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Comparative assessment of time-related bioactive glass and calcium hydroxide effects on mechanical properties of human root dentin

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Short title: Bioactive glass vs calcium hydroxide

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Abstract

Suspensions of micro- or nanoparticulate SiO_2-Na_2O-CaO-P_2O_5 bioactive glasses could potentially be used as dressings in traumatized front teeth with open apices as an alternative to calcium hydroxide. These materials have a disinfecting capacity similar to $Ca(OH)_2$, but bear the advantage of bioactivity. However, as bioactive glasses are alkaline biocides such as $Ca(OH)_2$, they may also negatively affect mechanical dentin properties over time. This was assessed in the current study using standardized human root dentin bars. Specimens were immersed in 1:20 (wt/vol) suspensions of nanometric bioactive glass 45S5 or calcium hydroxide for 1, 10, or 30 d. Control specimens were immersed in pure saline for 30 d (N = 20 per group). Subsequently, modulus of elasticity (E) and flexural strength (FS) of the specimens were determined. Results were compared between groups using one-way ANOVA and Scheffé's post-hoc test. $Ca(OH)_2$ caused a significant ($P < 0.001$) 35% drop in mean FS values compared to the control treatment after 10 d. No further change was observed between 10 d and 30 d. The negative effect of bioactive glass on FS failed to reach statistical significance. No effects of either material on dentin E values were observed. It was concluded that the $Ca(OH)_2$ suspension affected the dentin more than the bioactive glass counterpart; however, the effect was self-limiting and probably restricted to superficial dentin layers, as suggested by the mere decrease in FS but not in E values.

Introduction

Calcium hydroxide has successfully been used as a dressing of the necrotic pulp space for almost a century (1). In dental traumatology, calcium hydroxide has been applied during the treatment of (front) teeth with devitalized pulps and wide-open apices (2, 3). However, this so-called apexification process has two disadvantages: first: it takes a long time until a hard-tissue barrier has formed and second, a calcium hydroxide dressing over an extended period of time may increase the risk of tooth fractures (4).

Recently, suspensions of bioactive glass powders of the SiO_2-Na_2O-CaO-P_2O_5 type have been suggested as topical root canal disinfectants (5). Similarly to calcium hydroxide, the gold standard material for that purpose, bioactive glasses affect their environment via the continuous release of alkaline species in a wet environment (6, 7). However, in contrast to calcium hydroxide, these glasses do not only release calcium but also phosphate, sodium and silica. They do not simply dissolve, but depending on liquid exchange in the system slowly change into pure inert calcium phosphate particles (8). Furthermore, bioactive glasses cause calcium phosphate precipitations in their environment (9). Consequently, these materials transform from reactive local antiseptics into a bioactive hard-tissue like structure over time. This feature makes bioactive glass suspensions interesting in dental traumatology. However, little is known regarding the effect of bioactive glasses on root dentin stability (10).

It was the aim of the current study to compare the effect of calcium hydroxide and nanometric bioactive glass 45S5 suspensions on the mechanical integrity of human root dentin under controlled laboratory conditions.

Materials and methods

Preparation of dentin specimens

Intact human upper third molars with fully formed roots were selected from the department's collection of extracted teeth. The current research protocol was according to the Guideline for Good Clinical Practice (ICH, Geneva, Switzerland) and did not alter the treatment plan of any of the involved patients, who gave informed consent that their extracted teeth could be used for study purposes. The institutional ethics committee approved the procedures. Teeth were stored for less than one year in 0.1% thymol solution immediately after extraction. The absence of caries and cracks was verified under a stereo dissecting microscope (Leica Wild M3Z, Wild, Heerbrugg, Switzerland) with an internal light source (intralux 4000, SOWO-DENT, Birmensdorf, Switzerland). Teeth were mounted on stubs and longitudinally sectioned using a saw microtome (LEICA SP 1600, Leica Microsystems, Glattbrugg, Switzerland) with a diamond-coated internal-hole blade under continuous water flow. In a first step, longitudinal tooth slices of 1.2 mm thickness were obtained. These slices were then clamped in a specially designed micro-vice to remove the outer dentin and cementum with a second rectangular cut. The third cut was made parallel to the second at a distance of 0.8 mm towards the root canal, yielding a plane-parallel dentin bar of 0.8 mm x 1.2 mm. The bars were finally cut to a length of 10 mm using a diamond saw. One hundred and forty bars were selected and stored in sterile 0.9% saline solution until further use.

Test and control treatments

Nanoparticulate bioactive glass with a 45S5 composition (45 wt% SiO_2, 24.5 % Na_2O, 24.5 % CaO and 6 % P_2O_5), were prepared as described before (11). Calcium hydroxide powder ($Ca(OH)_2$, Merck, Darmstadt, Germany) was obtained from a commercial source. Suspensions were prepared by mixing 50 mg of material with 1 mL of unbuffered physiological saline solution. Pure saline solution (1 ml) was used as a control. The 140

dentin bars were randomly assigned to 7 groups of 20 specimens each using a computer algorithm (www.random.org). Specimens were immersed individually in Eppendorf tubes containing the respective supensions or pure saline for different exposure times in an incubator at 37°C (Fig. 1). The tubes were agitated manually every third day.

After treatment the specimens were thoroughly rinsed with ultrapure water and soaked for three days individually in 1 ml of saline. The dentin bars were then subjected to mechanical testing.

Three-point bending test

Three-point bending tests were performed using a universal testing machine (Z010, Zwick, Ulm, Germany). The dentin bars were kept moist with physiological saline solution during all manipulations. Before testing, the width and depth of each bar was measured using a sliding caliper. For the testing apparatus a specimen holder with two cylindrical supports with a radius of 1 mm and a span of 7 mm was used. Specimens were placed with the greater bearing surface centered on the support (i.e. with the tubules parallel to the cross-head). The cross-head speed of the testing machine was set to 0.5 mm min^{-1}, and the bars were tested until failure. The modulus of elasticity E was calculated from the slope (m) of the load-displacement curves within the linear elastic region using the formula

$$E = \frac{l^3 m}{4bh^3} \qquad \text{(a)}$$

with the support span width l, the width b and the height h of the specimen. The flexural strength (FS) was calculated according to the formula

$$\sigma = \frac{3FL}{2bh^2} \qquad \text{(b)}$$

with F representing the load at fracture. Registration of the load at fracture and calculation of modulus of elasticity as well as flexure strength were performed by means of a software program (testXpert; Zwick).

Data analysis

Data distribution was even as assessed by depicting the data sets as box plots (not shown). Consequently, parametric tests were applied to compare FS and E values between groups: one-was analysis of variance (ANOVA) followed by Scheffé's test for multiple group comparison. The alpha-type error was set at $P < 0.05$.

Results

A significant ($P < 0.001$) effect of calcium hydroxide on flexural strength was observed after 10 d compared to a 30-d control treatment in physiological saline. The FS mean value dropped by more than 35% compared to the control treatment after 10 d in calcium hydroxide, with no further effect after 30 d (Fig. 2). The negative effect of bioactive glass on FS values was only marginally significant after both 10 d and 30 d ($P = 0.07$ and 0.08, respectively). Nevertheless, a drop in mean FS compared to the saline control of 20% was observed after 10 d. Again, this value remained stable between 10 d and 30 d (Fig. 2).

On the other hand, no effects of the experimental treatments with calcium hydroxide or bioactive glass on modulus of elasticity of the dentin specimens was observed (Table 1).

Discussion

The current study showed that both calcium hydroxide and bioactive glass suspensions had some impact on flexural strength (FS) of standardized human root dentin specimens, while the modulus of elasticity (E) of the specimens remained unaffected.

Using standardized specimens bears the advantage that the outcome variables E and FS can be compared with results from other studies, which tested the effects of endodontic materials on similar dentin bars (10, 12-14). On the other hand, the model that was used in this study differs from the clinical situation in many respects. First and foremost, the dentin specimens are exposed to the test suspensions from all surfaces. Furthermore, the medications are applied in excess or, in other words, the medication to dentin ratio is in favour of the medication. These restrictions indicate that the current results cannot be extrapolated to the clinical situation. On the other hand, some interesting basic observations could be obtained using the current standardized laboratory approach. The finding that only FS and not modulus of elasticity were affected by the calcium hydroxide treatment suggests that only the superficial dentin layers were attacked by the alkaline biocide. FS is mainly a function of alterations on the surface of a specimen, while E values represent the bulk properties of a material (15). The relatively limited effect of calcium hydroxide is further highlighted by the fact that a plateau was reached after 10 d despite the fact that excess material was in the system. The effect of bioactive glass was only marginally significant, and the clinical implications of this remain to be shown. The lesser effect of the bioactive glass suspension, despite the fact that its initial pH is comparable to that of the calcium hydroxide counterpart, can be explained by the lesser alkaline capacity of the glass suspension compared to calcium hydroxide (16). It should be realized, however, that it is highly probable that all alkaline materials including mineral trioxide aggregate (or Portland cement) slightly reduce mechanical properties of root dentin (17). In their study, White and co-workers exposed standardized bovine root segments to calcium hydroxide or mineral trioxide aggregate suspensions for five weeks and observed reductions in load at fracture values by 32% and 33%, respectively. These values compare with the peak reduction in root dentin flexural strength induced by calcium hydroxide of 35% observed in the current investigation (load at fracture is the variable that is affected by the treatment, see formula (b) above). Andreasen

and co-workers observed reductions of fracture strength by 50% induced by a calcium hydroxide dressing in whole sheep mandibular incisors. In contrast to the current study, however, they observed a plateau of the calcium hydroxide effect only after 90 days (18). Similarly, using extracted human maxillary incisors, Rosenberg and co-workers observed a reduction in fracture strength of 45% induced by calcium hydroxide (19). The plateau of the effect in their study, however, was reached after 28 days. Obviously, differences in outcomes between such laboratory investigations can be explained by differing experimental designs. In our study, a plateau was probably reached relatively quickly because, as indicated above, the specimens were exposed to the medications from all sides, and thus, the dentin-medication interaction was expedited.

The mechanism by which alkaline biocides affect dentin is unclear. It has been surmised that calcium hydroxide might disrupt the link between hydroxyapatite crystals and the dentin matrix (18). In this context, however, it is interesting to note that the destructive effects of sodium hypochlorite, especially if used in its concentrated form, on the dentin matrix and consequently on mechanical dentin properties are by far greater than those of calcium hydroxide (12, 17). When human root dentin specimens of the exact same dimensions as those used in the current study were exposed to 5 ml of 5% NaOCl for merely 1 h, both FS *and* E were reduced by ≥ 50%, indicating a matrix destruction in deeper dentin layers (13). This was confirmed histologically. However, clinical studies linking the usage of concentrated hypochlorite as an endodontic irrigant to the occurrence of root fractures are missing and should be performed.

Conclusion

Immersion of standardized human root dentin specimens in a nanometric bioactive glass 45S5 suspension resulted in a slight drop in flexural strength compared to a control treatment in physiological saline. Calcium hydroxide treatment resulted in a 35% mean flexural strength reduction, which remained stable after 10 d. Neither experimental treatment resulted in reduced modulus of elasticity values.

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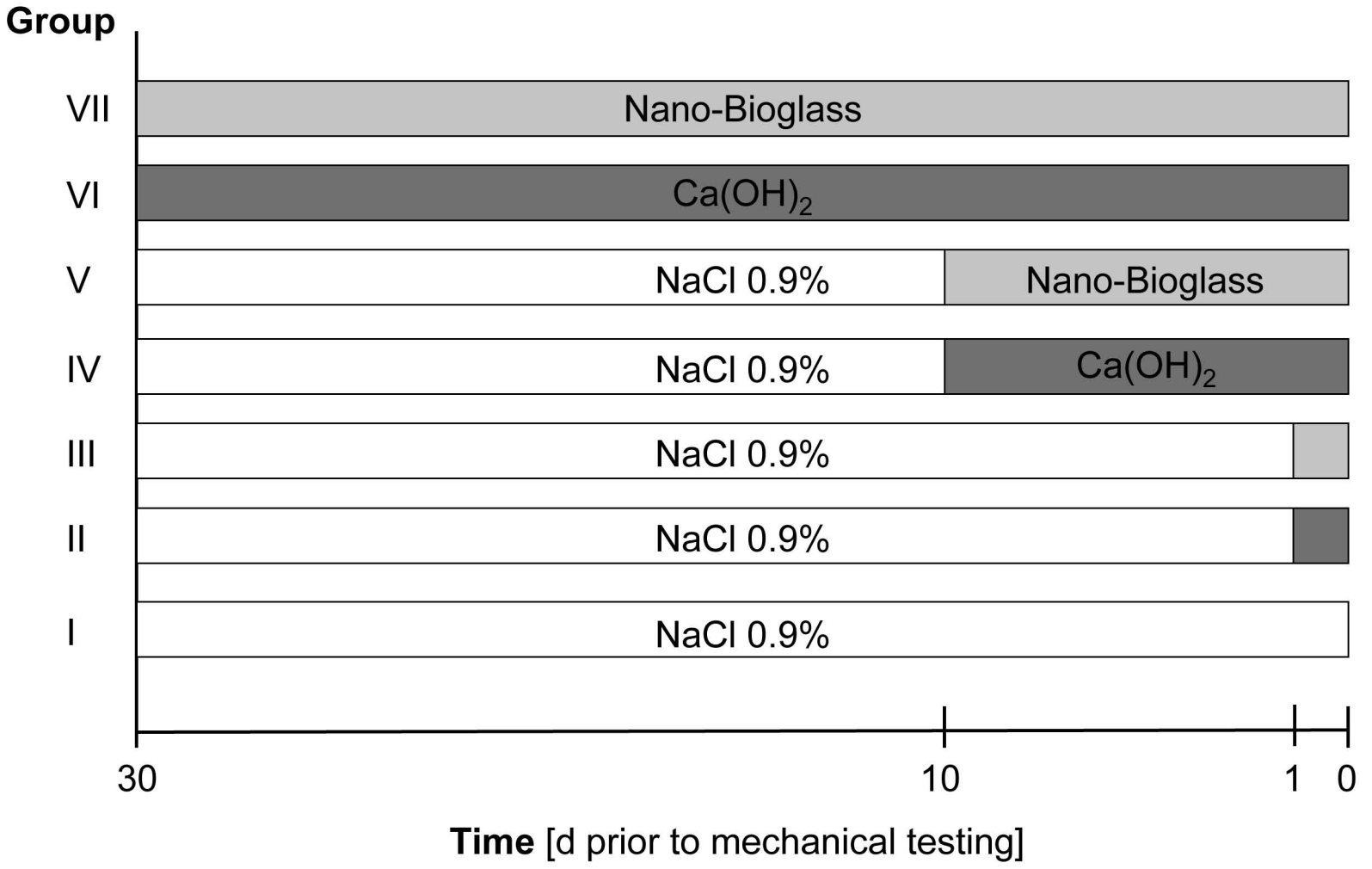
Caption

Fig. 1. Line graph depicting the changes in mean FS of standardized human root specimens (N = 20 specimens per group) immersed in either a calcium hydroxide or a bioactive glass 45S5 suspension over time. Error bars indicate standard deviations.


Group
VII
Nano-Bioglass
VI
Ca(OH)2
V
NaCl 0.9%
Nano-Bioglass
IV
NaCl 0.9%
Ca(OH)2
III
NaCl 0.9%
II
NaCl 0.9%
I
NaCl 0.9%
30
10
1
0
Time [d prior to mechanical testing]

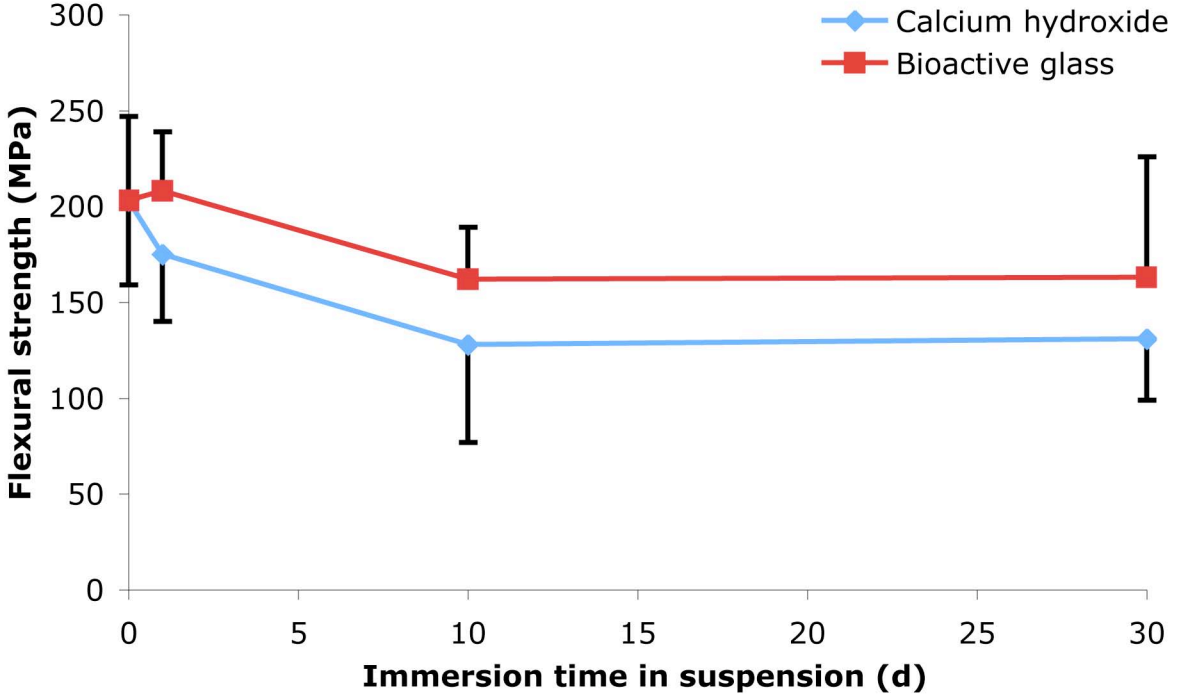

Calcium hydroxide
Bioactive glass
Flexural strength (MPa)
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Immersion time in suspension (d)

Table 1. Flexural strength [MPa] and modulus of elasticity [GPa] of standardized human root dentin bars exposed to test and control treatments depicted in Fig. 1. Values indicate means and standard deviations.

	I	II	III	IV	V	VI	VII
Flexural strength	203±44^{A}	175±35^{AB}	208±31^{A}	128±51^{B}	162±27^{AB}	131±32^{B}	163±20^{AB}
Modulus of elasticity	11±2^{a}	11±2^{a}	11±2^{a}	13±2^{a}	11±2^{a}	13±2^{a}	12±2^{a}

Same superscript letters indicate that there was no statistically significant difference at the 0.05 level between groups for the assessed variable.