The alkaline and antibacterial effect of seven $Ca(OH)_2$ liners in vitro

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The alkaline effect of seven $Ca(OH)_2$ liners was studied, using extractions with unbuffered 0.9% NaCl solutions and buffered bacterial growth media. The antibacterial effect was studied by cultivating *Streptococcus mutans* in the presence of liner specimens. All liners made the NaCl solutions strongly alkaline (pH >10), although MPC® and Reolite® released much less Ca than did Dycal®, Life®, Procal®, Renew®, and Reocap-E®. The weaker alkaline effect of MPC and Reolite was shown by the extractions with the growth media. Consequently, these two liners were not able to prevent the growth of *Str. mutans*. No growth was observed in the presence of the other five liners, and the media remained alkaline during the 12-h incubation period. Regrowth of the bacteria grown in the presence of these effective liners showed that only Dycal could be considered bactericidal, whereas the four other liners acted bacteriostatically. □ *Calcium hydroxide; dental cavity lining*

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One of the most important reasons for using $Ca(OH)_2$-containing liners is their expected alkaline reaction, which results in unfavorable conditions for possible remaining microorganisms (1, 2). Especially the $Ca(OH)_2$-based material Dycal® has been shown to have a strong effect on bacteria in carious dentin (3, 5). This was explained to be a result of the alkaline reaction of the liner. In another study (6) no alkaline effect was observed for the lining material Reocap®, which should be very similar to Dycal in composition.

The purpose of the present study was to test in vitro the strength of the alkaline reaction of different $Ca(OH)_2$ liners and their effect on bacterial growth.

Materials and methods

The tested brands (Table 1) are two-component materials that harden after mixing. Approximately 0.4-mm-thick round samples with a diameter of 3 mm were made in a plastic mold. The materials were handled in accordance with the manufacturer's instructions, and the specimens were left to harden in a moist atmosphere at room temperature (22 ± 2°C) for at least 48 h. The Ca content of the hardened specimens was determined by atomic absorption spectrophotometry (Perkin-Elmer 460, Perkin-Elmer Corp., Norwalk, Conn., USA) in accordance with the *Perkin-Elmer Manual* for 1976. For these determinations the liner samples were hom-

Table 1. The tested $Ca(OH)_2$ liners and their Ca content ($n = 2$)

Brand name	Batch no.		Manufacturer	Code	Ca content (μg/mg)
	Base	Catalyst			
Dycal®	102479	102479	Caulk, USA	A	157.9
Life®	01148	01150	Kerr, USA	B	119.6
MPC®	41173	41316	Kerr, USA	C	118.9
Procal®	70171	70171	3M, USA	D	81.1
Renew®	028012	028102	SS White, USA	E	135.3
Reocap-E®	030681		Vivadent, Liechtenstein	F	137.4
Reolite®	540980	560980	Vivadent, Liechtenstein	G	109.1

ogenized in a mortar, and aliquots were dissolved in hot, concentrated hydrochloric acid. The Ca contents of the different liner samples (Table 1) varied from 81 to 158 μg/mg. To test the alkaline effect of the liners, 1 ml of 0.9% NaCl was added to each sample ($n = 3$), and the tubes were agitated (500 rpm) for 12 h at 37 ± 1°C, whereafter the solutions were changed. This was repeated during 72 h, resulting in six supernatants from each liner sample. The pH and the Ca contents of the solutions were measured.

After the 72-h period, 3 ml 0.9% NaCl was added to each specimen, and the tubes were stored at 37°C without agitation. The pH of the solutions was measured after 1, 2, and 3 h. The dry weight of the specimens was determined before and after the dissolution procedure.

To test the effect of the liners on bacterial growth, specimens similar to those described above were made. The microorganism used was *Streptococcus mutans* ATCC 25175, which was pregrown in a modified Jordan medium (5 g Trypticase, 5 g yeast extract, 4 g glucose, 4 mg $MgSO_4 \cdot 7\,H_2O$, 0.2 mg $FeSO_4 \cdot 7\,H_2O$, 0.09 mg $MnCl_4 \cdot 4\,H_2O$ in 1 l distilled water) until the logarithmic growth phase, centrifuged (4000 *g*, 10 min, +4°C), washed once with the medium, and suspended in fresh medium ($A_{540} = 0.5$) for the experiments. The liner specimens were preincubated in plastic tubes in the Jordan medium (750 μl/tube and specimen) overnight at 25°C without agitation. The experiments were started by adding 500 μl of the *Str. mutans* suspension or the medium without bacteria (controls) to each medium–liner tube. The tubes were incubated at 37°C with agitation (50 and 500 rpm), and the pH was measured at the start and at various time intervals up to 12 h.

For determination of the possible bactericidal effect of the liners, samples (100 μl) from the solutions agitated 500 rpm for 4 h were subjected to regrowth in fresh Jordan medium (2 ml) for 3 h.

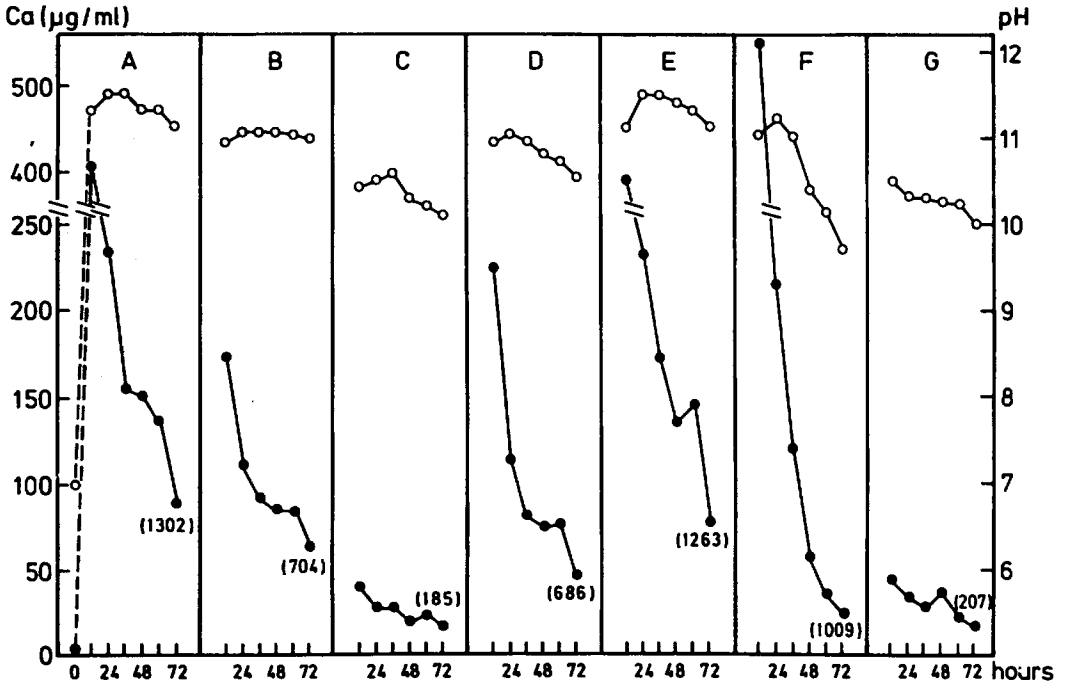

Fig. 1. Mean calcium release of liner specimens ($n = 3$) and pH of the supernatants resulting from subsequent extractions (12 h) with 0.9% NaCl for 72 h at 22°C (500 rpm). Open circles = pH; closed circles = calcium (μg/ml). Numbers in parentheses show the total amount of released Ca (μg/ml).

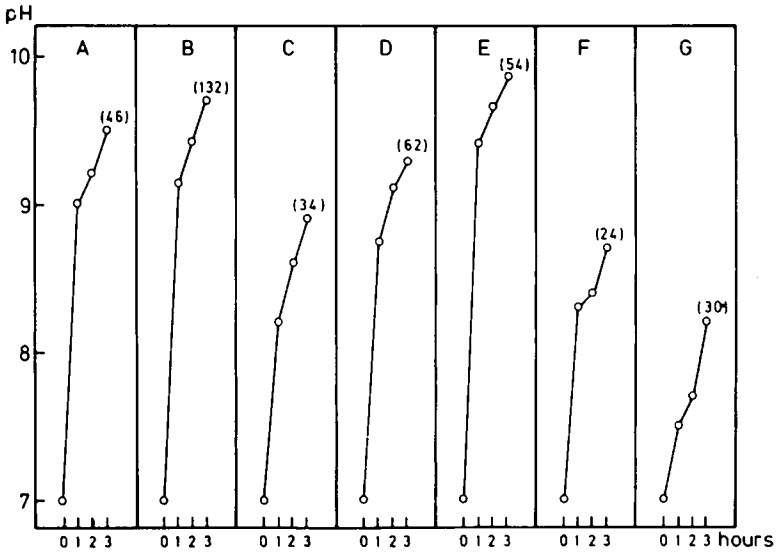

Fig. 2. Mean pH (n = 3) of 0.9% NaCl solutions (3 ml) incubated with liner specimens at 22°C without agitation after 1, 2, and 3 h. Numbers in parentheses as in Fig. 1.

All growth experiments were set up in triplicate with appropriate controls, and the growth was studied by measuring the pH and the turbidity (in Klett Units) of the media and the amount of bacterial protein from 5-ml samples of the media. The bacteria were centrifuged (3000 *g*, 10 min, +4°C), and the amount of total bacterial protein was determined by dissolving the bacteria in 1 N NaOH (100°C, 1 h). The amount of protein was estimated in accordance with Lowry et al. (7). Some liners dissolved partly and made the media turbid during the 500-rpm agitation. In these cases the growth was determined by measuring the pH and the bacterial protein only.

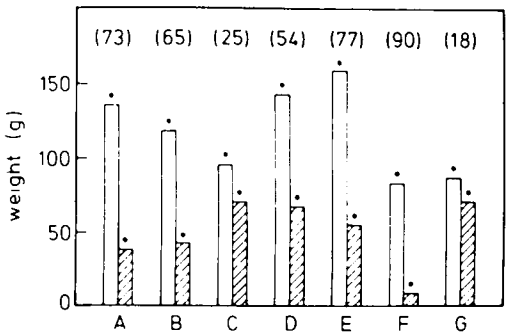

Fig. 3. Mean dry weight of liner specimens before (open bars) and after (hatched bars) the dissolution procedure. The dots mark the range of the three values. The percentage decrease in weight is also given.

Results

The Ca content of the solutions, which were changed every 12 h, generally decreased during the 72-h period (Fig. 1). The liners could be arranged into two groups in accordance with the amount of totally released Ca: Dycal, Life®, Procal®, Renew®, and Reocap released three to five times more Ca than MPC® and Reolite®: The first supernatant of the first group contained about 10 times more Ca than that of the second group. Fig. 1 also shows that the liner samples were able to raise the pH of each fresh solution to a value exceeding 10. In connection with Reocap only, the pH of the last three solutions remained slightly below this value.

Adding 3 ml NaCl to the 72-h samples and storing them without agitation (Fig. 2) gave a rise in pH for all materials during 3 h, but there were clear differences in the pH levels between the supernatants of the liner samples. The lowest pH was shown by Reolite, followed by Reocap and MPC.

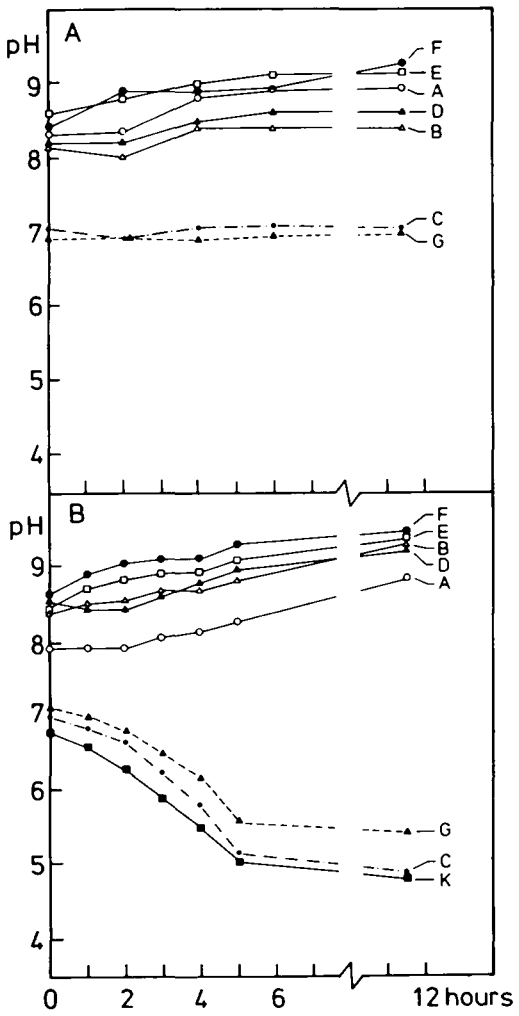

Fig. 4. Growth of *Streptococcus mutans* (change in pH) in the presence of liner specimens during 12 h at 37°C with 500-rpm agitation. 4A. No bacteria in the media (control). 4B. With *Str. mutans* in the media. The codes of the liners are explained in Table 1. K represents *Str. mutans* grown without any liner specimens (control).

During the extractions the weight of the specimens of Dycal, Life, Procal, Renew, and Reocap-E had decreased by at least 50%, whereas the MPC and Reolite samples had only decreased by about 20% (Fig. 3).

When the effect of the liners on bacterial growth was tested by agitating the test tubes gently (50 rpm) during the incubations, Life, MPC, and Reolite showed no effect; Procal, Renew, and Reocap-E inhibited slightly the growth; whereas no growth was observed in connection with Dycal. When the test tubes were subjected to strong agitation (500 rpm), the liners could be divided into two groups in accordance with their effect on the growth. Dycal, Life, Procal, Renew, and Reocap-E kept the growth medium alkaline for 12 h and inhibited the bacterial growth, whereas MPC and Reolite had no effect on the growth as judged by the change in the pH of the media (Fig. 4) and the total bacterial protein data (not shown). The specimens of Procal and Renew showed a trend to release material that made the growth medium turbid. Consequently, the turbidity was not used as an indication of bacterial growth in these cases. When samples of the bacterial growth solutions (agitated at 500 rpm) of the 'effective' liners were subjected to regrowth for 3 h in fresh media without any liners, these remaining liner brands could again be divided into two groups in accordance with the growth observed. Only Dycal caused a decrease in the amount of viable bacteria during the 4-h incubation period as judged by the bacterial protein data (Fig. 5) and by the pH and turbidity changes. For Life, Pro-

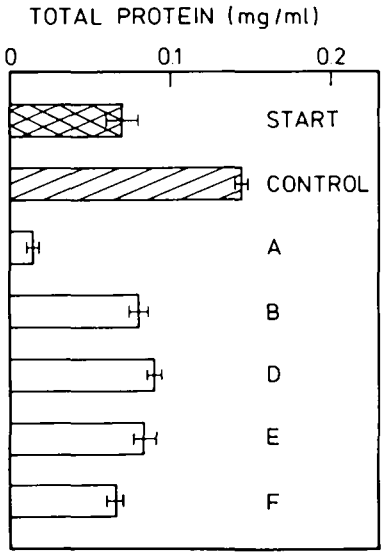

Fig. 5. Regrowth of bacteria in fresh Jordan medium (growth expressed as total protein in 3 h) in the presence of liners A, B, D, E, and F ($n = 3$). The amount of bacteria at the start of the growth and after 4 h of growth without liner specimen (control) is also shown.

cal, Renew, and Reocap-E the amount of viable bacteria appeared to remain almost unchanged during the 4-h incubation (Fig. 5).

Discussion

All tested liners were able to make an NaCl solution strongly alkaline during repeated changes of the solution. This is contrary to the finding of Knappwost (6), who did not observe any alkaline effect when Reocap was tested. It is noteworthy that even the very small amounts of Ca released by MPC and Reolite were enough to make the solutions strongly alkaline. The only difference that could be detected between the liners was the slightly weaker alkaline effect of Reolite compared with that of the other brands, when in the last part of the dilution extraction test Reolite raised the pH of the fresh NaCl solution more slowly than the other materials. Thus extractions with unbuffered solutions do not seem suitable for studying the alkaline effect of liners. Testing the materials in bacterial growth media indicated, however, that the alkaline effect of MPC and Reolite was clearly weaker than that of the other liners. This was in agreement with the much smaller release of Ca from MPC and Reolite than from the other liners. Since there were no great differences in the original Ca content of the different materials, the smaller release of Ca seemed to be partly due to slower dissolution of the specimen, indicated by weight changes in the dissolution experiment, and probably partly due to more stable chemical binding of the Ca itself. No attempt was made, however, to analyze the liner materials more profoundly. The bacterial regrowth experiment with the remaining five brands indicated that only Dycal could be considered bactericidal, whereas the effect of Life, Procal, Renew, and Reocap-E may be considered bacteriostatic under the present experimental conditions. The bactericidal effect of Dycal cannot be fully explained by the amount of Ca released, since a similar release was shown by Renew and Reocap-E. The results with Dycal confirm the earlier found antibacterial effect of this brand (3–5) but does not confirm the suggestion (4) that Procal and Reocap will behave clinically in a similar fashion as Dycal.

It may be concluded that, although the alkaline effect of the various liners was very similar according to the extraction experiments with the unbuffered saline solutions, these do not simulate the clinical conditions well. Moisture at the cavity floor under the lining is probably buffered, which apparently will influence the alkaline effect of the liner. In this case there are probably differences in the alkaline effect of liners, as indicated by the results when the partly buffered growth medium was used. In accordance with those results and the results of the bacterial growth experiments, the liner brands could be arranged into three groups. The alkaline and antibacterial effect was weak for MPC and Reolite, moderate for Life, Procal, Renew, and Reocap, and strong for Dycal.

In addition to the alkaline effect, there are many other properties of liner materials which have to be considered in clinical work. The greater dissolution in saline of the liner specimens that had an effect on the bacteria compared with the ineffective liners may be of some consequence clinically. In contrast to the free specimens of this experiment, however, the base layer is confined between the filling and the pulpal floor. This certainly has a reducing effect on the dissolution of the base. Furthermore, the favorable effects of the $Ca(OH)_2$ material are of greatest importance during the first months after the operative procedure and will decrease with time. On the other hand, the acid resistance of the liner should, beyond doubt, be noted when the acid etch technique is used in connection with composite fillings. Although one would expect that this property would be opposite to the alkaline effect, this seems not necessarily to be the case. In an earlier study (8), in which the acid resistance of the same brands as in this study was tested, the resistance decreased in the following order: MPC, Dycal, Renew, Reocap, Life, Reolite, and Procal. Thus it seems to be possible to choose a material with both good alkaline effect and acid resistance.

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