

Hardness Changes of Tissue Conditioners in Various Storage Media: An in Vitro Study

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ABSTRACT:

The aim of the present study was to evaluate the effects of storage media on the longitudinal hardness changes of tissue conditioning materials. Four tissue-conditioning materials were used for fabrication of 80 disc-shaped specimens and divided in four groups, stored in four storage media. The specimens underwent artificial ageing corresponding to 30 nights of extra-oral storage. Hardness measurements were obtained at nine intervals between 8 and 240 hours after specimen fabrication. To test the effects of storage media on hardness we employed multivariate modelling (Bonferroni correction: $\alpha=0.05$). The materials exhibited varying hardness changes, most pronounced when stored in ambient air.

INTRODUCTION

Tissue conditioning materials were initially introduced as temporary liners in 1961 by Chase.^{1,2} Fifty years later, they still remain in service as multipurpose materials used for functional impressions in removable^{3,4} and maxillofacial prosthodontics⁵, temporary liners^{6,7} or to enhance healing of distorted or inflamed tissues.^{8,9} These materials are available in powder/liquid form and are highly plasticizing acrylic resins that undergo gelation after mixing.¹⁰ Gelation leads to a compliant, viscoelastic polymer gel.^{11,12} The powder consists mainly of a polymer, usually poly-ethyl methacrylate (PEMA), and other co-polymers.¹³ The liquid contains an aromatic ester (usually butyl-phthalyl butyl glucolate) that acts as plasticizer and ethyl-alcohol in varying concentration of 6-30% wv for control of gelation speed.¹³⁻¹⁶ In vivo use of these materials induces changes in the viscoelastic behavior, as well as alterations in color and surface integrity, rendering the material more prone to fungal colonization and growth.¹⁷⁻²¹

Denture cleansing solutions have been used to prevent Candida colonization of tissue conditioners.^{22,23} The chemical composition of storage media such as denture cleansers is known to influence the viscoelastic properties of tissue conditioning materials, including hardness.²³⁻²⁷ Notably, hardness is regarded as a measure of favorable viscoelastic properties preservation.²⁸ Change in hardness is also one of the main reasons for replacing tissue conditioners at short intervals, commonly every two weeks.¹⁸ Loss of alcohol as well as leaching of the plasticizer are considered to be the main reasons for such hardness changes.^{15,16,25,28,29} Plasticizer loss ranges from 0.30 mg/g to 8.7 mg/g within two weeks and has been suggested as the main cause of hardness changes.³⁰ Moreover, viscoelastic changes have

been noted in tissue conditioning materials containing little or no alcohol.³¹ These changes have also been attributed to plasticizer loss, which is expected to be greater in vivo than in vitro.³⁰ According to Wilson, ethyl alcohol diffusion is a phenomenon that takes place within the first 12 hours,³² while Jones *et al*³⁰ concluded that ethyl alcohol was completely lost within 24h after storage in water at 37°C. Mante *et al*²⁵ examined changes in hardness of tissue conditioners under different storage conditions and concluded that ethanol solution caused severe softening of the tissue conditioners, while denture cleansers had a mild effect on hardness. Aloul and Shen²⁸ showed that tissue conditioners that had greater initial concentration of ethanol were more sensitive to alcohol loss, in terms of hardness change, while the role of plasticizer was more significant when alcohol content was low. The common denominator of the scientific evidence is that both plasticizer and ethanol loss are significant factors in viscoelastic changes of tissue conditioning materials.²⁸ Furthermore, application of coating agents that may prevent or reduce plasticizer and ethanol loss, is considered to enhance the longevity of favorable viscoelastic properties.^{29, 33-35} For example, Mante *et al*²⁵ applied a soft reline sealant (Permaseal) as coating on tissue conditioners and concluded that hardness remained close to pre-immersion values. Malmström *et al*²⁹ used two different coating agents (Monopoly and Permaseal) on a tissue conditioner applied on dentures, in vivo. In that study both coatings were associated with significantly reduced hardness change of the tissue conditioner compared to the non-coated materials. These investigators attributed this finding to the reduction of plasticizer leaching and ethanol loss in the coated specimens.

Along these lines, the aims of this in vitro study were to: a) examine the longitudinal hardness changes of four commercially available tissue conditioning materials stored in various

storage media, and b) quantify and compare the effects of storage media on the hardness changes.

MATERIALS AND METHODS

SPECIMEN PREPARATION

Four commercially available tissue-conditioning materials were used for the fabrication of 4 sets of 20 specimens each. Set 1 comprised of specimens made from Coe-Comfort (CC), set 2 from Tissue Conditioner without the coating agent provided by the manufacturer (TC), set 3 from Tissue Conditioner with application of the coating agent (TCC) according to manufacturer's instructions, and set 4 from Visco-Gel (VG). The materials were used according to manufacturer's instructions. A Plexiglas matrix was used to fabricate 80 disc-shaped specimens of 20mm in diameter and 10 mm in thickness. The matrix was pressed between two glass surfaces to obtain smooth surface on the specimens, and avoid material excess.

STORAGE MEDIA

The total 80 specimens were divided in four groups of 20, and therefore each group consisted of five specimens of each conditioning material. Group A was stored in tap water, group B was stored in a denture cleanser solution (Protefix), group C was stored in another denture cleanser solution (Corega Extradent), and group D was placed on the bench in room temperature for storage in ambient air under dry conditions. The solutions were replaced every 8 hours for 10 consecutive days, thus the specimens underwent artificial ageing corresponding to 30 nights of extra-oral storage. Information about the chemical composition of the tested soft liners is presented in Table 1.

Table 1. Product information of the four tested tissue conditioning materials.

Product	Manufacturer	Powder/ Liquid ratio	Powder*	Liquid†	Coating
Coe-Comfort – CC36	GC Corporation, Tokyo, Japan	0.90 (Wt)	PEMA 100%	BB (87.3%) DBP (4.5%) EtOH (8.2%)	n/a
Visco-Gel – VG36,37	Dentsply DeTrey, Germany	1.25 (Wt)	PEMA (86.2%) PMMA (13.8%)	BPBG DBP EtOH Citrate Ester Plasticizer	n/a
Tissue conditioner – TC38-40	GC Corporation, Tokyo, Japan	2.4 g/ 2 mL	PEMA 100%	EtOH (15%), DBS (plasticizer)	n/a
Tissue conditioner-coated – TCC38-40	GC Corporation, Tokyo, Japan	2.4 g/2 mL	PEMA 100%	EtOH (15%), DBS (plasticizer)	ethyl acetate (80%) Co-polymer of 50% MMA and 50% styrene (20%)

*PEMA- polyethyl methacrylate, PMMA- polymethyl methacrylate; †BB- benzyl benzoate, DBP- dibutyl phthalate, BS- benzyl salicylate, BPBG- butyl phthalyl butyl glycolate, EtOH- ethyl alcohol, DBS: dibutyl sebacate.

HARDNESS MEASUREMENTS

Shore A hardness is typically measured in elastomers such as tissue conditioners, according to ASTM D2240⁴¹ and is quantified on a scale ranging from 0 (least hardness) to 100 (most hardness). A Shore A durometer (S1 durometer) was used to obtain hardness measurements at intervals of 8, 16, 24, 48, 56, 80, 120, 160 and 240 hours after specimen fabrication. These intervals correspond to 1, 2, 3, 5, 7, 10, 15, 20 and 30 nights of extra-oral storage. Shore A hardness measuring utilizes a hardened steel rod with diameter 1.1-1.4 mm with a truncated 35° cone edge of 0.79 mm diameter. The rod creates an indentation on the surface of the specimen, under applied force of 8.064 N⁴¹. This technique is based on measurement of indentation hardness, which is a measure of a sample's resistance to permanent plastic deformation due to a constant compression load from a sharp object. All measurements were taken with the platform of the durometer being parallel, in complete and even contact with the measured surface. Minimum ten measurements were obtained for each specimen, with 3 minutes interval between measurements to allow for specimen relaxation. Previous studies^{25,29} did not report the number of repeated measurements per specimen. In this experiment at least ten measurements were considered adequate for the evaluation of hardness on the total surface of the specimens. A larger number of measurements (up to 22) was obtained for specimens that exhibited mean Shore A of less than 5. Our conduct of multiple measurements -although arbitrary in number and limited by the specimen surface- is likely to reduce site-specific random error of each set of measurements.

STATISTICAL ANALYSES

Descriptive statistics in tabular and graphical format were used for data summarization and presentation. Mean, standard deviation (sd) and standard error of the mean (se) at each time point were computed for hardness values of each combination of tissue conditioner and storage medium. Standard errors were corrected for clustering by specimen. A panel of bar graphs was created to illustrate these hardness estimates. As a measure of absolute hardness change we computed end-

to-start differences in Shore A hardness values, however we did not perform formal hypothesis tests between baseline and final measurements. 'Percent change' was obtained for end-to-start measurements, as an estimate of relative (percent) hardness change.

To statistically test the effects of tissue conditioner and storage medium type on hardness we employed generalized estimating equation (GEE) modeling. The selection of a GEE analysis over traditional approaches (ANOVA, MANOVA) was dictated by the unequal number of observations (Shore A measurements) in some strata, and completely missing data in one stratum (material=TCC, storage=Air, time=5 days). Repeated-measures analyses of variance would require equal number of observations and data completeness, which would not permit use of all available data. An identity link function was used, facilitating the interpretation of beta coefficients on the Shore A scale. Clustering of observations by specimen was accounted for, and an exchangeable correlation matrix was specified. Assuming no homogeneity of effects, the GEE equation included disjoint indicator terms for each tissue conditioner, storage medium and time point, as well as all pairwise interaction terms between these three variables.

Inferences with regard to quantification of effects were based on the interpretation of the GEE-derived 'main effects', which correspond to mutually- and time-adjusted effects of tissue conditioner material and storage medium on specimen hardness. With our experimental set-up of five specimens per material-solvent we had >80% power to detect differences of >7 on the Shore A scale (assuming standard deviation of the difference=5), using an alpha level of 0.05. Formal hypothesis testing of these effects entailed subjecting the model-derived estimates of tissue conditioners and storage media to *post hoc* pairwise contrasts based on GEE score tests⁴². While these contrasts can be considered homogeneity tests and therefore be subject to a more lenient significance threshold, for this analysis we applied a conservative Bonferroni correction for an experiment-wise α level of 0.05, resulting in a P-value threshold of 0.004. All analyses were conducted with the statistical package SAS® 9.2 (SAS Institute Inc., Cary, NC, USA).

Table 2. Shore A hardness values (mean, standard error) of specimens at start (t1) and experiment end (t30), corresponding differences and standard errors [diff (se)], by soft liner and ageing medium.

	Corega			Protefix			Water			Ambient air		
	t1	t30	diff (se)	t1	t30	diff (se)	t1	t30	diff (se)	t1	t30	diff (se)
VG	7.2(1.0)	11.6(0.8)	4.4(0.7)	8.6(0.4)	11.2(2.2)	2.6(0.9)	12.0(0.5)	11.0(1.0)	-1.1(0.7)	9.1(1.3)	21.6(1.5)	12.5(0.8)
CC	0.5(0.2)	1.9(0.6)	1.4(0.2)	1.0(0.4)	3.2(2.2)	2.2(0.5)	2.1(0.5)	1.0(0.5)	-1.2(0.4)	1.4(0.2)	8.4(1.9)	7.0(0.4)
TC	21.4(0.9)	40.9(1.4)	19.5(0.9)	17.9(1.9)	37.8(2.1)	19.9(1.1)	12.5(7.9)	22.8(3.8)	10.2(2.3)	19.1(0.5)	39.5(3.1)	20.4(1.2)
TCC	25.7(1.6)	42.7(3.1)	17.1(1.3)	23.2(1.0)	44.4(1.0)	21.2(0.7)	25.2(1.7)	30.3(1.4)	5.1(0.8)	24.4(1.6)	46.5(2.7)	22.0(1.3)

t: VG: Visco-Gel, CC: Coe-Comfort, TC: Tissue Conditioner uncoated; TCC: Tissue Conditioner-coated.

Table 3. Generalized estimating equation (GEE)† results of Shore A hardness longitudinal changes on soft liner type and storage medium.

Regressors	beta coefficient (Shore A)	Standard error	95% confidence limits
Soft liners			
CC	Referent ^A		.
VG	10.8 ^B	0.7	9.4, 12.1
TC	23.5 ^B	2.1	19.4, 27.7
TCC	30.1 ^B	1.4	27.3, 32.8
Ageing media			
Ambient air	Referent ^C		.
Protefix	-1.9 ^D	0.6	-3.1, -0.6
Corega	-2.4 ^D	0.3	-3.0, -1.8
Water	-3.8 ^D	0.9	-5.6, -2.0
Experimental time (days)			
1	Referent		
2	2.5	0.3	1.9, 3.1
3	3.1	0.7	1.8, 4.5
5	3.9	0.8	2.3, 5.5
7	3.7	0.6	2.6, 4.8
10	3.4	0.6	2.3, 4.6
15	4.2	0.6	3.1, 5.4
20	3.9	0.5	3.0, 4.8
30	7.4	0.8	5.8, 9.0

†The Model included terms for soft liner type, storage medium, experimental time, and pairwise interactions between soft liner, storage medium and experimental time; constant and coefficients of interaction terms are not shown. A/B and C/D superscripts denote groups that were not statistically significantly different after post-hoc pairwise comparisons with Bonferroni correction (experiment-wise alpha=0.05; 12 pairwise post-hoc tests; critical P<0.004)

RESULTS

Baseline hardness measurements [Shore A mean (sd)] for each material were: VG-9.2 (3.5); CC- 1.0 (1.8); TC- 16.8 (9.3); TCC- 24.7 (3.7). Corresponding values at the end of the experiment (t=240hours, or 30days extra-oral storage time) were: VG- 14.0 (6.0); CC- 2.5 (4.0); TC- 35.3 (9.7); TCC- 41.1 (8.5). Using these crude estimates, TC showed the greatest absolute hardness increase (TC- 18.5 or 110%), followed by TCC (TCC- 16.4 or 66%). VG and CC exhibited smaller baseline values and net increases (VG- 4.8 or 52% and CC- 2.5 or 150%); however, CC had the highest percent hardness increase. Baseline and end measurements, along with their corresponding net differences by soft liner material and by storage medium are presented in *Table 2*. It was evident that the storage of both TC and TCC in ambient air and Protefix produced the highest increases in hardness, of about 20 Shore A units. CC presented the least degree of hardening, whereas storage in water resulted in a slight reduction of hardness for VG and CC, of about 1 Shore A unit.

The differences in absolute hardness values and the longitudinal patterns of change are evident in the figure. First, CC's hardness virtually remained unchanged across the experiment, and VG increased only slightly. Protefix resulted in near-linear increases in hardness for TC and TCC, the effect of water however, was markedly different. In all three materials that underwent hardness changes a sharp decrease in hardness was noted after day 5, followed by a plateau between days 5-10. This plateau ended with a rapid increase in hardness that took place between the last two measurements. Noteworthy, this plateau appears later (between days 7 and 20) in specimens stored in tap water.

Results of the GEE modeling and *post hoc* tests are presented in *Table 3*. CC exhibited significantly less hardening compared to the other three tissue conditioners. VG exhibited less hardening compared to TC and TCC, but this difference was smaller and not significant using the conservative threshold after Bonferroni correction (P=0.03 for both contrasts). Considering the storage media, water had the least and air had the most impact on hardness increase. Protefix and Corega had virtually identical effects (P=0.43), and both resulted in significantly less hardening compared to ambient air (P<0.001). An overall hardening trend over time was observed, however a mild plateau was observed between experimental times (days) 5 and 10 for specimens stored in Corega and Protefix solutions and between experimental times (days) 7-20 for specimens stored in water. This observed "average" effect was mostly due to the effect of water, which resulted in the significant decrease in hardness after the fifth day that was mentioned above.

DISCUSSION

Hardness change has been previously used as an indicator of serviceability of applied tissue conditioners, while hardness change and patient discomfort are the main reasons for regular replacement of these materials, at short intervals.^{25,43} The present longitudinal hardness examination indicated that CC underwent the least absolute hardness change in the four storage media, followed by VG. Tissue conditioner specimens (TC and TCC) exhibited substantial hardness increase, which was more pronounced on the coated specimens (TCC).

All materials tested presented different patterns and degrees of hardening, and this can be attributed to their different chemical composition and properties. Coating with a sealing agent has been shown to increase initial hardness and provide longevity of viscoelastic properties by inhibition of plasticizer and ethanol loss.^{25,29,33} In this study, coating of Tissue Conditioner specimens (TCC) was associated with both increased initial hardness and increased hardness changes, although the latter effect was not statistically significant when juxtaposed to our conservative hypothesis-testing cri-

teria. Thus, the coating agent provided by the manufacturer (GC Corporation), failed to inhibit hardness change less than 66%. This was in contrast with recent findings of Singh *et al*³⁵ who reported a beneficial effect of coating in terms of softness preservation and surface integrity. More research is warranted to confirm whether the coating agent used in this study on TCC specimens improves the longitudinal preservation of softness of the Tissue Conditioner. Our analyses indicated that storage media, with the exception of tap water, had virtually similar effect on hardness changes and significantly lesser effect compared to air. A previous investigation²⁸ suggested that plasticizer leaching is the main cause of recovery rate change of tissue conditioning materials, since these materials have a low initial ethanol concentration. These authors suggested storage under dry conditions, despite the fact that CC exhibited significant degradation of viscoelasticity. In our study, storage in ambient air caused the most pronounced increase on the hardness of all tested materials. TCC specimens underwent the highest adjusted changes while CC specimens presented the most modest. It is suggested that when stored under dry conditions, plasticizer leaching is prevented, and the main cause for hardness change is ethanol loss²⁵.

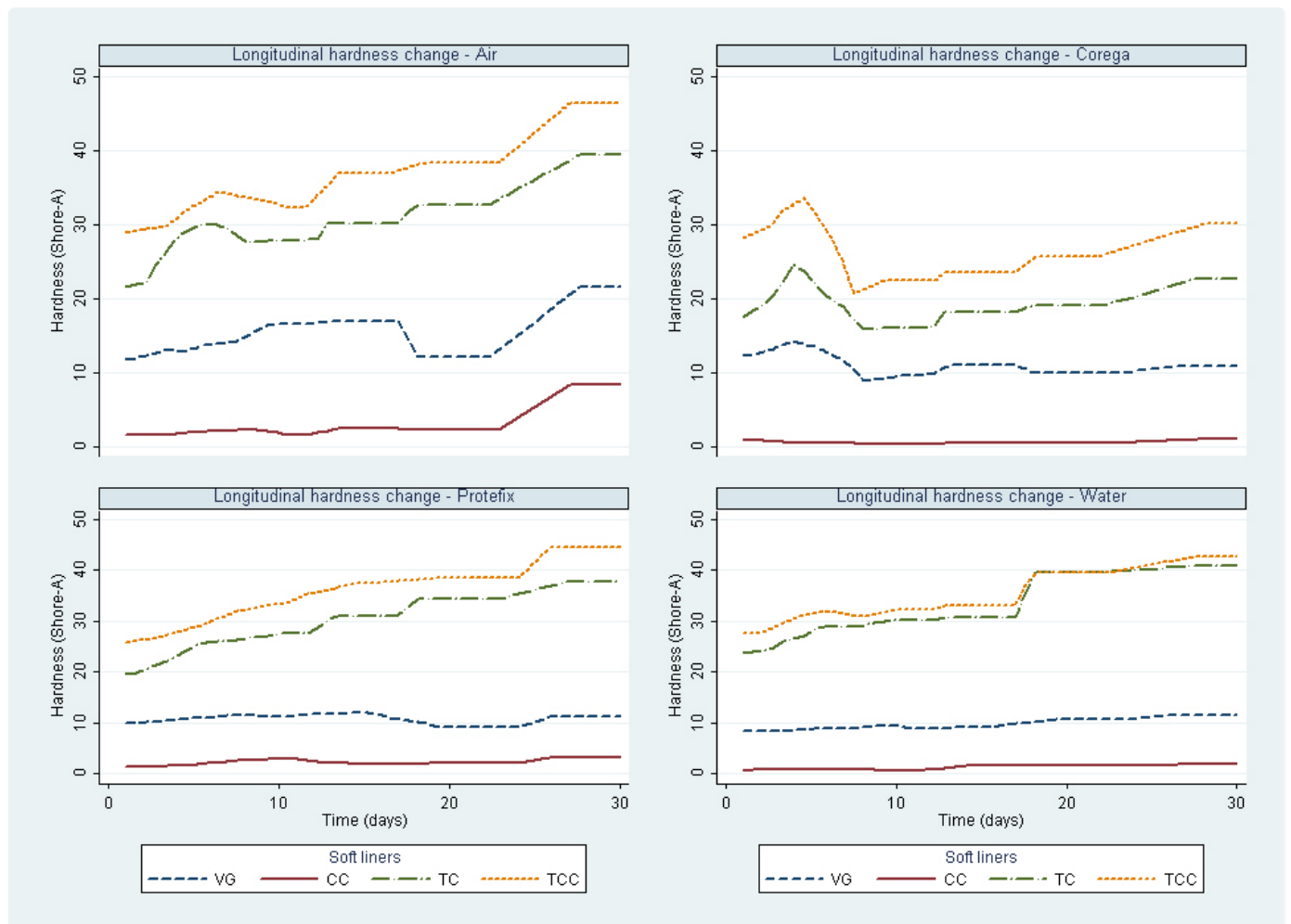


Figure 1. Longitudinal hardness changes on the Shore A scale of the four tissue conditioners aged in the four storage media, illustrated by line plots (mean and 95% confidence limits shown).

The fact that all the materials demonstrated increased hardness when stored under dry conditions, was attributed predominantly to ethanol loss.^{10,28} Storage in tap water, caused an initial increase in hardness which was more pronounced in all tissue conditioning materials (TCC, TC and VG), but CC. This increase in hardness at the early stages of the experiment was attributed to the combined initial loss of ethanol as well as plasticizing ester.^{15,16} After five days a decrease in the rate of hardness development was noted on most of the specimens stored in water (*Figure 1*), which was attributed to water.²² By that time point, water molecules had invaded the amorphous viscoelastic gel of the specimens stored in solutions, providing a “cushion” mechanism against the penetration of the stainless steel rod. This water related decrease in the rate of hardness development²² led to a plateau in hardness measurements. The plateau represented a balance between the softening effect provided by water and the continuing plasticizing ester loss. This pattern continued until about day 20. Between the last two measurement (day 20 and day 30), the plasticizing ester concentration was reduced dramatically and a rapid increase in hardness was recorded. This finding supported the results of Murata *et al*, that immersion in water results in moderate hardening and reduction in flow properties.⁴⁴ Despite the fact that Protefix and Corega Extradent had very similar composition, the hardening pattern produced was different, with Protefix acting more similar to water.. Both products contain carbon sodium and hyperoxide molecules that produce free oxygen radicals as well as other acidic molecules like citric acid. The above mentioned chemical structures can dissolve microbial plaque and stains. A significant difference between the two solutions is that Corega Extradent contained an enzyme which functions as a protease, breaking peptide bonds contained in microbial plaque. The incorporation of this enzyme might account for the different hardening pattern between the two products.

Of particular note are the hardness values obtained in the present experiment which were substantially lower compared to previous investigations.²⁵⁻²⁹ For example, initial hardness for CC was 1 on the Shore A scale, while the range reported in Mante *et al*²⁵ was 12-17. This difference is attributed to the varying thickness of the specimens prepared. In this experiment the thickness was set at 10 mm, while in the above mentioned study the thickness was only 4 mm. Yoeli and Zeltser⁴⁸ confirmed that greater specimen thickness results in a lower Shore A hardness value. In fact, thickness of 10 mm is compatible with ASTM D2240 standard.⁴¹ To our knowledge, there is no evidence for material or specimen specific relaxation time. The authors recognize that the used interval of 3 minutes between measurements was adopted arbitrarily. This time was considered adequate to allow relaxation of the specimen. Future research should focus on the development of clinically relevant immersion protocols and the improvement of temporary tissue conditioners

CONCLUSIONS

Our findings support:

1. The soft liners tested exhibited varying degrees of hardening, with TC/TCC presenting the most and VG/CC the least hardening in absolute terms.
2. Storage in ambient air had the most substantial effect on hardness increase, whereas water had the most modest effect.
3. Longitudinal hardness changes were not linear. Especially for specimens stored in aqueous solutions, a plateau was noted before hardness increased rapidly.
4. Coated specimens (TCC) had higher initial hardness values and demonstrated a more pronounced hardening compared to the non-coated specimens.

MANUFACTURERS' DETAILS

- Permaseal; Austenal, Inc. Chicago, IL
- Monopoly; Austenal, Inc. Chicago, IL
- Coe-Comfort; GC Corporation, Tokyo, Japan
- Tissue Conditioner; GC Corporation, Tokyo, Japan
- Visco-Gel; Dentsply DeTrey, Germany
- Protefix; Queisser Pharma, Flensburg, Germany
- Shore A durometer; S1 durometer, Instron, MA, USA
- Corega Extradent; GlaxoSmithKline, Middlesex, UK

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