

The cytotoxic effect of denture base polymers

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The cytotoxic potential of autopolymerized pour and dough type resins and heat cured resins was studied by in vitro cell culture techniques. Human epithelial cells (NCTC 2544) were grown in Eagle's minimal essential medium on the surface of the polymer disks. The cell multiplication on the surface of the specimens was measured. One heat cured resin and one pour type resin demonstrated a slight cytotoxic effect. The other polymers gave a moderate cytotoxic effect. The study did not indicate any difference in the cytotoxicity of the polymers when manufactured by alternate processing methods.

Key-words: Dental materials; biological effects; prosthetics

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The contact of dentures with the oral mucosa may lead to «denture stomatitis». In this condition increased redness of the denture bearing area is exhibited, and in some cases, a burning sensation is felt. The etiology is usually ascribed to fungal infection with *Candida* species, poorly fitting dentures and unbalanced occlusion (2, 12, 13, 17). Similar symptoms may also be provoked by sensitization reactions to components of the denture base materials (4, 6).

Another possible contributing factor is the local effect of toxic leachable substances from the denture base materials. Cured denture resins may leach out residual monomers and other chemically reactive, toxic components

(1, 3, 16). The amount of unreacted substances remaining in the denture base materials after the processing procedures are completed, may be related to the material used and to the processing method of the denture (1, 15).

In vitro cell culture techniques have been used to evaluate the cytotoxicity of various dental materials, among them acrylic denture polymers (5, 7, 8, 9, 14). The technique of measuring the growth rate of cells in direct contact with the materials is a very sensitive parameter of cytotoxicity. The present study applies this method to assess to what extent the processing procedure, the type of polymer and the storage conditions influence the cytotoxicity of the cured denture base materials.

MATERIALS AND METHODS

Test specimens

Circular disks with a diameter of 40 mm and a thickness of 4 mm were prepared from the denture base resins listed in Table 1. The mixing ratio used was according to the manufacturer's recommendation. The mixing of powder and liquid was performed in a rotating mixer angled at 30°C to diminish the occurrence of voids.

The heat-cured disks were subjected to two processing methods. Polymerization scheme 1: 73°C for 30 min., up to 100°C during 30 min. and then 100°C for 30 min. Polymerization scheme 2: 73°C for 9 hrs. Duraflow was processed according to scheme 1 only.

The autopolymerizing dough and pour type resins were also processed by two methods. Polymerization scheme 3: 21°C for 1 hr. Polymerization scheme 4: 45°C and a pressure of 2 kp for 30 min. No centrifugal force was used for the pour type resin.

The disks were polymerized in tin-foiled flasks with the test-surface against a glass surface. The flasks and the disks were treated aseptically. The disks were stored under various conditions prior to testing, i.e. freshly made or in saline for 1-4 days, or in a humid atmosphere in sealed containers for two weeks.

Circular glass disks of the same dimensions as the resin disks served as controls in all experiments.

Cells

Human epithelial cells (NCTC 2544), obtained from American Type Culture Collection (MD., USA), were propagated in Eagle's minimal essential medium (Eagle's MEM), supplemented with 10 % calf serum (5).

Table 1. *Denture base resins used in the present study*

Code	Heat cured resins	Manufacturer
C	SR 3/60	Ivoclar, Liechtenstein
DF	Dura-Flow	Myerson Corp., MA., USA
Autopolymerized pour type resins		
D	Swe-Flow	Swedia, Sweden
B	Palacast	Kulzer & Co., W.-Germany
Autopolymerized dough type resins		
E	Quick 20 Pink	Ivoclar, Liechtenstein
F	Quick 20 Clear	Ivoclar, Liechtenstein

Growth measurements

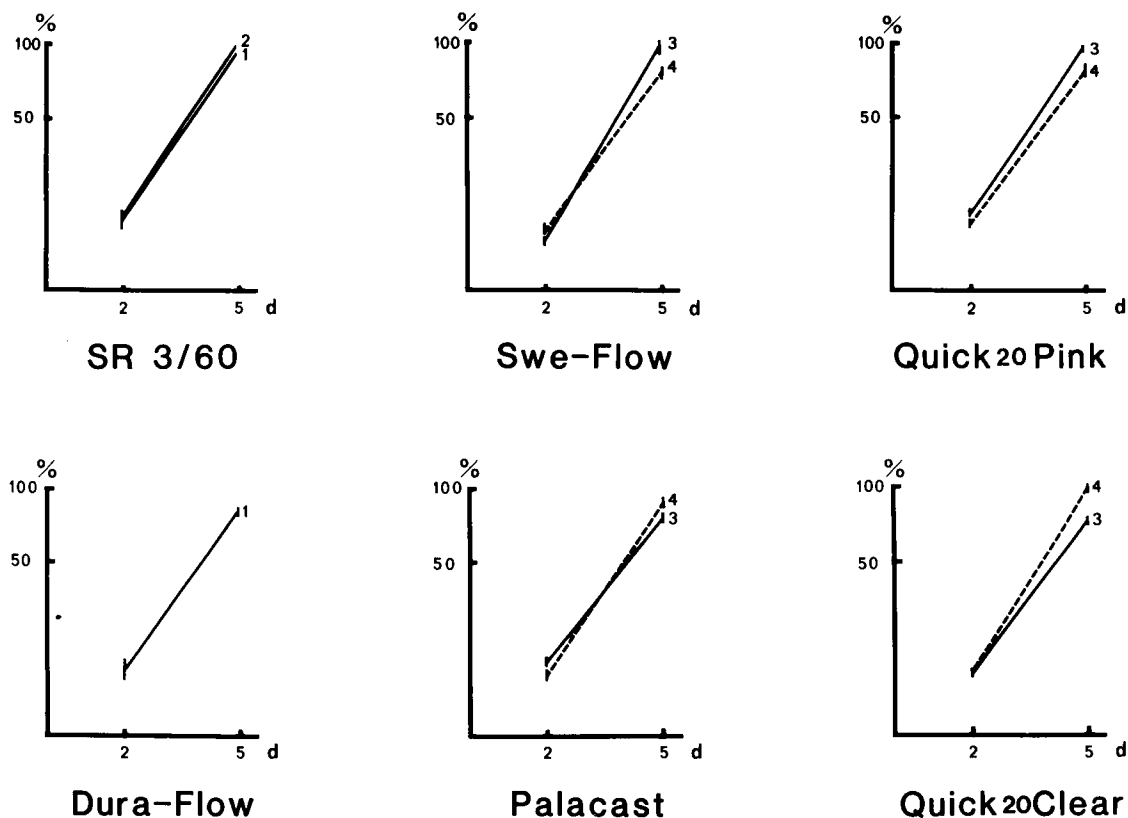
The test and control specimens were attached centrally to the bottom of Petri dishes (60 mm diam.) with sterile silicone grease (Midland Silicone Ltd., UK). Cells (0,6 x 10⁶) in 10 ml Eagle's MEM was added to the Petri dishes, and incubation was carried out at 37°C in a humidified atmosphere of CO₂ in air.

Cell multiplication on the surface of the disks was measured by counting the cells from 2 parallel cultures after specified incubation periods. The specimens were rinsed in saline, transferred to new Petri dishes, and 2 ml of a 0.25 % solution of trypsin in versen was added (5). After 5 min. incubation at 37°C, the cells were suspended in saline and counted in a Celloscope 101 (Ljungberg A. B., Sweden).

RESULTS

Growth measurements

The cells growing *around* the test specimens had a normal morphology as evaluated by light microscopy, and no zone of growth inhibition was seen in any culture.



HEAT CURED POUR TYPE DOUGH TYPE

Fig. 1. Cell growth on 2 - 3 weeks old denture base materials processed by polymerization schemes 1 - 4 (see methods for details). The bars represent two parallel cultures with each material after 2 and 5 days incubation. The 100 % value refers to cell growth on glass control specimens after 5 days incubation. (D = Swe Flow; B = Palacast; F = Quick 20 Clear; E = Quick 20 Pink; C = SR 3/60; DF = Duraflow)

Alternative processing methods

The two alternative ways of processing the various resins did not seem to significantly influence the cytotoxicity of the test specimens (Fig. 1). The differences observed were within the experimental errors of these systems.

Polymerization type

The growth rate of the cells did not appear to be related to the general classification of the resin types (Fig. 2). The relative growth rates on Swe-Flow

(pour type resin), Quick 20 Pink and Quick 20 Clear (dough type autopolymerized resins) were at a fairly constant level of 40 to 60 per cent of the controls. Palacast (pour type resin) gave only a minor decrease in growth rate, whereas SR 3/60 (heat-cured resin) caused an intermediary growth rate (Fig. 2).

Storage conditions

The specimens tested above had been stored in closed containers in a humid atmosphere for two weeks before the testing was carried out. When the cell

growth on freshly made (less than 2 hours old) specimens was compared with that on specimens that had been stored in 10 ml distilled water for 1 to 4 days, the cell growth on SR 3/60 (heat-cured) and Swe-Flow (pour type resin) did not vary much. Quick 20 Pink (dough type) showed a decrease in cytotoxicity with time.

DISCUSSION

The present technique of testing the cytotoxic potential of dental materials has several limitations. When used to compare the toxicity of materials of basically different composition such as silicate cement, composites and zinc oxide/eugenol cement, the results obtained are not in accordance with clinical experience and specific usage tests (11). In vitro cell culture techniques may prove to be of more value when comparing the cytotoxic potential of materials of fairly similar composition, such as in the present experiments with denture base materials. The method also makes it possible to test the specimens in a condition comparable to the clinical application without disaggregating the material. In the first series of experiments with the specimens being polymerized by alternative processing methods, the differences were slight, indicating that this parameter had little influence on the amount of residual cytotoxic substances in the set specimens. This is somewhat in contrast to the report of McCabe & Basker (10), who found that by changing the processing method, the amount of residual monomer was reduced to a level sufficiently low to avoid clinical manifestations in a patient with allergic reactions to a denture base material. However, the amount of a chemical that may elicit allergic reactions is much less than what is necessary for a cytotoxic reac-

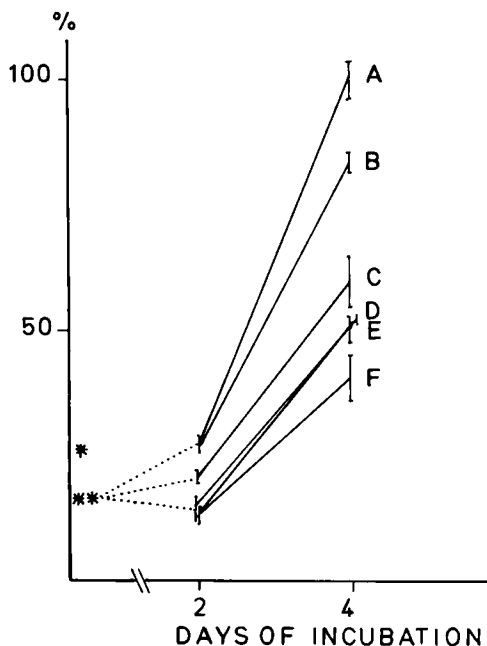


Fig. 2. Cell growth on the surface of 2-3 weeks old denture base materials processed according to schemes 1 and 3. The vertical bars represent the range of cell growth in two parallel cultures of each material. The 100 % value refers to the number of cells on the glass control specimens after 4 days incubation. (A = glass; B = Palacast; C = SR 3/60; D = Swe Flow; E = Quick 20 Pink; F = Quick 20 Clear)

tion. The amount of residual monomers in autopolymerized resins is calculated to be about 2-5 per cent, and around 0.5 per cent in heat-cured resins (1, 15). It was therefore expected that the inhibition in cell growth could be correlated with the general classification of the resins into dough and pour types, autopolymerized resins and heat-polymerized resins, respectively.

The results indicated that this was true for the two dough types and one of the heat-cured materials. However, the two pour type resins tested demonstrated different toxicity. Also, one of the heat-cured materials gave a growth inhibition comparable to the dough type autopolymerized resins. Thus, in the present experiments, the differences

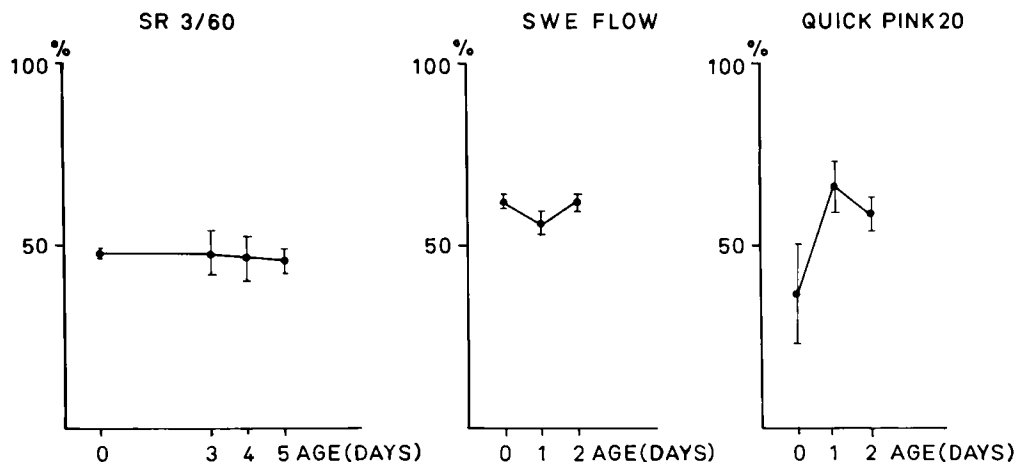


Fig. 3. Cell growth on denture base materials as a function of the age of test specimens stored in distilled water at 37°C. The vertical bars represent range and average cell count of three parallel cultures incubated for 4 days. The 100 % value refers to cell growth on the glass control specimens.

between brands appeared to be greater than the corresponding difference between type of denture base material. There might be several explanations for this finding. The cytotoxicity of the individual components on a weight basis had been shown to vary (16). The polymerization system could be composed in different ways for the brands investigated, so that the amount of residual monomers and other unreacted substances such as amines vary from one brand to the other. This is at least so for Palacast, where the manufacturer states that it «does not contain tertiary amines».

After the first day, there was a relatively small shift in cytotoxicity with time of the denture base materials, indicating that the amount of residual components that leach out with time is a fairly constant property of each brand tested. Except in the case of dough type materials the present experiments thus demonstrate that two weeks storage in a humid atmosphere or several days of storage in water does not significantly reduce the cytotoxic potential of the materials. In conclusion, the present set of experiments

have indicated that classification of denture base resins by the method of polymerization (dough and pour types, autopolymerized and heat-polymerized resins) need not be related to the cytotoxic potential of residual leachable substances in the cured denture bases.

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