

Do Resin Cements Alter Action Potentials of Isolated Rat Sciatic Nerve?

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ABSTRACT

Objectives: The purpose of this study was to explore the effects dual-cure resin cements on nerve conduction.

Methods: Panavia F, RelyX ARC, and Variolink II polymerized either by light-emitting diode (LED) or quartz tungsten halogen (QTH) were used in the study (n=10). The conductance of sciatic nerves of 50 rats were measured before and after contact with the specimens for 1 h.

Results: The time-dependent change in nerve conductance and the comparison of LED versus QTH showed that differences between groups are significant ($P<.05$). For both polymerization techniques, pair-wise comparisons of resin cements showed that the nerve conductance between groups is different ($P<.05$). RelyX ARC elicited irreversible inhibition of compound action potentials (more than 50% change) and Panavia F and Variolink II polymerized by LED and QTH did not alter nerve conduction beyond physiologic limits.

Conclusions: Resin cements may alter nerve conductance and even lead to neurotoxic effects. (Eur J Dent 2011;5:199-205)

Key words: Resin cement; Light-emitting diode; Quartz tungsten halogen; Neurotoxicity; Rat; Sciatic nerve.

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INTRODUCTION

A wide range of dental cements exists for luting of posts, inlays/onlays, composite/ceramic laminates, and fixed prostheses. Over the last decades, enthusiasm for the use of resin cements and resin modified glass ionomer cements was great for endodontic treatment and luting of restorations. At the margin of the restoration, cements may come into contact with soft tissues and fluids; since they may have adverse effects on the pulp, the biocompatibility of dental cements seems as

important as their mechanical and physical properties.^{1,2}

Dual-cure resin composites were developed to add the advantageous properties of chemically polymerizing and light-polymerizing materials, thereby providing sufficient polymerization in deeper zones with shorter polymerizing times.^{3,4} Nevertheless, resin-based materials and adhesives release several components into the oral environment such as considerable amounts of triethylene glycol dimethacrylate (TEGDMA), 2-hydroxy-ethyl-methacrylate (HEMA), and unbound resin components in the early phase after polymerization. The cements then continue to release materials due to degradation or erosion.¹ These leachable materials cause apoptosis⁵⁻⁸ and have shown genotoxic and mutagenic effects⁹⁻¹² and, therefore, may potentially have adverse effects on the pulp, gingiva, and apical tissue.¹³⁻¹⁷

In the context of biocompatibility, another critical issue that needs to be considered is whether these materials have neurotoxic effects. Recent evidence suggests that dentin bonding agents could significantly alter action potentials of nerves, thereby leading to irreversible adverse effects in the pulp.^{18,19} The success of polymerization depends on the thickness of the filling material, the wavelength of the excitation light, the power density, and time of irradiation. Tuning between excitation wavelength and the photoinitiator system has a decisive effect on the degree of polymerization. There are various types of light-curing units (LCU) in the dental market such as quartz tungsten halogen (QTH) and light-emitting diodes (LED) curing units. QTH units have been used to polymerize composite resin, but the drawback of the halogen unit is a declining irradiance over time due to aging lamps and filter. Increasingly, as an alternative to QTH, LED curing units are being used in dental practice. The LED has the advantages of extended lifetimes over 10000 h, little degradation of light output over time, preventing and minimal overheating, resistance shock, or vibration. The spectral output of LEDs consists of the absorption peak of camphorquinone (CQ; 400-500 nm, peak at 470 nm), the most used photoinitiator in resin composites. Comparative studies demonstrated that the type of LCU is an important factor for both curing efficiency and generated heat. However, toxicity of the composites with dif-

ferent curing methods has had relatively little investigation. The determination of the composite's possible toxic effect is a matter of interest. In view of the great variety of LCUs and resin materials currently in use, the question is which combinations cause the least toxic effects. At present, no evidence exists on the possible neurotoxic effects of resin cements. Therefore, the purpose of this study was to explore the effects of dual-cure resin cements polymerized by two different techniques on action potentials of isolated rat sciatic nerves.

MATERIALS AND METHODS

Sample preparation

Three different dual-cure resin cements – Panavia F (Kuraray Medical Inc., Tokyo, Japan), RelyX ARC (3M ESPE, Seefeld, Germany), and Variolink II (IvoclarVivadent, Schaan, Liechtenstein) – were used in this study (Table 1). Disc-shaped samples were prepared by using a teflon mold in 10 mm diameter and 1 mm thickness. Two Mylar bands were used to cover the unpolymerized resin cements, and the tip of light-curing units were placed directly over the Mylar band. Twenty samples were prepared from each cement and randomly allocated into two groups (n=10/group) according to the light-curing unit used:

1. QTH/40seconds (Optilux 501, Kerr, Orange, CA);
2. LED/20 seconds (Elipar Freelight II, 3M/ESPE, Seefeld, Germany).

Before photoactivation, the irradiance of both curing units was confirmed with QTH and LED radiometers (Kerr/Demetron, Orange, CA).

Animals and care

Fifty locally bred male Wistar rats weighing 300-330 g were used in this study. Prior to experiments, the animals were cared for according to the policies and principles established by the Animal Welfare Act and the NIH Guide for Care and Use of Laboratory Animals (Publication # 86-23). All surgical procedures were performed under general anesthesia using a mixture of ketamin (Ketalar, Parke-Davis; 30 mg/kg i.m.) and xylazine (Rompun, Bayer; 10 mg/kg i.m.).

Measurement of compound action potentials

Bilateral sciatic nerves of the animals were dissected from the spinal cord to the knee,

cleaned free from adherent tissue under a dissection microscope, and placed in a three-chambered recording Pyrex bath containing Tyrode solution (8 g/l NaCl, 0.2 g/l KCl, 0.2 g/l CaCl₂, 0.1 g/l MgCl₂, 1 g/l NaHCO₃, 0.05 g/l NaH₂PO₄, and 1 g/l glucose) kept at 37°C and continuously aerated with O₂ and CO₂ (95% and 5%, respectively).¹⁸⁻²⁰ To keep the nerve tissue fresh and vital, the animal was kept under general anesthesia in a 25°C incubator while an experiment was carried out using the first sciatic nerve. The nerves were perfused with the Tyrode solution with a constant rate of 2 ml/min at 37°C, and had a pH value of 7.4. The nerves were mounted between the stimulating and the recording electrodes, and continuous pulses were delivered (pulse width: 0.049 msec; pulse rate 2.0) by a 0811A Pulse Generator (Hewlett Packard, Melrose, MA, USA) through a Schwarzer AK1W161B Stimulus isolation unit (Schwarzer GmbH, Munich, Germany). The nerve signals were digitalized by a Biopac MP30 Data Acquisition System (Biopac Systems Inc., CA, USA) and displayed in a computer with corresponding software (Biopac Student Lab Pro 3.6.6.; Biopac Systems Inc., CA, USA) at a sample rate of 100 kHz.¹⁸⁻²⁰ These values were used to establish the basal response (control) of each nerve before it was brought into contact with one of the test materials. The amplitude of the evoked compound action potentials (cAPs), the time required for initiation of compound action po-

tentials (TcAP), depolarization (Dp), and repolarization (Rp) were recorded before and for up to 60 minutes after contact with the resin cements.

Statistical analysis

Using percentage change of data in relation to control measurements, within-group comparisons for 0-10 min. (T1) and 50-60 min (T2) data were performed by Friedman tests, followed by Wilcoxon tests at 95% confidence levels. To explore the effects of LED versus QTH on nerve conduction, between-group comparisons were performed by Mann-Whitney U tests at 95% confidence level. Between-group pair-wise comparisons of cements were also undertaken by Kruskal Wallis test followed by Mann-Whitney U tests at 95% confidence levels.

RESULTS

Within-group comparisons

Descriptive statistics of tests groups and P values for within-group comparisons are presented in Tables 2 and 3, respectively. The differences in C-T2 for cAPs were significant in all groups (P<.05), although some groups showed similarity between C-T1 and T1-T2 data (P>.05). Likewise, differences in C-T2 for TcAP were significant between groups (P<.05), except for Variolink-QTH (P=.959). In addition, the differences in C-T1 and T1-T2 were significant in most groups (P<.05),

Table 1. Resin cements used in the study.

Materials	Ingredients	Batch No	Manufacturer	Abbreviation
Panavia F	10-Methacryloyloxydecyl dihydrogen phosphate Hydrophobic aromatic dimethacrylate Hydrophobic aliphatic dimethacrylate Hydrophilic aliphatic dimethacrylate Silanated silica filler Silanated colloidal silica dl-Camphorquinone Silanated barium glass filler Initiators Accelerators Pigments Sodium fluoride	41168	Kuraray Dental INC.	Panavia
RelyX ARC	Fillers 60-70% Triethylene glycoldimethacrylate %10-20 Bis-GMA %10-20 Silanated filler %1-10 Functionalized dimethacrylate polymer %1-10	20061204	3M, ESPE	RelyX
Variolink II	Paste of dimethacrylates, inorganic fillers, ytterbiumtrifluoride, initiators, stabilizers and pigments 10-14% Bis-GMA 5-7% Triethylene glycoldimethacrylate 5-7% Urethanedimethacrylate < 1% Benzoylperoxide	J03326	Ivoclar Vivadent	Variolink

although similar data was obtained for the Variolink-QTH group ($P>.05$) (Table 3). The differences in T1-T2 and C-T2 data for Dp were significant in all groups ($P<.05$), except for C-T2 data of Panavia-QTH ($P>.05$). Some groups showed similarity between C-T1 data ($P>.05$) (Table 3). The differences in C-T2 for Rp values were significant only for Panavia-QTH and Variolink-QTH ($P<.05$) and some groups showed similarity between C-T1 and T1-T2 data ($P>.05$).

Between-group comparisons

Between-group comparisons of LED versus QTH on nerve conduction (cAP, TcAP, Dp, and Rp) showed that the differences between groups were significant ($P<.05$), except for TcAP and Dp of RelyX ($P=.353$ and $P=.247$, respectively) during T1, Dp of Rely X ($P=.912$) during T2, and Rp of Variolink during T1 ($P=.075$). P values for between-group

comparisons are presented in Table 4. In the LED group, the differences between groups in T1 and T2 were significant for most variables ($P<.05$), except TcAP, Dp, and Rp data of Panavia and Variolink in T1 in and RelyX and Variolink in T2 ($P>.05$). In the QTH group, the outcome was similar for most variables in T1 ($P>.05$), whereas significant differences were observed at the termination of the experiments ($P<.05$).

DISCUSSION

The mechanism of neural response and nerve conduction basically depend on depolarization-repolarization of nerve activities, which are known to be related to precise regulation of sodium and potassium gateways. In the context of biomaterial-nerve tissue reactions, alterations of depolarization and repolarization are crucial, as they influence signal transduction through the nerve.

Table 2. Descriptive statistics and percentage change of data acquired from sciatic nerves before (control) and after contact with resin cements during T1 and T2.

cAPs		Control			T1				T2				
		TcAP	Dp	Rp	cAPs	TcAP	Dp	Rp	cAPs	TcAP	Dp	Rp	
Pan-LED	Mean±SD	1.09±0.02	1.62±0.03	0.81±0.01	0.81±0.03	0.94(0.02)	1.53±0.04	0.77±0.02	0.76±0.02	0.75±0.02	1.41±0.03	0.59±0.04	0.82±0.03
	Minimum	1.05	1.59	0.78	0.75	0.90	1.44	0.73	0.70	0.72	1.35	0.53	0.77
	Maximum	1.12	1.69	0.83	0.87	0.97	1.58	0.82	0.79	0.78	1.47	0.67	0.87
	% change	100	100	100	100	85.95	94.27	95.27	93.46	68.91	87.16	72.71	101.72
Pan-QTH	Mean±SD	0.37±0.01	0.73±0.01	0.36±0.01	0.36±0.01	0.37(0.01)	0.73±0.01	0.37±0.01	0.36±0.01	0.32±0.01	0.70±0.00	0.35±0.01	0.34±0.00
	Minimum	0.35	0.71	0.34	0.34	0.35	0.72	0.35	0.34	0.31	0.67	0.34	0.33
	Maximum	0.40	0.75	0.38	0.39	0.41	0.75	0.38	0.39	0.34	0.72	0.36	0.36
	% change	100	100	100	100	100.79	100.38	101.86	99.35	87.14	95.11	97.72	94.28
RelyX-LED	Mean±SD	0.08±0.03	1.32±0.03	0.59±0.01	0.72±0.02	0.13(0.00)	1.35±0.02	0.60±0.01	0.74±0.01	0.20±0.00	1.27±0.03	0.54±0.01	0.73±0.03
	Minimum	0.03	1.26	0.57	0.68	0.13	1.31	0.58	0.71	0.20	1.20	0.50	0.68
	Maximum	0.12	1.35	0.61	0.75	0.13	1.37	0.61	0.76	0.21	1.35	0.57	0.79
	% change	100	100	100	100	197.62	102.28	101.85	102.69	305.19	96.60	91.85	100.34
RelyX-QTH	Mean±SD	0.001±0.03	1.41±0.05	0.62±0.03	0.79±0.03	-0.05(0.0)	1.41±0.01	0.66±0.02	0.75±0.0	-0.05±0.0	1.51±0.02	0.72±0.02	0.79±0.02
	Minimum	-0.05	1.34	0.57	0.75	-0.06	1.39	0.62	0.73	-0.10	1.47	0.69	0.75
	Maximum	0.04	1.50	0.68	0.83	-0.05	1.45	0.69	0.76	-0.03	1.56	0.75	0.83
	% change	100	100	100	100	48.31	100.09	105.33	95.94	61.53	107.22	115.28	100.74
Vario-LED	Mean±SD	0.04±0.00	2.16±0.07	1.05±0.04	1.11±0.04	0.04(0.00)	2.05±0.04	0.98±0.02	1.06±0.03	0.05±0.00	2.05±0.05	0.95±0.02	1.09±0.05
	Minimum	0.02	2.05	0.98	1.03	0.04	1.98	0.92	1.00	0.05	1.99	0.91	1.03
	Maximum	0.04	2.25	1.11	1.15	0.04	2.14	1.02	1.12	0.05	2.15	0.99	1.21
	% change	100	100	100	100	96.11	95.17	93.74	96.14	130.43	94.95	90.76	98.57
Vario-QTH	Mean±SD	2.98±0.04	1.57±0.01	1.07±0.01	0.49±0.01	3.00(0.03)	1.57±0.01	1.07±0.01	0.49±0.02	2.68±0.02	1.57±0.01	1.26±0.01	0.30±0.02
	Minimum	2.89	1.53	1.05	0.46	2.97	1.54	1.04	0.45	2.64	1.55	1.24	0.28
	Maximum	3.04	1.59	1.09	0.52	3.07	1.61	1.09	0.56	2.75	1.59	1.29	0.35
	% change	100	100	100	100	100.96	100.17	99.97	100.63	89.99	100.20	118.18	61.43

T1: Data acquired during 0-10 minutes; T2: Data acquired during 50-60 minutes; Pan-LED: Panavia F polymerized by LED; Pan-QTH: Panavia F polymerized by QTH; RelyX-LED: RelyX ARC polymerized by LED; RelyX-QTH: RelyX ARC polymerized by QTH; Vario-LED: Variolink II polymerized by LED; Vario-QTH: Variolink II polymerized by QTH.

Any alteration during depolarization or repolarization and initiation of a cAP (TcAP) could be interpreted as alteration in the function of Na-K gates in the cell membrane. In the present study, TcAP, Dp, and Rp of all cements showed changes in the range of 5-10%, although the Dp of Panavia-LED in T2 and Rp of Variolink in T2 had 27.29% and 38.57% decrease, respectively.

Any material causing more than 50% alteration of cAPs without recovery (irreversible inhibition of nerve conductance) should be interpreted as neurotoxic.¹⁸ In the present study, a change of 197% was observed for RelyX-LED for T1, which continued to rise up to 305.19%. Likewise, reduction of cAPs by 51.69% were observed for RelyX-QTH group and recovered only by 13.22% (Table 2). This result suggests that RelyX – polymerized either by LED or QTH – has a high potential of eliciting irreversible inhibition of cAPs of rat sciatic nerves. The drastic change in cAPs could be attributed to 10-20% TEGDMA content of RelyX, which also has a strong potential of leading to apoptosis.⁵⁻⁸ How-

ever, the dose-dependent neurotoxic effects of TEGDMA is unknown and remain as an open field for investigation.

Ideally, resin cement is expected to have converted all of its monomer to polymer upon polymerization, but under conventional irradiation, all dimethacrylate monomers suffer from considerable amount of residual monomer with a degree of conversion between 55%-75%.^{21,22} Although RelyX ARC cement has higher degree of conversion in comparison with Panavia and Variolink,²³ the TEGDMA content in Variolink is 7-10% and it is not present in Panavia. Therefore, in the context of cytotoxicity, a low degree of conversion is not always an indicator of release of toxic substances due to inadequate development of the polymer network. In the present study, the percentage changes in cAPs of other resin cements could not be inferred as neurotoxic effects but rather reversible inhibition of cAPs, as full recovery was not observed.²⁰ It seems that Panavia and Variolink polymerized by QTH had the lowest effect on nerve conductance.

Table 3. P values for within-group comparisons.

	cAP			TcAP				Dp			Rp		
	CT1	C-T2	T1-T2	C-T1	C-T2	T1-T2	C-T1	C-T2	T1-T2	C-T1	C-T2	T1-T2	
Pan-LED	0.005	0.005	0.005	0.005	0.005	0.007	0.005	0.005	0.005	0.007	0.508*	0.005	
Pan-QTH	0.878*	0.005	0.005	0.859*	0.005	0.005	0.359*	0.285*	0.007	0.799*	0.007	0.022	
RelyX-LED	0.005	0.005	0.005	0.007	0.037	0.007	0.037	0.005	0.005	0.011	0.799*	0.109*	
RelyX-QTH	0.005	0.037	0.093*	0.959*	0.008	0.005	0.047	0.005	0.005	0.028	0.646*	0.013	
Vario-LED	0.139*	0.005	0.005	0.013	0.013	0.575*	0.009	0.007	0.017	0.047	0.386*	0.444*	
Vario-QTH	0.169*	0.005	0.005	0.575*	0.959*	0.959*	0.878*	0.005	0.005	0.959*	0.005	0.005	

*: not significant; C:Control; T1: Data acquired during 0-10 minutes; T2: Data acquired during 50-60 minutes; Pan-LED: Panavia F polymerized by LED; Pan-QTH: Panavia F polymerized by QTH; RelyX-LED: RelyX ARC polymerized by LED; RelyX-QTH RelyX ARC polymerized by QTH; Vario-LED: Variolink II polymerized by LED; Vario-QTH: Variolink II polymerized by QTH.

Table 4. P values for between-group comparisons.

		T1				T2			
		cAP	TcAP	Dp	Rp	cAP	TcAP	Dp	Rp
LED	Pan-RelyX	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.631*
	Pan-Vario	0.00	0.912*	0.247*	0.218*	0.00	0.00	0.00	0.28*
	RelyX-Vario	0.00	0.001	0.001	0.007	0.00	0.436*	0.579*	0.529*
QTH	Pan-RelyX	0.00	0.94*	0.226*	0.406*	0.028	0.00	0.00	0.023
	Pan-Vario	0.88*	0.762*	0.226*	0.545*	0.049	0.001	0.00	0.00
	RelyX-Vario	0.00	0.762*	0.131*	0.112*	0.023	0.004	0.226*	0.00

*: not significant; T1: Data acquired during 0-10 minutes; T2: Data acquired during 50-60 minutes; PAN: Panavia F; Vario: Variolink II

However, it should be taken into account that polymerization of these cements by LED resulted in approximately a 30% change in cAPs at the termination of the experiments.

Comparison of LED with QTH showed that the differences were statistically significant for cAPs of all cements and for most variables. Nerve conductance of Panavia and Variolink polymerized by QTH showed a more stable time-dependent behavior of nerve conductance in comparison with LED-polymerized specimens of these cements. Overall, QTH-polymerized specimens tended to show better results, although a clear advantage of QTH over LED was not discernable in the present study.

CONCLUSIONS

The present results suggest that RelyX polymerized by either LED or QTH leads to irreversible effects on nerve conductance. Panavia and Variolink polymerized by both techniques lead to reversible alteration of nerve function. These cements polymerized by QTH show a more stable nerve conductance over time in comparison with LED-polymerization.

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