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Original Research Article

Cytotoxicity of Different Impression Materials

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Abstract

In the moment it is on the market you can meet different types of impression materials differing in their properties and chemical composition. *The purpose* of the work was to test the cytotoxicity of various types of materials for taking impressions. *Material and methods*: Samples of 7 different materials (alginates, silicones, impression compound, zinc oxide eugenol, and acrylic) were tested on cell culture Vero CCL-81 in direct contact for a period of 2 hours. *Results*: One alginate material Elastic Cromo (71.35%) and Impression Compound (80.42%) haven't negative influence on the cell cultures. Others significantly inhibit the development of cell cultures (Image 34.25%, Zetaplus 11.45%, Stomaflex Putty 8.02%, Repin 10.37%, FITT 28.92%). *Conclusion*: Most of the impression materials tested have cytotoxic properties.

Keywords: impression materials, alginate, silicones, zinc oxide, eugenol, cytotoxic acrylics.

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Introduction

At the moment, almost every prosthetic procedure begins with the impression taking. On the market it is possible to find a lot of matrices designed for impressions, differing in chemical composition and properties [1]. The most popular is the alginate impression mass (delivery in powder), which prior to use is mixed with water. The powder is not a homogeneous substance, but a mixture of sodium alginate, gypsum dihydrate, tri-sodium phosphate and diatomaceous earth as filler. The cross-linking reaction consists in converting sodium ions through calcium in the structure of alginate [2].

The second commonly used material for taking impressions for crowns and bridges are silicones. Among them they are two groups of products hardened by additions or condensations. In both cases, it is necessary to mix the two pastes, base mass and catalyst prior to use. The material polymerizes in the temperature of the oral cavity in 3-5 minutes [1, 3].

One of the older impression materials is masses based on the reaction of zinc oxide with eugenol. They are used to perform impressions on individual trays for removable dentures [1, 4].

The use of thermoplastic materials on the basis of waxes and resins is also necessary for making off the edge of individual trays. They are calling non elastic materials - among which we stand out impression compound. During their heating, the material becomes soft, and after reducing the temperature it is hardening, on the basis of physical process [1, 5].

A separate group of impression materials are materials that contain acrylic resins, called tissue conditioners. They are used, among others, to take functional impressions on old prostheses. After mixing the powder with the liquid, there is no chemical reaction, but the absorption of liquid by the powder. The powder is a poly (ethyl methacrylate) and the liquid is a mixture of ethyl alcohol and a plasticizer. The time to remove functional impression from the patient mouth can be longer in this case and takes from minutes to even 1-2 days [6, 7].

The time of contact between each of the above-mentioned materials with the human body is relatively short, which is why they are all included in the 1st group of medical device. Therefore, before being placed on the market, they must be tested for their biocompatibility. One of the most popular normative tests is the cytotoxicity test on cell cultures. It gives an answer to the question whether the material will interact with the human or not [1,3].

At the moment, there is a small amount of publications in the literature that cover the cytotoxicity of impression materials. Among them are articles discussing individual groups of materials, for example alginate or silicone materials. This is mainly due to the fact that such materials are for short-term use. Unfortunately, it is not taken into account, that in the case of a dentist, he or she is staying in the long-term effects of this material. Individual groups of materials, examined by various authors, are difficult to compare in a precise way, because they used a variety of research methods and various cell cultures. Therefore, it seems advisable to display the cytotoxicity of various impression materials in one study.

The purpose of this work was to present the cytotoxicity of various impression materials using the MTT method direct contact.

MATERIALS AND METHODS

The following impression materials were used for testing: alginate: Elastic Cromo (SpofaDental), Image (Dux), condensation silicone Stomaflex Putty (SpofaDental), Zetaplus (Zhermack), based on zinc oxide with eugenol - Repin (SpofaDental), as thermoplastic mas - Impression Compounds (Kerr) and for functional impressions - FITT (Kerr). Compositions of individual materials are presented in Table 1.

Table-1: Composition of impression materials used in the study

material	Batch number	Mixing ratio	composition
Elastic Cromo	6627278	20 g powder/ 40 ml water	Diatomic earth, sodium alginate, calcium sulphate dihydrate, tri sodium phosphate, potassium hexafluorotitanate aroma, trace of phenolphthalein and thymolphthalein, propylene glycol
Image	6645787	18g powder/ 48 ml water	Diatomic earth, potassium alginate, calcium sulphate dihydrate, tetra sodium pyrophosphate, potassium hexafluorotitanate Mint aroma, Vaseline oil
Stomaflex Putty/ Stomaflex Catalyst	putty:6877371, catalyst:6891786	6.5 g base/ 0.13 g catalyst	Base: Polysiloxanes hydroxy terminated, calcium carbonate Catalyst: silica, amorphous, crystalline-free, Nonylphenol, ethoxylated Dibutyltin dilaurate, tetraethyl silicate, ethyl silicate methyl silicate
Zetaplus Indurent gel	Putty: 280241 Catalyst:275661	6.5 g base/ 0.13 g catalyst	Base: Polysiloxanes hydroxy terminated, crystobalite Catalyst: trimethoxypropylsilane dioctyltin oxide,ethyl silicate, carvone
Repin		2 ml base/ 2 ml catalyst	Base: Zinc oxide, mineral oil, mint aroma Catalyst: Eugenol, colophony, oil, amorphous silica,
Impression Compound Red	7050223	One compound melted in water 55 C	Stearic acid, copal resins, talc, iron oxide red
FITT	6989932	1.4g powder/1 g liquid	Powder: poly(ethyl methacrylate), titanium dioxide Liquid: ethanol, dibutyl phthalate

Samples preparation

Samples are mixed according the manufactures information and filled in the acid-resistant steel moulds, 6 mm in diameter. Ten samples were prepared for each material, of which six were used for further tests. The material was tested 24 hours after preparation of the sample. The alginate materials were stored in polyethylene bags wrapped in a water-infused paper towel to protect them from dry out.

Performed tests

The impression materials discs test articles were evaluated using the MTT (methylthiazolyldiphenyltetrazolium bromide). The viability assay was performed on VERO CCL-81 cell line. Cells were maintained in MEM medium containing 4% FBS in 37°C in humified atmosphere enriched by 5% CO2. Before exposition cells were suspended on 96-well plate in density 1x104 cells/100 μl in MEM/well. The cells were cultivated for 24 hours to obtained 80% of confluency, and then were exposed to tested samples, positive and negative controls during

2, 4, and 24 hours. Cell medium was removed and 6x 100 μ l of the sample, positive control, negative control, blank samples were added to individual wells. After the incubation time with samples, MTT test was applied using in final step 2-isopropanol (100 μ g/l/well) with simultaneous shaking. The absorbance was detected at 570 nm. The negative control (MEM) and blank sample (MEM with 4% FBS) both demonstrated no cytotoxic effect, thus cell oxidoreductive potential was undisturbed. The positive control (Sodium lauryl sulphate) demonstrated significant cytotoxic impact even after the shortest time of incubation (2h). The viability was less than 20% after 2h exposition and was less than 10% after 4 and 24 hours.

The test article extract was prepared in 1x MEM cell growth medium (MEM supplemented with 10% fetal bovine serum extract) at the sample to extraction medium ratio of 6.0 cm 2/mL and extracted at 37 ± 1 °C for 72 ± 2 hours. The sample was unchanged by the extraction procedure and the extract was found to be clear and free of particulates.

DATA ANALYSIS

According the obtained results the following data analysis was performed. The results obtained from spectrophotometric measurements defined as the viability were calculated according to the formula:

Viability $\% = (100 \times OD570 \text{ test sample})/OD570 \text{ blank}$

In the equation are used mean values of all measured optical densities at 570 nm of respective samples. The basic interpretation is based on the following observation, the lower the viability % value,

the higher the cytotoxic potential of the test sample is. If viability is reduced to <70% of the blank, it has a cytotoxic potential. The results are shown in Tab 2 for tested samples and in Tab. 3-9 for control samples. Material was tested after 2 hours, because impression procedure takes few minutes and the contact time with patient mouth is very short.

RESULTS

Results from the performed tests were collected in below tables.

Table-2: Results from the cytotoxicity tests of impression materials after 2 hours

	Elastic Cromo	Image	Zetaplus	Stomaflex	Impression compound	Repin	FITT
	2 hours	2 hours	2 hours	2 hours	2 hours	2 hours	2 hours
Concentration	100%	100%	100%	100%	100%	100%	100%
OD_{570}	0.452	0.424	0.077	0.014	0.352	0.043	0.125
	0.4	0.334	0.024	0.016	0.342	0.027	0.081
	0.533	0.293	0.198	0.018	0.51	0.031	0.076
	0.488	0.184	0.218	0.018	0.584	0.028	0.081
	0.415	0.018	0.13	0.018	0.604	0.017	0.045
	0.401	0.082	0.035	0.018	0.64	0.02	0.174
Mean	0.448	0.223	0.114	0.017	0.505	0.028	0.097
Viability %	71.35%	34.25%	11.45%	8.02%	80.42%	10.37%	28.92%

Table-3: Cytotoxicity of control solutions for Elastic Cromo after 2 hours

	PC (Sodium	n lauryl sulph	ate dill in MI	NC(MEM)	Blank (MEM in	with 4% FBS)	
Concentration	0.05	0.10	0.15	0.20	100%	100%	100%
	mg/ml	mg/ml	mg/ml	mg/ml		2 nd row	11 th row
OD ₅₇₀	0.121	0.078	0.07	0.05	0.512	0.716	0.532
	0.12	0.08	0.075	0.058	0.428	0.769	0.441
	0.113	0.075	0.069	0.059	0.439	0.841	0.514
	0.119	0.07	0.069	0.06	0.563	0.718	0.534
	0.125	0.085	0.058	0.058	0.555	0.643	0.594
	0.11	0.085	0.075	0.05	0.55	0.597	0.636
Mean	0.118	0.079	0.069	0.056	0.508	0.628	
Difference in %						13.71%	
Viability v %	18.79	12.58	10.99	8.92	80.9	100%	

Table-4: Cytotoxicity of control solutions for Image after 2 hours

Table-4: Cytotoxicity of control solutions for finage after 2 nours										
	PC (Sodium	n lauryl sulph	ate dill in Ml	NC(MEM)	Blank	with 4%				
						(MEM in	FBS)			
Concentration	0.05	0.10	0.15	0.20	100%	100%	100%			
	mg/ml	mg/ml	mg/ml	mg/ml		2 nd row	11 th row			
OD_{570}	0.121	0.078	0.07	0.05	0.512	0.716	0.532			
	0.12	0.08	0.075	0.058	0.428	0.769	0.441			
	0.113	0.075	0.069	0.059	0.439	0.841	0.514			
	0.119	0.07	0.069	0.06	0.563	0.718	0.534			
	0.125	0.085	0.058	0.058	0.555	0.643	0.594			
	0.11	0.085	0.075	0.05	0.55	0.597	0.636			
Mean	0.118	0.079	0.069	0.056	0.508	0.628				
Difference in %						13.71				
Viability v %	18.79	12.58	10.99	8.92	80.9	100%				

Table-5: Cytotoxicity of control solutions for Zetaplus after 2 hours

		m lauryl sulp		NC(MEM)	lank	with 4%	
	`				(MEM in	FBS)	
Concentration	0.05	0.10	0.15	0.20	100%	100%	100%
	mg/ml	mg/ml	mg/ml	mg/ml		2 nd row	11 th row
OD_{570}	0.182	0.1	0.075	0.07	0.812	0.828	1.022
	0.165	0.122	0.072	0.073	0.89	0.987	1.325
	0.16	0.103	0.078	0.07	0.961	1.351	1.496
	0.174	0.095	0.082	0.065	0.902	0.708	1.034
	0.172	0.095	0.062	0.079	0.781	0.807	0.859
	0.174	0.121	0.08	0.07	0.547	0.69	0.836
Mean	0.171	0.104	0.075	0.071	0.816	0.995	
Difference in %						10.06	
Viability v %	17.18	10.45	7.54	7.13	81.99	100%	

Table-6: Cytotoxicity of control solutions for Stomaflex after 2 hours

	PC (Sodiu	m lauryl sulp	hate dill in I	MEM	NC(MEM)	Blank	with 4%
					(MEM in	FBS)	
Concentration	0.05	0.10	0.15	0.20	100%	100%	100%
	mg/ml	mg/ml	mg/ml	mg/ml		2nd row	11 th row
OD_{570}	0.112	0.066	0.06	0.045	0.121	0.199	0.222
	0.137	0.052	0.058	0.046	0.159	0.184	0.171
	0.124	0.051	0.056	0.051	0.263	0.231	0.227
	0.124	0.049	0.055	0.04	0.271	0.219	0.264
	0.131	0.058	0.049	0.041	0.269	0.209	0.245
	0.11	0.06	0.058	0.045	0.18	0.192	0.181
Mean	0.123	0.056	0.056	0.045	0.211	0.212	
Difference in %						2.99	
Viability v %	58.02	26.42	26.42	21.23	99.53	100%	

Table-7: Cytotoxicity of control solutions for Impression Compound Red after 2 hours

	PC (Sodium	lauryl sulphate	e dill in MEM	•	NC(MEM)	Blank	with 4%
						(MEM	FBS
Concentration	0.05 mg/ml	0.10 mg/ml	0.15 mg/ml	0.20 mg/ml	100%	100% 2 nd	100%
						row	11 th row
OD_{570}	0.121	0.078	0.07	0.05	0.512	0.716	0.532
	0.12	0.08	0.075	0.058	0.428	0.769	0.441
	0.113	0.075	0.069	0.059	0.439	0.841	0.514
	0.119	0.07	0.069	0.06	0.563	0.718	0.534
	0.125	0.085	0.058	0.058	0.555	0.643	0.594
	0.11	0.085	0.075	0.05	0.55	0.597	0.636
Mean	0.18	0.079	0.069	0.056	0.508	0.628	
Difference in %						13.71	
Viability v %	18.79	12.58	10.99	8.92	90.9	100%	

Table-8: Cytotoxicity of control solutions for Repin after 2 hours

	PC (Sodium	lauryl sulpha	te dill in MEM	NC(MEM)	Blank (MEM in	with 4% FBS)	
Concentration	0.05 mg/ml	0.10 mg/ml	0.15 mg/ml	0.20 mg/ml	100%	100% 2 nd row	100% 11 th row
OD_{570}	0.15	0.05	0.05	0.04	0.373	0.412	0.285
	0.149	0.059	0.05	0.035	0.248	0.425	0.241
	0.138	0.5	0.056	0.035	0.3	0.269	0.247
	0.138	0.061	0.059	0.041	0.26	0.261	0.228
	0.14	0.059	0.059	0.045	0.29	0.219	0.22
	0.127	0.055	0.055	0.046	0.248	0.18	0.254
Mean	0.14	0.056	0.055	0.04	0.287	0.27	
Difference in %						8.98	
Viability v %	51.84	20.73	20.36	14.81	106.26	100%	

Table-9: Cytotoxicity of control solutions for FIIT after 2 hours

	PC (Sodiun	n lauryl sulph	ate dill in MI	NC(MEM)	Blank	with 4%	
					(MEM in	FBS)	
Concentration	0.05	0.10	0.15	0.20	100%	100%	100%
	mg/ml	mg/ml	mg/ml	mg/ml		2 nd row	11 th row
OD_{570}	0.175	0.07	0.055	0.054	0.306	0.216	0.294
	0.18	0.07	0.06	0.06	0.285	0.357	0.371
	0.169	0.068	0.06	0.068	0.26	0.37	0.405
	0.169	0.055	0.071	0.068	0.272	0.318	0.436
	0.188	0.07	0.065	0.055	0.285	0.271	0.379
	0.16	0.068	0.066	0.065	0.29	0.205	0.403
Mean	0.174	0.067	0.063	0.062	0.283	0.335	
Difference in %						13.69	
Viability v %	51.88	19.97	18.78	18.48	84.37	100%	

Quality check of assay

Elastic Cromo: In case of 2 hour incubation the mean OD_{570} of blanks is 0.651 and is therefore greater than 0.2. The left (absorbance values in 2^{nd} row) and the right (absorbance values in 11^{th} row) mean of blanks in the 96-well plate differ from the mean of absorbance of all blanks (OD_{570}) by 13.71%, which is less than 15%.

Image: In case of 2 hour incubation the mean OD_{570} of blanks is 0.628 and is therefore greater than 0.2. The left (absorbance values in 2^{nd} row) and the right (absorbance values in 11^{th} row) mean of blanks in the 96-well plate differ from the mean of absorbance of all blanks (OD_{570}) by 13.71%, which is less than 15 %.

Zetaplus: In case of 2 hour incubation the mean OD_{570} of blanks is 0.995 and is therefore greater than 0.2. The left (absorbance values in 2^{nd} row) and the right (absorbance values in 11^{th} row) mean of blanks in the 96-well plate differ from the mean of absorbance of all blanks (OD_{570}) by 10.06%, which is less than 15 %.

Stomaflex Putty: In case of 2 hour incubation the mean OD570 of blanks is 0.212 and is therefore greater than 0.2. The left (absorbance values in 2^{nd} row) and the right (absorbance values in 11^{th} row) mean of blanks in the 96-well plate differ from the mean of absorbance of all blanks (OD₅₇₀) by 2.99%, which is less than 15 %.

Impression Compound Red: In case of 2 hour incubation the mean OD_{570} of blanks is 0.628 and is therefore greater than 0.2. The left (absorbance values in 2^{nd} row) and the right (absorbance values in 11^{th} row) mean of blanks in the 96-well plate differ from the mean of absorbance of all blanks (OD_{570}) by 13.71%, which is less than 15 %.

Repin: In case of 2 hour incubation the mean OD_{570} of blanks is 0.270 and is therefore greater than 0.2. The left (absorbance values in 2^{nd} row) and the right (absorbance values in 11^{th} row) mean of blanks in

the 96-well plate differ from the mean of absorbance of all blanks (OD_{570}) by 8.98%, which is less than 15 %.

FITT: In case of 2 hour incubation the mean OD_{570} of blanks is 0.335 and is therefore greater than 0.2. The left (absorbance values in 2^{nd} row) and the right (absorbance values in 11^{th} row) mean of blanks in the 96-well plate differ from the mean of absorbance of all blanks (OD_{570}) by 13.69%, which is less than 15 %.

DATA ANALYSIS

The viability of the cells after 2 hours application of the test samples *Elastic Cromo* and Impression Compound Red were >70%. According to obtain results these two materials haven't cytotoxic effect. For the rest of materials values were lower (Image 34.25%, Zetaplus 11.45%, Stomaflex Putty 8.02%, Repin 10.37% and FITT 28.92%). It means, that these impression materials affected on the cell cultures. The cytotoxic effect of positive control has been proven in all concentrations. Negative control proved no cytotoxic potential.

DISCUSSION

The cytotoxic effect of alginate impression materials has been described by Pithonat all. For Jeltrate Chromatic Ortho, Orthoprint and Cavex Orthotrace authors indicate, that all alginate impression materials adversely affect cell culture [8].

The same author with another group of researchers in one of his subsequent works has tested a larger group of 14 alginate impression materials. They obtained similar results, all materials inhibited the growth of cell cultures. Some of the tested materials had a stronger impact and other weak (even a 2-times difference) [9].

Sydiskis and Gerhard tested different kinds of impression materials: silicones, alginate and polysulfides, like a conclusion from the research was cytotoxicity off all of them all them and some of the components were also cytotoxic [10].

In the case of the current work, one of the tested materials Elastic Cromo does not have cytotoxic properties, whereas the other alginate Image affects cell cultures. The explanation of these facts can be in different composition between these two materials. Elastic Cromo has cherry aroma and Image mint. Essential oils (mint) potentially can inhibit the development of cell cultures [11]. In the composition of alginates, it is possible to find potassium hexafluorotitanate, and zinc compounds, which can also significantly influence cytotoxicity [12].

Most authors are also in agreement about the fact of cytotoxicity of condensation silicones, whereas a harmful factor the by-product of the condensation reaction ethanol is distinguished [13, 14]. Also, tin organic compounds [15], and terta etoxysilane [16], are known for their toxicity.

The exception of the cytotoxicity of condensation silicone (C silicones) was published is the work of Jae-Sung Kwon at all, who tested 3 types of addition silicone and one condensation, Xantopren. Obtained results indicate that this material has low influence on the cell cultures during test in extract and with direct contact for all fibroblast [17].

The same Koreans authors noted at the same time, those addition silicones, so far have not been thought to inhibit the growth of cellular culture, and may have an effect on them, due to the surfactants contained in these materials. These compounds are added to the silicone impression materials to reduce their surface tension, when taking the impression in a humid oral environment. As it is known, that silicones belong to strongly hydrophobic compounds [17].

The same authors in the next paper, develops the effect of surfactants on the cytotoxicity of silicone impression materials. They added different concentrations of sodium lauryl sulfate (SLS) to the impression mass (concentration form 0, 1, 2, 4, 8 and 16 wt%). The obtained results indicate that the material after the addition of SLS is cytotoxic. Additionally this substance was isolated by ion chromatography. The eluted amount was directly proportional to the increase in its concentration in the silicone material [18].

F. Boraldi at all demonstrated cytotoxicity of addition silicones (Elite HD, Express Putty and Express Light Body) and polyether (Impregum Penta and Permadyne Penta L) [19].

Results from current study conducted in two C silicones Zetaplus and Stomaflex Putty are in accordance with the literature. Even during short period (2 hours) both material are high cytotoxic for VERO CCL-81 cultures.

In literature it is not possible to find any publication about the cytotoxicity of impression compounds, although they belong to one of the oldest materials for taking impressions. The first patents on the use of this type of materials could be found at the beginning of the 20th century [1, 3]. The obtained results indicate that these types of materials are not toxic. They consist of raw materials of natural origin: tree resins, stearic acid, talc. After 2 hours, the viability of cell cultures is quite high and amounts to 80%.

Another group of materials, which are pastes containing zinc oxide and eugenol, they are very widespread in dentistry, and possess very large spectrum of use: to taking impressions, for temporary filling and cementation. They are used because of the bactericidal properties of eugenol [20, 21]. Main mechanisms are that eugenol induces cell lysis through the leakage of protein and lipid in the cell [22].

Material based on ZnO-Eugneol is characterized by strong cytotoxic properties. However, this material does not penetrate the dentin barrier thicker than 0.5 mm, without irritating the pulp [20, 21].

Very interesting is the work of S. D. Meryon, who investigated the cytotoxicity of various types of materials containing zinc oxide and eugenol. The conclusion from his research is that powders of zinc oxide combined with clove oil released significantly more eugenol and were more toxic than pure eugenol with pure zinc oxide [23].

Also, the material Repin (based on ZnO-Eugenol) has strong cytotoxic properties during a 2-hour exposure to the cell viability of the cells is 10.37%.

The last of the impression materials tested in our study was FITT, which belongs to the group of acrylics- tissue conditioners. However, it does not have monomers in its composition. In the literature on the cytotoxicity of acrylic resins, as one of the main factors, which can cause cytotoxicity, irritancy or allergies is residual methyl methacrylate [24, 25].

N. Okita at all made investigation about tissue conditioners. They concluded that all tested materials seemed to be more cytotoxic than self-curing denture base resins [26]. The same conclusion was introduced by A. Atay at all, who tested different kinds of soft and hard relining materials. One of them GC belongs to the family of tissue conditioners and possess cytotoxic properties [27].

For the cytotoxicity of this type of material in the main extent can be associated with large concentrations of ethyl alcohol (from 10-20%), which is rapidly leaching. In the second steps, from the material migrates unconnected plasticizer, it can also adversely effect on cell culture [28]. In this study, FITT material also possessed cytotoxic properties (cell survival 28%)

CONCLUSION

In current study we tested seven impression materials belonging to different groups, two of them (Elastic Cromo and Impression Compound) did not have cytotoxic properties during a direct contact within 2 hours with cell culture. Others in a strong way were distracting to cells.

Clinical Significance

During the removal of impressions, the dentist has a contact with various impression materials, which can affect the organs of man in different ways. Therefore, it seems necessary to use appropriate tools of personal protection, such as appropriate gloves, goggles and masks.

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