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A CELL CULTURE METHOD FOR SCREENING THE BIOCOMPATIBILITY OF DENTAL MATERIALS

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SUMMARY

A cell culture technique for use as a standard procedure for biocompatibility testing of dental materials was evaluated, eluates of different materials were tested on L-929 cells. Toxicity was evaluated by the slope of the dose response curves. The technique was easy to perform, was not expensive and yielded measurable, reproducible and statistically treatable results. This test method should be considered as part of a standardized programme for biocompatibility testing of dental materials.

INTRODUCTION

Many different cell culture techniques for testing the biocompatibility of dental materials have been published but only a few have been adopted into national or international standardized testing protocols (American Dental Association, 1979; Fédération Dentaire Internationale, 1980). The reason is that a standard cell culture method has to fullfill special requirements (Autian and Dillingham, 1978):

- it should be reasonable in cost,
- all components should be easily available through commercial sources,
- the procedure has to be so clear and easy, that it can be performed by average laboratory personnel,
- the recording should be done systematically, either by grading or measuring,
- the results must be reproducible, statistically significant, and quickly available,
- the results should have a definite relationship to animal and human tissue compatibility.

The purpose of this investigation was to further develop and evaluate a cell culture technique for biocompatibility testing of dental materials which meets these specifications.

MATERIALS AND METHODS

Thirty eight test materials (Table 1) were investigated. These were mixed according to the manufacturer's instructions and separate specimen were aged for 1 hour and 24 hours at 37°C and 100% relative humidity. Samples of the aged materials, each weighing 4.0 g, were in-

cubated in BME cell culture Medium (GIBCO, Nr. A988450/D132) at 37°C. After 24 hours the medium was decanted, filtered under sterile conditions and diluted with conc. BME medium according to the following scheme: 1:0, 1:1, 1:7, 1:31.

TABLE 1: Test materials

A: Summary of the 38 test materials

impression materials	filling materials
silicones	amalgams
polysulphides	composites
ZnO/E pastes	phosphate cem.
tooth pastes	silicophosphate cem.
endodontic materials	copper cement

B: Specifications of materials specially mentioned

Code-Nr.	Name	Manufacturer	batch-Nr.
G-66	Guttapercha	DeTrey/W.Ger.	RA 4
G-80	Chloropercha	OY Dental/Finnl.	10,6
G-91	Jodoform	A.Haupt/W.Ger.	n.i.+
G-92	N 2	Dr.Sargenti	n.i.+
G-100	Diaket	ESPE/W.Ger.	75324
G-162A	Ledermix	Cyanamid/W.Ger.	557/9771

+No information (n.i.) was available about batch-Nrs for G-91 and G-92. Both materials were purchased Feb. 1977

Cultures of L-929 mouse fibroblasts were grown in BME medium, supplemented with foetal calf serum (GIBCO, No. HL590101S) to 5%. One ml of a cell suspension containing $2x10^5$ cells/ml together with one ml of the diluted test solution was put into a sterile test tube. These cell suspensions were incubated at 37°C, in an atmosphere of 5% CO_2 and 100% relative humidity for 72 hours. For the control cultures the cell suspension was mixed with regular medium instead of the test solution. One set of the controls was kept at 4°C (0 hour control), the other set was incubated like the test cultures (72 hours control). The test cultures were performed with 10 replicates, the control cultures with 20.

The growth of the cultures was evaluated by the LOWRY protein determination (Oyama and Eagle, 1956). The relative growth of the test cultures was calculated as the percentage of the maximum growth, represented by the 72 hours controls (Dillingham et al., 1973). The 95% confidence interval for the average relative growth was calculated by a computer programme (Schmalz, 1981). For each material and each aging period dose response curves were derived with the different concentrations of the extracts as doses. From the regression of each curve according to the equation $y=e^{-ax}$, the slope (-a) was used as an indicator for the biocompatibility of the respective material. The slopes were compared for significant differences by the Fischer-test (Miller, 1966).

RESULTS

The linear calibration curve of the LOWRY protein determination against cell count (Fig. 1) indicates good agreement between both methods which corresponds to results in other laboratories (Dillingham et al., 1973). The growth curve of the 72 hours control cultures (Fig. 2) shows that in our system the test is performed during the logarithmic growth phase, therefore monitoring the influence of toxicants upon growth kinetics. This is a better test than one in which only cell lysis is measured (Autian, 1970). Typical dose response curves are demonstrated in Figures 3 and 4. The endodontic filling material N2 (G-92) proved to be one of the most toxic substances in the test series showing very steep dose response curves (Fig. 3). A chloropercha sealer (G-80) was only slightly toxic and decreases in toxicity after 24 hours aging (Fig. 4).

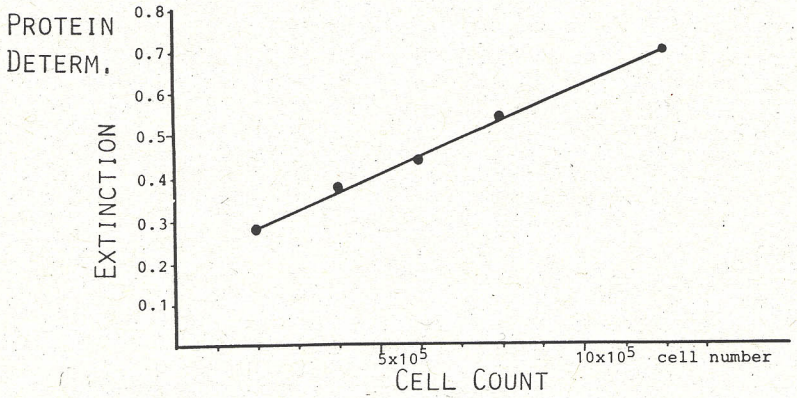

Fig. 1. Calibration curve of LOWRY protein determination against cell count

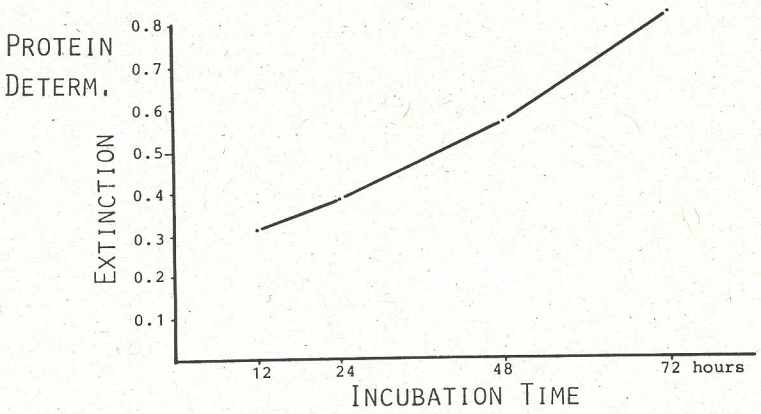

Fig. 2. Growth curve of the 72 hours control cultures

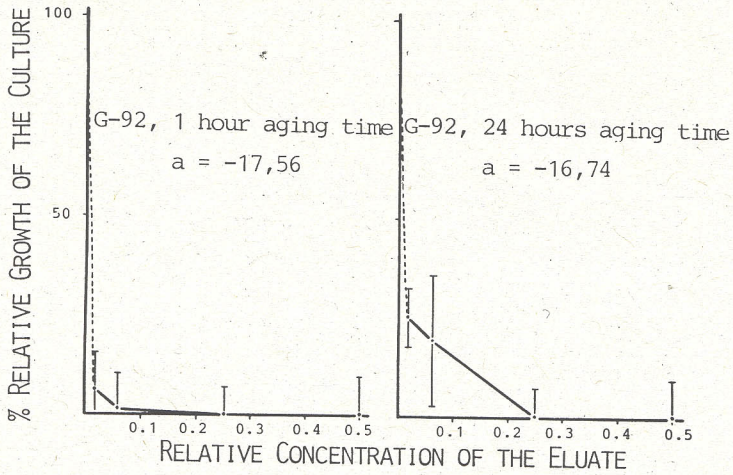

Fig. 3. Influence of material G-92, defined in table 1, upon the growth of L-929 cells. a=slope; regression model: $y=e^{-ax}$

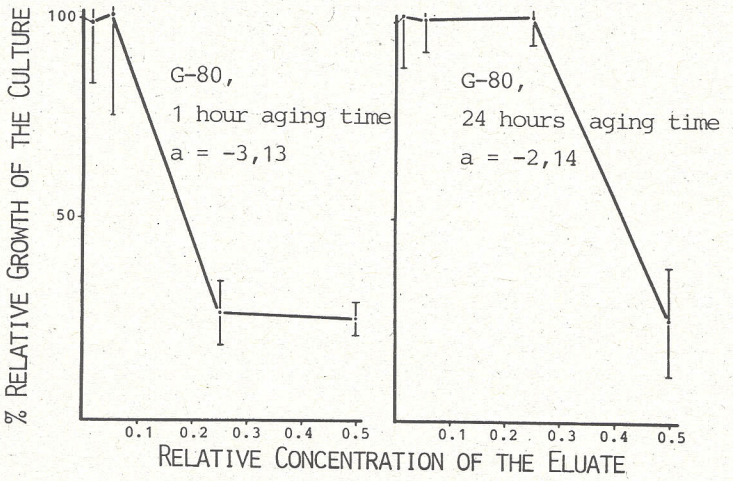

Fig. 4. Influence of material G-80, defined in table 1, upon the growth of L-929 cells. a=slope; regression model: $y=e^{-ax}$

The reliability of our results was evaluated by comparing them with corresponding reports in the literature about in vitro and in vivo findings. I am well aware that because of the lack of standardization, different results were reported for the same materials (Langeland and Klötzer, 1971; Schmalz, 1981). However a critical review of the literature (Schmalz, 1981) shows with two exceptions (silicophosphate- and copper cement), that there is good agreement between these results and those of other investigators and fewer discrepancies than with the diffusion test (Autian, 1970) using the same materials.

The reproducibility of the results obtained by our test method was evaluated by repeating the same experiment with 5 materials in the same laboratory, but with different personnel, different commercial sources for the cells and with a time lapse of 12 months between the respective experiments. The results (Tab. 2) show that β=1.00 and α=0.3 almost optimal reproducibility, optimum being characterized by β=1 and α=0 (Moran, 1971). The 95% confidence intervals are high because only a small number of experiments were done.

TABLE 2. Test of reproducibility

test materials	slope of the dose response curves 1	2
G-92	-17.56	-16.40
G-91	-14.25	-15.10
G-162A	- 7.23	- 8.41
G-100	- 7.04	- 6.81
G-66	- 2.96	- 2.10

statistical analysis (Moran, 1971)

general equation: $y = \beta x + \alpha$ — calculated equation: $y = 1.00 x + 0.03$

95% confidence limits: 0.2 - 5.3 (for β)

Materials, defined in Table 1, were aged one hour, 1 = result of initial test; 2 = result of similar test 12 months later

According to the Fischer-test the least significant difference between two slopes was 3.5 (95% confidence), where the original data for the slopes ranged from 0 to -18.

DISCUSSION

Examining this method in the light of the criteria for standardized cell culture testing published by Autian and Dillingham (1978), the test under discussion is lower in cost than the Cr^{51} release test (Spangberg, 1973) which was proposed for the same purpose (Fédération Dentaire Internationale, 1980), because the latter needs scintillation equipment and radiochemicals.

All components of our test are easily available. Problems of safety when handling radiochemicals needed with the Cr^{51}-test are avoided. The test was performed by average laboratory personnel. Working with gamma-emitters like Cr^{51} is more complicated and requires specially trained personnel.

The recording of the experimental data by photometric readings is more objective than the diffusion method described by Autian (1970), in which the cell response is graded by the investigator. The method generates dose response curves which are more reliable than the single data points obtained by the diffusion method and the Cr^{51}-release test. In our hands there were larger variations in the results when cells were counted, probably because of problems in taking aliquots.

The data are available within 4 days, which is longer than with the Cr^{51}-test (1 day) and the diffusion test (1 day), but this length of time still seems reasonable.

It may be argued that a disadvantage of this method is, that there is no direct cell/material contact (Spangberg, 1973). However, based on earlier investigations (Schmalz, 1978) in which the results of in vitro tests on the materials themselves and different eluates were compared, it is thought that acute toxicity testing of dental material eluates is justified if the elution is performed in the nutritient medium for the cell culture. Using eluates instead of the material itself, antibiotics in the nutritient cell medium are avoided and dose response curves are easily established.

The results of this investigation suggest that the test method described might be a useful addition to established standard testing procedures.

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