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Cytotoxicity of Dental Impression Materials

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Elastometric rubber impression materials have been used in dentistry for a long time. These materials can be characterized by the following properties: accuracy (Peutzfeld, 1989; Hung et al. 1992; Jonson and Craig, 1985), dimensional stability (William et al. 1984; Fano et al. 1992), elasticity (Huget and Murray, 1993; Blomberg et al. 1992; Klooster et al. 1991), tear strength (Craig and Sun 1994), rigidity and reproduction of details (Jamani et al. 1989; Pratten and Novetsky 1991) . These materials are also easy to disinfect (Herrera and Merchant 1986; Johnson et al. 1988; Pratten et al. 1990), electroplate (Payne and Jeganathan 1994) , and allow for simple beading and boxing (Clear and Hansen 1996). Although there are many advantages for clinical use, several disadvantages, such as adverse tissue response and allergic reactions, have been reported (Hensten-Pettersen and Jacobsen 1991; Dahl et al. 1990). Also, foreign body reaction caused by accidental inhalation or swallowing may induce inflammation of the lung or the sinuses (Winstock and Warnakulasuiya 1986; Marshak et al. 1987; Sivers and Johnson 1988; Cameron et al. 1996). These materials are commonly used in dentistry, yet little information on the cytotoxic effect on humans is currently available (Meryon 1987; Kaga et al.1991; Sydiskis and Gerhardt 1993). There are also no guidelines to regulate the concentration of compounds used in dental impression materials. The purpose of this study was to evaluate the cytotoxicity of a nine commercial impression materials and one experimental material on human gingival fibroblasts.

MATERIALS AND METHODS

The impression materials, types of material, manufacturers and lot number are listed in Table 1. The composition of the experimental materials was as follows: rubber base (KE 106) (60%), catalyst (15%), accelerator (12%), Aerosil R972 (6%), talc extrafine powder (6%), pigment (1%).

Fibroblasts derived from human gingival area were used to evaluate the cellular response to the impression materials. Cells were grown in a 37℃ humidified 5% CO_2 atmosphere in 89 % α-minimum essential medium supplemented with 10 % fetal bovine serum and 1% PEN-STREP solution (penicillin: 10,000 units/ml,

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Table 1. Dental impression materials used

Material	Types of material	Manufacturer	Lot No.
Exaflex injection type (light body)	Addition type Silicone rubber	GC America Inc. Chicago, IL	Base 012596A catalyst: 012696A
Exaflex regular type (medium body)	Addition type Silicone rubber	GC America Inc. Chicago, IL	Base 052196A Catalyst 052396A
REPROSIL HF (light body)	Addition type Silicone rubber	Detrey Dentsply, Konstanz, Germany	Base 9501170
Experimental material (light body)	Addition type Silicone rubber	KE106 Shinetu chemical Co. Japan	Base 705257 Catalyst 706250
SA-100 (medium body)	Addition type + Alginate Silicone rubber	Buffalo Dental Mfg. Co., Inc. N.Y.	961201
Coltex fine (light body)	Condensation type Silicone rubber	Coltene/Whaledent Inc., Mahwah, N J	Base FH23 Catalyst FE95
Coltex medium (medium body)	Condensation type Silicone rubber	Coltene/Whaledent inc.,Mahwah, N J	Base CI42 Catalyst CJ63
Rapid liner (light body)	Condensation type Silicone rubber	Coltene/Whaledent inc., Mahwah, N J	FL81
XANTOPREN VL plus (light body)	Condensation type Silicone rubber	Bayer Dental Leverkusen, Germany	Base 020749 Catalyst 5059K
PERMADYNE (medium body)	Polyether rubber	ESPE Seefeld/Oberbay, Germany	Base 0305W251 Catalyst 0616W216

streptomyci Israel). Cells were checked daily for growth, and the medium was changed twice a week. The culture reached confluence in seven days and was then subcultured by 1:2 splits until the experiments were initiated. When enough cells were collected, the cells were prepared at a density of $5x10^4$cells suspension in 2ml media per well, and in order to save time during manipulation, just one plate was tested for two kinds of impression materials; in other words, only 10 wells were used per 24- well cell culture plate. Each kind of impression material was mixed according to the manufacturer's instructions within 1 min, and 0.5 ml of mixture was injected into each well as soon as possible with a 2.5ml plastic syringe. Five wells per impression material were prepared, and 5 wells which were not inoculated with any impression material were used as the control group. After finishing the inoculation, the culture plates were returned to the incubator. Ten minutes later, the plate was taken out of the incubator, and the set impression materials were removed, then 10μl cell suspension was mixed with 40μl Trypan blue to stain the dead cells. Then 2 μl mixed cell suspension was pipetted onto a glass counting plate and the viable (unstained) cells were counted under a refractive light microscope (Olympus Co., Japan). The number of viable cells was compared to the number in the control group, and the same procedure was repeated with an incubation period of 30 min. The difference in number of viable cells was compared for each impression material at both time intervals.

All of the data were analyzed using SAS/pc+ 6.04 statistical software (SAS/STAT 1986). The analytic method included multiple comparison analysis to compare the number of viable cells using the nine impression materials and the control material. Paired t-test was used to compare the effect of incubation time and different impression materials on the growth of human gingival fibroblast cells.

Table 2. Survival rates of human gingival fibroblast cells grown on impression materials at 10 min and 30 min incubation time (N=5)

Materials	Incubation time (10 min)	Survival rate (%)	P_1	Incubation time (30 min)	Survival Rate (%)	P_2	P_3
Control	18.8±2.5	-		17.8±1.0	-		NS
Exaflex injection type	9.8±2.1	52.2	**	5.8±1.5	32.6	**	*
Exaflex regular type	17.0±1.9	90.4	NS	11.0±1.8	61.8	**	*
Erprosil HF	14.2±4.3	75.5	*	10.0±3.0	56.2	**	NS
Experimental material	14.6±1.4	77.7	*	11.6±1.4	65.2	**	NS
SA-100	11.8±3.1	62.8	**	6.8±1.7	38.2	**	*
Coltex fine	9.2±2.0	48.9	**	7.0±1.9	39.3	**	NS
Coltex medium	12.8±4.0	68.1	*	6.2±2.5	34.8	**	*
Rapid liner	12.6±1.0	67.0	**	11.8±3.1	66.3	**	NS
Xantopren VL plus	12.2±2.6	64.9	**	9.8±2.9	55.1	**	NS
Permadyne	15.0±3.2	79.8	NS	9.2±4.5	51.7	**	*

5×10^4 cells were co-treated with or without (control group) impression materials. After incubation for 10 min or 30 min, the cells were collected and counted using the trypan blue exclusion method. The unit is 10^4 cells. The value is mean ±SD.
P_1 and P_2 were used to compare the numbers of viable cells growing on the impression materials and the control material at 10 min and 30 min incubation time, respectively. P_3 was used to compare the number of viable cells at both incubation times for each impression material. * <0.05 ** <0.01
NS: nonsignificant

RESULTS AND DISCUSSION

There are many methods for evaluating biocompatibility of dental materials. However, the cell culture technique seems to be the most popular. For this method, fibroblast is the most commonly used tissue type for this purpose (Wataha et al. 1993; Bumgardner & Lucos 1993; Schedle et al 1993). In order to closely reproduce the clinical conditions under which irritating components are released from the beginning of the setting reaction, and to ensure that each culture well contained exactly equal volumes of impression materials, a 2.5 ml plastic syringe was used to inject the mixed material into the culture well as soon as possible. For these data cytotoxicity was calculated by dividing the number of viable cells for the impression materials into the number of viable cells for the control material to give a survival rate. Therefore, a high survival rate indicates low cytotoxicity of the impression materials. Table 2 shows that all of the materials tested had some degree of cytotoxicity. The results of multiple comparison analysis showed that at an incubation of 10 min, except for Exaflex regular type (90.4%) and Permadyne (79.8%), there were significantly lower numbers of viable cells for all the impression materials, compared to the control material. The lowest survival rate was for Coltex fine (48.9%), followed by Evaflex injection type (52.2%). At an incubation time of 30 min, survival rates for all impression materials were significantly lower than for the control material. The lowest survival rates were for Evaflex (32.6%) and Coltex medium (34.8%). The highest survival rates were for Rapid liner (66.3%) and Experimental material (65.2%). Survival rates were consistently lower at 30 min incubation compared to 10 min incubation time. Except for Rapid liner and Xantopren VL plus, there were significantly lower numbers of viable cells at 30 min incubation compared to 10 min incubation for all impression materials. There was no significant difference between survival rates for the control material at both incubation times. The survival rate for Exaflex regular type decreased by the most (90.4% at 10 min compared to 61.8% at 30 min incubation). The lowest difference in survival rates was for Rapid liner (67.0% at 10 min compared to 66.3% at 30 min incubation).

The by-products of condensation polymerization may be related to the cytotoxicity. Some of the light body materials (Exaflex injection type, Coltex fine, SA-100) revealed higher cytotoxicity. The other components of the materials, such as the initiator, the accelerators or the metal ions are potentially cytotoxic. The vinyl polysiloxane impression material usually contains platinum as a catalyst and palladium as a hydrogen gas absorber. In 1995 Schedle et al. verified both of the metal ions had cytopathogenic effects on murine L-929 fibroblasts, human gingival fibroblasts, and human tissue mast cells. Although Exaflex injection and regular type impression materials contain the same components, the injection type material has a lower ratio of filler, and therefore a relatively higher proportion of irritating compounds which may also be cytotoxic. In 1990, Hensten-Pettersen et al. reported that the polyether impression material (Impregum) could be classified as strong to extreme sensitizers. However, in the current study, the cytotoxicity of the polyether impression material (Permadyne) did not reveal the highest cytotoxicity. This may have been because some of the components in Permadyne were chemically different to Impregum, but it still showed high cytotoxicity at 30 min incubation. Sydiskis et al. (1993) used the polyether, polysulfide, vinyl polysiloxane, zinc oxide, eugenol, and hydrocolloidals impression materials to determine the cytotoxicity to green monkey kidney cells, by observing the cell morphology change, comparing the growth rate and calculating the zone ofinhibition under agarose overlay culture medium. All materials showed some degree of cytotoxic effect. Although the materials used and methodology was different, the results were consistent with the findings of the current study. In our study, however, the methods used were much closer to real clinical conditions. The methodology used in the current study differed from the recommended method of Federation Dentaire Internationale (FDI), which stipulates that the setting material block be placed on the culture cell layer for observation. However, for a dental impression procedure, when the material has set, it is removed from the mouth, so this method does not correspond to real dental clinic conditions. We acknowledge our method may be useful for further research in similar or related studies.

In conclusion, our results showed that even a 10 min exposure of human gingival fibroblast cells to various impression materials had a cytotoxic effect. It is very important, therefore, for the dentist to select an impression material with low cytotoxicity, a short setting time and care must be taken to avoid leaving behind any impression material in the oral cavity.

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