

Dentin Barrier Test with Transfected Bovine Pulp-Derived Cells

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Growth kinetics of SV40 large T-antigen-transfected bovine pulp-derived cells on dentin were investigated. These cells were used in a dentin barrier test device, and the system was evaluated by testing a set of dental filling materials. Cells (120 cells/mm²) were seeded on dentin slices and incubated for up to 21 days. Cell proliferation was recorded using MTT assay. For cytotoxicity tests 3500 cells/mm² were seeded on dentin discs, which were then incorporated into the dentin barrier test device. After 72 h preincubation test materials were applied. After a 24 h exposure with or without perfusion of the pulpal part of the test device, cell survival was evaluated using MTT assay. The cells revealed similar growth kinetics on dentin slices and on tissue culture plates. In cytotoxicity tests the cells were more sensitive toward the test materials than previously used three-dimensional cultures of human foreskin fibroblasts and as anticipated from clinical experience. Further improvement is expected by using three-dimensional cultures of pulp-derived cells.

According to national and international regulations, dental filling materials have to be evaluated for biocompatibility before being applied to patients. For this purpose, animal experiments or cell culture tests are available. Animal experiments for cytotoxicity testing of dental materials are time-consuming, expensive, and subject to extensive public discussions. Cell culture methods have important technical advantages compared with animal experimentation. They are better standardized and reproducible. Furthermore they are fast and easy to perform at relatively low costs (1). However extrapolation of results from cell culture cytotoxicity tests of dental filling materials to data obtained from clinical usage tests may be problematic. This was demonstrated with zinc oxide-eugenol (ZnOE). This material elicits a strong reaction *in vitro* (2), but it does not damage the pulp in the same way when applied in the dental cavity with an intact dentin layer *in vivo* (3). It was postulated that the dentin layer is responsible for the different results obtained *in vivo* and *in vitro*. Consequently special cell

culture methods have been developed for testing dental filling materials, introducing dentin as a barrier between test material and target cells. We described a technique that was mainly based on commercially available components for constructing an artificial pulp chamber (4), thus fulfilling a basic requirement for standardized testing techniques. An additional advantage of this artificial pulp chamber was regarded to be the possibility of perfusing the pulpal part with nutrition medium, thus simulating *in vivo* pulpal blood flow. The target cells used so far in this test system were either L929 fibroblasts (4), a permanent cell line originally derived from the murine mamma, or primary human foreskin fibroblasts, which were grown three-dimensionally on nylon meshes (5).

One target for adverse effects of dental filling materials *in vivo* is the dental pulp tissue. However dentin barrier tests developed so far for evaluating the cytotoxicity of dental filling materials mainly use cells/cell lines, which are not derived from the pulp tissue and which do not represent the characteristics of this target organ. It has been reported that dental pulp cells show a different sensibility for the cytotoxicity testing of dental filling materials than mouse fibroblasts, which are often used for the toxicity testing of dental filling materials (6). On the other hand the use of pulp-derived cells is restricted because of limited availability, lack of standardization, and changes of protein expression pattern after prolonged subcultivation. Furthermore Johnson et al. (7) reported that assays using established cell lines seem to yield more reproducible results than assays using primary cell cultures. The disadvantages of primary cell cultures can be overcome using the 3T6 protocol with rodent pulp cells (8) or by using viral oncogenes. Transfection of primary cells with, for example, SV40 large T-antigen gene (9), HPV18 E6/E7 gene (10), or adenovirus (11) can lead to cells with an extended lifespan and with the potential of becoming an immortalized cell line.

In the present study the growth kinetics of SV40 large T-antigen-transfected clonal cells from the dental papilla of calf tooth germs on bovine dentin in comparison with cell culture plates are described. Next, these cells were seeded on bovine dentin discs and incorporated into the *in vitro* pulp chamber, as was described previously (4). The suitability of this approach was evaluated by testing the cytotoxicity of a series of dental filling materials, which had been used in previous tests with monolayers of L929 fibroblasts (4) and three-dimensional fibroblast cultures (5), respectively. The experiments were performed under static conditions or under perfusion conditions, whereby the pulpal part of the artificial

TABLE 1. Test materials

Test Material	Brand Name	Manufacturer	Lot. No.
Control: Silicone impression material	President regular	Coltène AG, Altstätten, Switzerland	EK 454
Resin-modified glass-ionomer cement	Vitrebond	3M Medica GmbH, Borken, Germany	1997 0202
Zinc-phosphate cement	Harvard Cement	Richter & Hoffmann Harvard-Dental GmbH, Berlin, Germany	2122396035 (powder) 2121096027 (liquid)
Glass-ionomer cement	Ketac-Fil	Espe GmbH & Co KG, Seefeld, Germany	004 19586
Cermet cement	Ketac-Silver	Espe GmbH & Co KG, Seefeld, Germany	FW0042425
ZnOE cement		Eugenol: Merck-Suchard, Hohenbrunn, Germany Zinc oxide: E. Wasserfuhr, GmbH, Bonn, Germany	818455 34920.027 A5

pulp chamber was perfused with cell culture medium (0.3 ml/h or 2 ml/h).

MATERIALS AND METHODS

Cell Culture and Preparation of Dentin Discs

Clonal SV40 large T-antigen-transfected cells, derived from calf dental papilla, were maintained in growth medium (MEM α , Gibco BRL, Germany, supplemented with 20% fetal calf serum, 150 IU/ml penicillin, 150 μ g/ml streptomycin, 0.125 μ g/ml amphotericin B, and 0.1 mg/ml geneticin) in a humidified atmosphere at 37°C in 5% CO₂. For determination of growth kinetics cells within passages 18 to 32 were used; for cytotoxicity experiments cells within passages 18 to 28 were used.

Dentin slices of 500 μ m thickness were cut from first incisors of freshly slaughtered bovines (3 to 4 years of age) with a wheel-saw (Leitz GmbH, Germany) under constant water flow. The smear layer on the pulpal side of the dentin discs was removed by applying 50% citric acid for 30 s. The dentin slices were rinsed with physiological saline and sterilized by autoclaving (121°C for 25 min), as described elsewhere (12).

Determination of Growth Kinetics

Bovine dentin slices were mounted into a metallic insert that was designed for use in an artificial pulp chamber. The cells were seeded at a density of 120 cells/mm² on the etched dentin surface. The free dentin surface for cell growth amounted to 12.6 mm². The inserts were placed in 12-well plates. The bottom of the culture plates was covered by 1.5% agarose to prevent attachment of the cells on the culture plate. As a reference, cells were seeded at a density of 120 cells/mm² in 96-well tissue culture plates (32 mm²; Falcon, Germany). Cultures were incubated at 37°C in 5% CO₂ in a humidified atmosphere for up to 21 days. Medium was changed every 48 h. Cell growth was determined using the MTT assay daily from day 1 to day 11, then every second day up to 21 days. Each measurement was performed in triplicate.

Cytotoxicity Testing

Bovine dentin slices were mounted into metallic inserts and placed in 12-well-plates covered by 1.5% agarose, as described. The cells were seeded at a density of 3500 cells/mm² on the etched

side of the dentin discs, incubated for 48 h (37°C, 5% CO₂), and transferred into an in vitro pulp chamber as described (4). A commercially available cell culture perfusion chamber (Minucells & Minutissue GmbH, Bad Abbach, Germany) made of polycarbonate with a base of 40 \times 40 mm and a height of 36 mm was modified. The original membrane that served as a substrate for cell growth was replaced by the metallic inserts containing the dentin discs with the cells. Thus, the cell culture chamber was separated into two compartments by the dentin disc resulting in a dentin barrier test device.

All chambers were perfused with 0.3 ml assay medium (growth medium with 5.96 g/L HEPES buffer) per hour for 24 h. Next perfusion was switched off and test materials were introduced into the upper chamber in direct contact with the "cavity" side of the dentin disk. Cytotoxicity of test materials was recorded after 24 h of incubation at 37°C (static condition) using the MTT assay. In further experiments, the pulpal part of the in vitro pulp chamber was perfused with assay medium (0.3 ml/h or 2 ml/h) during the incubation period (perfusion condition).

Test Materials

The materials used for cytotoxicity testing are listed in Table 1. They were mixed according to the manufacturers' instructions. Zinc-phosphate cement was mixed in two different liquid/powder ratios (1:1 and 1:2, wt/wt). Zinc oxide-eugenol cement was mixed in liquid/powder ratios (wt/wt) of 1:7.5, 1:4.5, and 1:2. Each material was tested 15 times; a nontoxic A-silicone impression material (President, Coltène) was used as negative control (100% cell viability).

MTT Assay

Cell viability was determined by enzyme activity (MTT assay). The dentin discs covered with the cells were removed from the metallic inserts and placed into 24-well plates containing 1 ml of prewarmed MTT solution (0.5 mg/ml growth medium) and incubated for 2 h at 37°C. Then the discs were washed two times with phosphate-buffered saline. The blue formazan precipitate was extracted from the mitochondria using 0.5 ml dimethyl sulfoxide on a shaker at room temperature for 30 min. Two hundred microliters of this solution were transferred to a 96-well plate, and the absorption at 540 nm (OD₅₄₀) was determined spectrophotometri-

cally. For cytotoxicity experiments the mean OD₅₄₀ of the negative controls was set to represent 100% viability. Results were expressed as a percentage of untreated control.

Scanning Electron Microscopy (SEM) Analysis

For SEM analysis transfected pulp-derived cells of passage 22 were seeded at a density of 120 cells/mm² on dentin slices mounted in metallic inserts, as described previously. After 48 h, and 7, 14, and 21 days the dentin slices were removed from the inserts and fixated in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4°C; post fixation was performed in 1% osmium tetroxide followed by critical point drying.

Statistics

Statistical analysis was performed using the nonparametric Mann-Whitney pairwise test followed by applying the error rates method, thereby adjusting the significance level α to $\alpha^*(k) = 1 - (1 - \alpha)^{1/k}$ (k = number of pairwise tests to be considered).

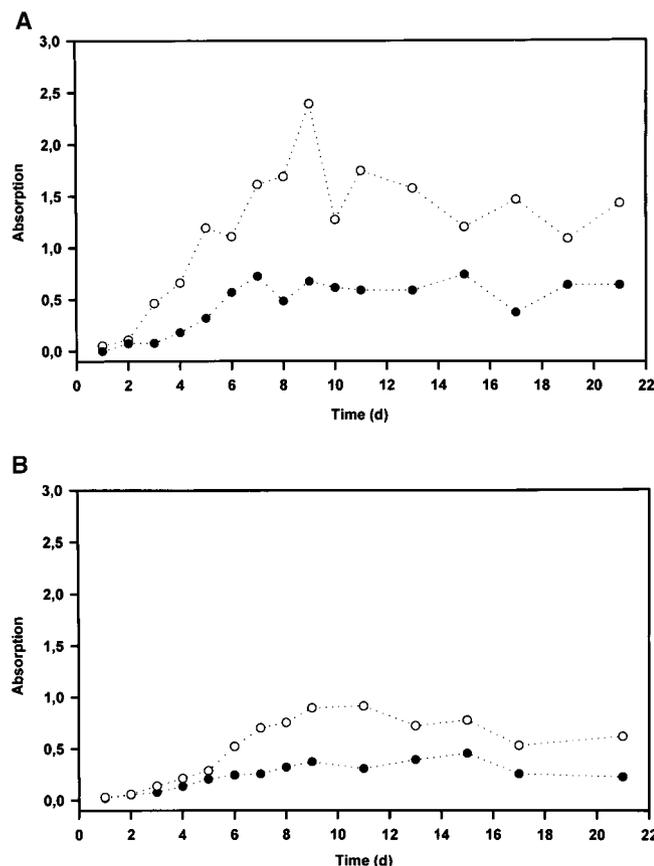


Fig 1. (A) Growth kinetic of SV40 large T-antigen-transfected pulp-derived cells from passage 22 on dentin (●) and on 96-well plates (○). The indicated values are the medians of three replicates. (B) Growth kinetic of SV40 large T-antigen-transfected pulp-derived cells from passage 32 on dentin (●) and on 96-well plates (○). The indicated values are the medians of three replicates.

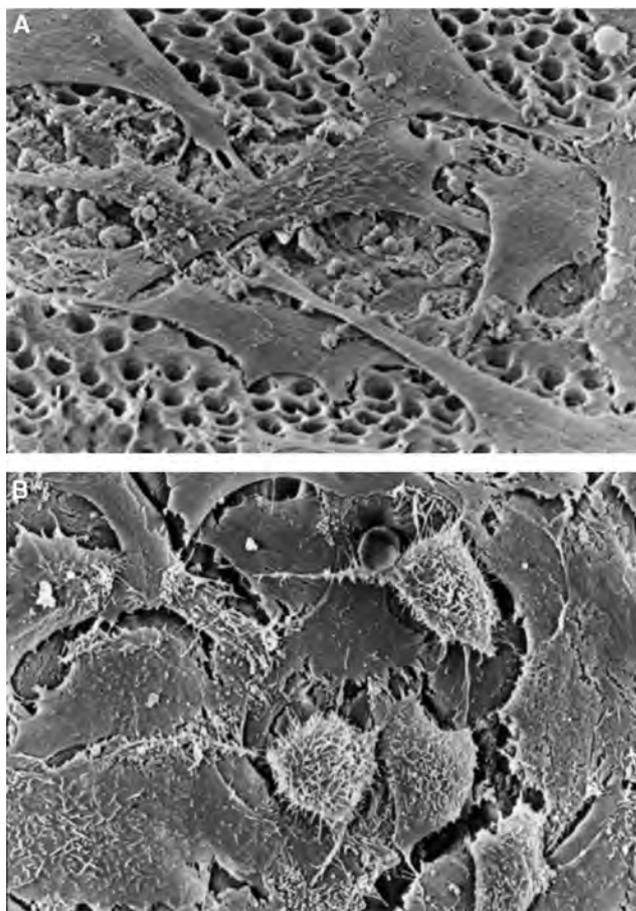


Fig 2. (A) SEM of transfected pulp-derived cells growing on dentin after 7 days. (B) SEM of transfected pulp-derived cells growing on dentin after 14 days.

RESULTS

Growth Kinetics

Cell proliferation of transfected bovine pulp-derived cells on bovine dentin slices and on the bottom of cell culture plates was determined photometrically after staining with MTT. The results of

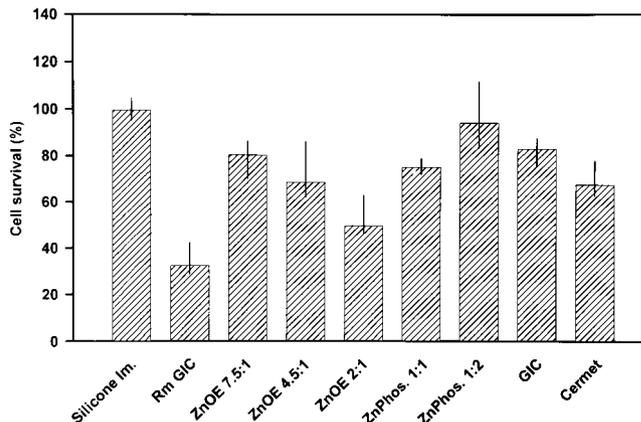


Fig 3. Cytotoxicity of dental materials on transfected bovine pulp-derived cells without perfusion. Indicated values are medians, 25% and 75% percentiles.

TABLE 2. Statistics of experiments without perfusion

	Silicone Im.	Rm GIC	ZnOE 1:7.5	ZnOE 1:4.5	ZnOE 1:2	ZnPhos. 1:1	ZnPhos. 1:2	GIC
Rm GIC	+++							
ZnOE 1:7.5	+++	+++						
ZnOE 1:4.5	+++	+++	-					
ZnOE 1:2	+++	+++	+++	+++				
ZnPhos. 1:1	+++	+++	-	-	+++			
ZnPhos. 1:2	-	++	+	+	++	+		
GIC	++	+++	-	-	+++	-		
Cermet	+++	+++	+	-	++	-	++	+

- = $p \geq 0.05$; + = $p \leq 0.05$; ++ = $p \leq 0.01$; +++ = $p \leq 0.001$.

Im. = impression material; Rm GIC = resin-modified glass-ionomer cement; ZnPhos. = zinc phosphate.

cell proliferation tests are presented in Fig. 1, *a* and *b*. The growth curves revealed similar growth characteristics for transformed pulp-derived cells on dentin slices and on tissue culture plates. After a short "lag phase," the cells showed an increase in cell number up to 9 days after seeding ("log phase"). A constant cell number was observed in cell cultures grown on dentin discs for the rest of the incubation period ("plateau phase"). Similar to these findings the absorption rates remained constant for the cells grown on tissue culture plates. In general absorption rates measured in cell cultures which were grown on dentin were smaller than those obtained from cell cultures in 96-well plates.

The growth characteristics of cells from different passages varied. Cells from early passages grew almost exponentially until day 9 after seeding and maintained the same cell density up to 21 days (Fig. 1*a*). Compared with these characteristics the proliferation rates of cells from higher passages were smaller in culture plates and on dentin slices (Fig. 1*b*).

SEM Analysis

Transfected pulp-derived cells of passage 22 were grown on bovine dentin slices for visualization in detail by SEM. Cells on the dentin surface were spread and flattened after 48 h, indicating cell attachment to the substrate. The processes of the cells reached into the opened dentinal tubules and cell-cell contacts could be observed (Fig. 2*a*). After 7 days in culture, a closed cell monolayer had formed. Again the dentin was covered by flattened cells with cell processes forming cell-cell contacts. After 14 days cells began to form multilayers. The cells at the surface of the multilayer became more polygonal, exhibiting an epithelial-like cell shape (Fig. 2*b*).

Cytotoxicity without Perfusion

The results of the cytotoxicity studies at static conditions are summarized in Fig. 3. Statistics of these experiments are shown in Table 2. With the exception of the zinc-phosphate cement at a liquid/powder ratio of 1:2 all materials were significantly more toxic to the transfected pulp-derived cells than the negative control, President, an A-silicone impression material ($p \leq 0.01$). Application of the zinc-phosphate cement at a liquid/powder ratio of 1:2 resulted in a cell viability rate of 94.3% ($p \geq 0.05$, compared with the negative control). The resin-modified glass-ionomer cement (Vitrebond) was the most toxic material and reduced cell survival rate to 32.5%; which means a highly significant difference in comparison with all other test materials ($p \leq 0.01$). Cell viability

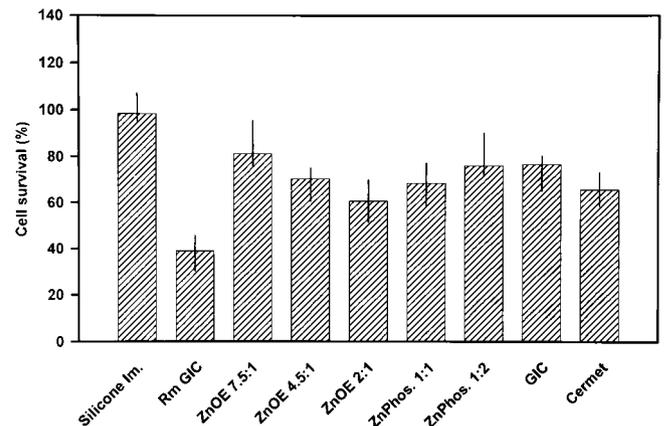


FIG 4. Cytotoxicity of dental materials on transfected bovine pulp-derived cells with perfusion (0.3 ml/h). Indicated values are medians, 25% and 75% percentiles. Im. = impression material; Rm GIC = resin-modified glass-ionomer cement; ZnPhos. = zinc phosphate.

rates after exposure to ZnOE cement ranged from 80.5% to 49.8%, depending on the mixing ratio. ZnOE (1:2) was significantly more toxic than the other test materials ($p \leq 0.01$) with the exception of the resin-modified glass-ionomer cement (Vitrebond). ZnOE (1:7.5) and ZnOE (1:4.5) evoked similar cell survival rates to the other test materials with the exception of the resin-modified glass-ionomer cement and zinc-phosphate cement (1:2).

Cytotoxicity after Perfusion Conditions of 0.3 ml/h

Cell survival rates of cytotoxicity experiments at perfusion conditions of 0.3 ml/h are summarized in Fig. 4; statistics of these experiments (Table 3) show similar results compared with static conditions. All test materials (also zinc-phosphate cement at a liquid/powder ratio of 1:2) significantly reduced cell survival rates, compared with the negative control ($p \leq 0.001$). The resin-modified glass-ionomer cement (Vitrebond) caused survival rates (39.0%) that were significantly lower than those of the negative control and all other test materials ($p \leq 0.001$). The toxicity of ZnOE cement depended on the mixing ratio, with a liquid/powder ratio of 1:2 being the most toxic mixing ratio.

Cytotoxicity after Perfusion Conditions of 2 ml/h

The results of the cytotoxicity studies at perfusion conditions of 2 ml/h are summarized in Fig. 5 and in Table 4, showing similar

TABLE 3. Statistics of experiments with perfusion (0.3 ml/h)

	Silicone Im.	Rm GIC	ZnOE 1:7.5	ZnOE 1:4.5	ZnOE 1:2	ZnPhos. 1:1	ZnPhos. 1:2	GIC
Rm GIC	+++							
ZnOE 1:7.5	+++	+++						
ZnOE 1:4.5	+++	+++	++					
ZnOE 1:2	+++	+++	+++	—				
ZnPhos. 1:1	+++	+++	++	—	—			
ZnPhos. 1:2	+++	+++	—	+	+++	+		
GIC	+++	+++	—	—	++	—	—	
Cermet	+++	+++	+++	—	—	—	+++	+

— = $p \geq 0.05$; + = $p \leq 0.05$; ++ = $p \leq 0.01$; +++ = $p \leq 0.001$.

Im. = impression material; Rm GIC = resin-modified glass-ionomer cement; ZnPhos. = zinc phosphate.

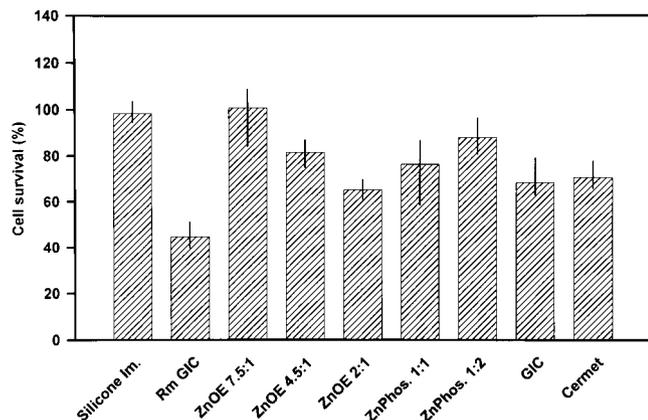


Fig 5. Cytotoxicity of dental materials on transfected bovine pulp-derived cells with perfusion (2 ml/h). Indicated values are medians, 25% and 75% percentiles. Im. = impression material; Rm GIC = resin-modified glass-ionomer cement; ZnPhos. = zinc phosphate.

results compared with static experiments and experiments with perfusion conditions of 0.3 ml/h. All experiments, with the exception of ZnOE (1:7.5) (100.9% cell viability), resulted in significantly lower survival rates compared with the negative control group ($p \leq 0.01$). Cell survival rate after exposure to the resin-modified glass-ionomer cement (Vitrebond) was 44.6%; which was a highly significant difference to all other materials tested ($p \leq 0.001$). Again the dependency of the toxicity of ZnOE cement on the mixing ratio could be observed.

Influence of Different Perfusion Conditions on the Cytotoxicity of Test Materials

Analysis of the influence of perfusion conditions on the cytotoxicity of test materials by the Mann-Whitney test showed no significant difference between static conditions and perfusion conditions of 0.3 ml/h, as is the case for the difference between static conditions and perfusion conditions of 2 ml/h ($p \geq 0.05$). The statistical difference between different perfusion conditions was significant ($p \leq 0.05$). After application of the most toxic materials—the resin-modified glass-ionomer cement (Vitrebond) and ZnOE cement at a liquid/powder ratio of 1:2—significantly more cells survived at perfusion conditions of 2 ml/h than under static conditions ($p \leq 0.01$).

DISCUSSION

For in vitro cytotoxicity testing of dental filling materials either permanent cell lines or primary cells are recommended. However controversy arises whether in vivo target cells (e.g. primary cells derived from the dental pulp (13)) or permanent cell lines should be used (1). Access to primary cells is difficult and limited; besides these cells are often poorly characterized. In contrast cell lines (e.g. L929 mouse fibroblasts) are stable and well-known in their biological characteristics and can be obtained from cell culture collections. However at the present time available cell lines do not represent the actual in vivo target cells for dental filling materials. Besides gingiva cells, pulp-derived cells are target cells in vivo. To combine the advantages of primary cells with those of established cell lines for cytotoxicity testing of dental filling materials, primary bovine pulp-derived cells were transfected with SV40 large T-antigen and cloned (14). Primary cells derived from the calf dental papilla reach senescence at passage 9 (unpublished data). Transfection of these cells with SV40 large T-antigen results in cells with an extended lifespan, thus increasing their availability for further characterization (14).

In the present study it was shown that the growth kinetics of SV40 large T-antigen-transfected bovine pulp-derived cells on bovine dentin is similar to the growth characteristics on cell culture plates with typical lag, log, and plateau phases. In general absorption rates measured in cell cultures that were grown on dentin were smaller than those obtained from cell cultures in 96-well plates. However these differences can be explained by the smaller surface available for cells growing on dentin, compared with 96-well plates. Cell attachment to dentin surfaces in vitro was reported previously by Safavi et al. (15) using SEM. This study did not describe the growth kinetics of cells on dentin discs. In contrast Schmalz et al. (12) described the growth characteristics of L929 mouse fibroblasts on bovine dentin. L929 cells revealed very similar growth characteristics on culture plates and on dentin. The mouse fibroblasts showed a relatively rapid decrease of living cells on dentin about 10 days after seeding. We did not observe this decrease of cell number in cultures of SV40 large T-antigen-transfected cells on dentin. After an initial increase in cell numbers the cell density was constant over the observation period, indicating the good attachment of these cells to dentin. Using SEM analysis Schmalz et al. (12) showed that L929 cells form a monolayer on dentin after incubation for 4 days. With SV40 large T-antigen-transfected bovine pulp-derived cells the formation of a multilayer can be observed after an incubation period of 21 days. This may allow cytodifferentiation within the cell multilayer at longer incubation times as described by MacDougall et al. (9).

TABLE 4. Statistics of experiments with perfusion (2 ml/h)

	Silicone Im.	Rm GIC	ZnOE 1:7.5	ZnOE 1:4.5	ZnOE 1:2	ZnPhos. 1:1	ZnPhos. 1:2	GIC
Rm GIC	+++							
ZnOE 1:7.5	-	+++						
ZnOE 1:4.5	+++	+++	+++					
ZnOE 1:2	+++	+++	+++	+++				
ZnPhos. 1:1	+++	+++	+++	-	-			
ZnPhos. 1:2	++	+++	+	-	+++	+		
GIC	+++	+++	+++	++	-	-	+++	
Cermet	+++	+++	+++	++	-	-	+++	-

- = $p \geq 0.05$; + = $p \leq 0.05$; ++ = $p \leq 0.01$; +++ = $p \leq 0.001$.

Im. = impression material; Rm GIC = resin-modified glass-ionomer cement; ZnPhos. = zinc phosphate.

Even though the transfected cells show an extended lifespan, compared with primary cultures, it can be observed that the proliferation rate slows down at higher passages. The process of immortalization of primary cells with viral oncogenes is a two-stage mechanism and crisis occurs within passages 30 to 40 (16). This phenomenon may account for the lower cell proliferation rates at passage 32 in the present study and was the reason to perform cytotoxicity tests with cells from passages 22 to not more than 28. It has been observed that once passage 45 or higher is reached, the proliferation rates of SV40 large T-antigen-transfected cells maintained in culture flasks are again similar to the proliferation rates at earlier passages (unpublished data).

Regarding the growth characteristics on bovine dentin further use of bovine pulp-derived cells transfected with SV40 large T-antigen in an in vitro pulp chamber for cytotoxicity testing of dental filling materials seems to be a suitable alternative to conventional permanent cell lines. Therefore these cells were grown on dentin slices and incorporated into a commercially available perfusion chamber modified for a dentin barrier test system. This system was evaluated by testing a series of dental filling materials and by comparing the results with those obtained from previous studies with L929 mouse fibroblasts and experiments with three-dimensional cultures of human foreskin fibroblasts, respectively. Furthermore the influence of different perfusion conditions (without perfusion, 0.3 ml/h, 2 ml/h), simulating the pulpal blood flow, was tested.

Independent of perfusion conditions all materials significantly reduced cell survival, compared with the negative control (President, silicone impression material). Only the application of zinc-phosphate cement (1:2) at static conditions and ZnOE cement (1:7.5) at perfusion conditions of 2 ml/h resulted in cell viability rates that were not significantly different from the control group. These results are contradictory to our previous findings with three-dimensional fibroblast cultures. In experiments with these tissue cultures only the resin-modified glass-ionomer cement Vitrebond significantly reduced cell survival rates, compared with the negative control group (cotton pellet soaked with medium). Compared with the silicone impression material the cermet cement (Ketac-Silver) was significantly more toxic under static conditions. At perfusion conditions of 0.3 ml/h the conventional glass-ionomer cement (Ketac-Fil) and the cermet cement (Ketac-Silver) evoked significantly lower cell survival rates compared with the silicone impression material.

Perfusion conditions of 5 ml/h generally lead to an increased cell death or cell disruption of the three-dimensional foreskin fibroblast cultures used in previous studies (5). Therefore in the present study perfusion conditions of 2 ml/h instead of 5 ml/h were chosen. Various studies describe a pulpal blood flow of 20 to 82.4

ml/min/100 g (17, 18). This corresponds to a perfusion of 0.6 to 5 ml/h/pulp, assuming a pulpal wet weight of 50 to 100 mg. Minuth et al. (19) recommend perfusion conditions of 1 ml/h for optimal differentiation of kidney cells. After application of the resin-modified glass-ionomer cement (Vitrebond) and ZnOE cement at a liquid/powder ratio of 1:2, which were the most toxic materials in the present study, significantly more cells survived at perfusion conditions of 2 ml/h than under static conditions, possibly indicating a transportation of toxic substances by the medium flow, which simulates the pulpal bloodstream.

The cytotoxicity of ZnOE cement toward transfected pulp-derived cells depended on the mixing ratio. Whereas ZnOE at a liquid/powder ratio of 1:4.5 was highly toxic toward L929 mouse fibroblasts (cell survival: 0% at static conditions and at perfusion conditions of 0.3 ml/h; 4, 20), this mixing ratio resulted in cell survival rates of 68.7% to 81.7% (depending on perfusion conditions) in experiments with transfected pulp-derived cells. The high toxicity of ZnOE cement observed with L929 mouse fibroblasts is in accordance with results from other cell culture experiments (2), but in disagreement with results from pulp studies on experimental animals (3). Therefore we could prove the cytotoxicity reducing effect of dentin in combination with transfected pulp-derived cells; that means cells that were obtained from one in vivo target tissue for dental filling materials.

On the other hand, transfected pulp-derived cells react more sensitively toward the test materials than previously used three-dimensional cultures of human foreskin fibroblasts (as described) and as anticipated from clinical experience. The reason for this discrepancy may be the use of unphysiological cell monolayers in this in vitro test system, which does not simulate the three-dimensional structure of the dental pulp tissue. In contrast to the limited morphological, physiological, and biochemical differentiation of monolayer cell cultures, three-dimensional cultures facilitate cell growth, which is similar to the in vivo situation (19). Thus further improvement in the in vitro pulp chamber methodology is expected by using three-dimensional cultures of pulp-derived cells.

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You Might Be Interested

A recent study in New York of patients advised to have elective surgery found that about 9% were advised that the proposed procedure was not medically necessary after a second opinion examination (*J Am Coll Surg* 185:451). Given the expense, discomfort, risk and the like of surgery, that does seem to indicate that a second opinion might not be a bad idea.

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