Descriptive statistics as means and standard deviations were used. Student –t- test of significance at 5 percent was performed for comparison between means of the control group and the test groups at 24hrs and 72hrs.

Paired t-test value was used for comparing between 24hrs and 72hrs incubation in each group.

3. Results:

In the WST-1 assay, the mean value of the survival cell rate of hMSCs in the test materials was significantly decrease in group II (t=2.735), whereas in group III and group IV, the mean value of the survival cell rate was increased (t=4.352, 8.926) after 24hrs of incubation when compared to the control group as showed in table (1).

In the WST-1 assay, the mean value of the survival cell rate of hMSCs cells in the test material was significantly increased in group II, group III and group IV (t= 21.221,8.915,6.544) after 27hrs of incubation as compared to the control group as showed in table (2).

Comparison between the mean value of survival cell rate at 24hrs and 72hrs of each group I and group III showed no significant difference while in group II and group IV showed significant difference. (t=21.220, 4.817) respectively as shownin table (3).

Table (1): Comparison between the test groups and the control group after 24hrs incubation

Parameter	Control Group I	Test groups		
		Acid (group II)	Alkaline (group III)	Sebum (group IV)
Mean ± SD.	0.99 ± 0.09	0.85 ± 0.13	1.54 ± 0.39	2.17 ± 0.41
t (p)		2.735 *(0.014*)	4.352 *(0.001*)	8.926*(<0.001*)

t: Student t-test (comparison between the test groups and control group)
*: Statistically significant at $p \leq 0.05$

Table (2): Comparison between the test groups and the control group after 72hrs

Parameter	Control Group I	Test groups		
		Acid (group II)	Alkaline (group III)	Sebum (group IV)
Mean ± SD.	1.0 ± 0.08	1.96 ± 0.12	1.81 ± 0.28	1.40 ± 0.18
t (p)		21.221*(<0.001*)	8.915* (<0.001*)	6.544* (<0.001*)

t: Student t-test (comparison between the test groups and control group)
*: Statistically significant at $p \leq 0.05$

Table (3):Comparison between the test groups and control group after 24hrs and 72hrs incubation.

	24hrs	72hrs	t	p
Control group (I)				
Mean ± SD.	0.99 ± 0.09	1.0 ± 0.08	0.324	0.753
Acid group (II)				
Mean ± SD.	0.85 ± 0.13	1.96 ± 0.12	21.220*	<0.001*
Alkaline group (III)				
Mean ± SD.	1.54 ± 0.39	1.81 ± 0.28	1.955	0.082
Sebum group (IV)				
Mean ± SD.	2.17 ± 0.41	1.40 ± 0.18	4.817*	0.001*

t: Paired t-test (comparison between 24hrs and 72hrs incubation in each group)
*: Statistically significant at $p \leq 0.05$.

4. Discussion:

The recent technology and methodology for fabrication of pigmented maxillofacial prostheses are important to improve patient's aesthetics and patient's quality of life [16,17].

Cosmesil intrinsic skin pigments shade was used to simulate the normal skin and racial skin tones,.It was suspended in silicone fluids to increase levels of color stability and pigment dispersion [18].The amounts of pigments employed for colorant maxillofacial prosthetic elastomers may be affected the structures and properties of these materials. Cell culture method was used to study the effect of pigmented cosmesil series M511 on the viability of tissue cells, because the amount of pigments may affect the structures of these materials and the facial prosthetics that resting on living human skin for extended periods, may absorb perspiration and sebum causing deterioration of prosthesis [3,19,20]

In vitro cytotoxicity tests were developed to simulate and predict biological reactions of the human facial skin tissues to the maxillofacial silicon materials. In recent years cytotoxicity of dental materials has been evaluated in a variety of ways like MTT assay and WST-1 which can be used for the measurement of cell proliferation and estimation of the number of viable

cells in culture [21,19]. The new cell proliferation Reagent WST－1 cell cytotoxicity assay was used in this study as it is sensitive and accurate assay for cell cytotoxicity and proliferation. The assay is highly convenient because it is performed in a single tissue culture well and requires no washing, harvesting or solubilization of cells. Adherent or suspension cells are cultured in a microplate and then incubated with WST－1 and the assay is monitored with a spectrophotometer. The assay principle is based upon the reduction of the tetrazolium salt WST－1 to formazan by cellular dehydrogenases. The generation of the dark yellow colored formazan is measured at 450 nm and is directly correlate to cell number [22, 23,15].

Human Mesenchymal stem cells (hMSCs) were selected in our study because the selection of cell type is important. With the progress of tissue engineering field,BMSCs are used in the protocol line of cytotxicity test because the maxillofacial silicon materials are in contact with the facial complex defect,movable tissue bed,graft and flap application.The effects on cells within that tissue should be observed and evaluated [24]. hMSCs are multipotent self-renewing progenitor cells with the ability to secrete growth factors, cells capable of differentiating into several cell lineages including osteoblasts, chondrocytes, and adipocytes [25], easy isolation and expansion, and unique anti-inflammatory and immune-modulatory properties [26.27].

Our results revealed that the survival cell rates of the test group II showed a significant decreased at the 24hrs incubation.This result may be contributed to the immersion of the pigmented disc specimens in acidic simulated solution (pH less than 7) which affect the surface of the specimens and then affect the cell proliferation viability showing a decreased number of living cells, the morphological functionality were inhibited and mitochondria activity changed lead to a variety of physiological responses [28,29].

After 72hrs incubation the survival cell rates of test group II was significantly increased which might be due to hydrophobic silica fillers present in the polymer matrix of cosmesil siliconM511 repelling water molecules and hence stop acidic solution absorption into substance [30,31].

The survival cell rates of group III and groupIV was significantly increased at 24hrs incubation. This result might be due to no interaction of alkaline or fatty solution with the surface of Cosmesil silicon M511 of the pigmented specimens [3].

After 72hrs incubation the cell viability of cosmesil silicon of group II was significantly increased suggesting that time factor may affect the cell activity. While in group IV the survival cell rate was significantly decreased after 72hrs compared to 24hrs incubation suggesting that time factor and the chemical composition of the simulated sebum solution could affect the cytotoxicity of the materials [3,11].

Conclusion:

hMSCs is recommended for better screening of the cytotoxic effect of cosmesil silicon elastomer. The pigmented cosmesil silicon M511 cross linking elastomer had no cytotoxic effect and more compatible when immersed in alkaline and acidic solution, while sebum showed minimal biocompatibility.

Clinical implication:

A clear cleaning is necessary for cosmesil silicon M511 from any sebum in contact with maxillofacial prosthesis.

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