Acta Odontologica Scandinavica, 2013; 71: 710–715

informa
healthcare

ORIGINAL ARTICLE

Solvent content and dentin bond strengths using water-wet, ethanol-wet and deproteinization bonding techniques

ANDRÉ LUIS FARIA-E-SILVA[1], JOSÉ EVERTON ARAÚJO[1], GILLIANE PEREIRA ROCHA[1], ALINE DA SILVA DE OLIVEIRA[2] & RAFAEL RATTO DE MORAES[2]

[1]*Department of Dentistry, Federal University of Sergipe, Aracaju, SE, Brazil, and* [2]*School of Dentistry, Federal University of Pelotas, Pelotas, RS, Brazil*

Abstract

Objectives. To evaluate the impact of solvent content in two-step, etch-and-rinse adhesives on the dentin bond strengths obtained via water-wet, ethanol-wet or deproteinization techniques. ***Materials and methods.*** A model photocurable Bis-GMA/HEMA blend was diluted in ethanol (7.5, 15 or 30 mass%) or acetone (15, 30 or 60 mass%) (low, medium or high solvent content, respectively). Viscosity of the solutions was measured with an oscillatory viscometer and data analyzed using ANOVA on Ranks (5%). Dentin bond strengths were evaluated using microshear bond test. After acid-etching and rinsing, the dentin was kept wet (water-wet), treated with ascending ethanol concentrations (ethanol-wet) or with 10% NaOCl solution (deproteinization). Composite cylinders built-up on the surfaces for the microshear test. Data from each bonding technique were submitted to two-way ANOVA and Fisher's LSD method (5%). Failure modes were classified under magnification and data analyzed using chi-square tests (5%). ***Results.*** Viscosity of ethanol-based agents was remarkably higher than acetone solutions. For the water-wet technique, lower bond strength was observed for the low compared with medium and high ethanol contents. For the ethanol-wet technique, the bond strength for both solvents types was low < medium = high solvent content. For the deproteinization technique, no significant differences were observed among groups. Significant differences in failure modes were observed between the bonding techniques. The ethanol-wet technique had more adhesive failures, whereas the other techniques showed a predominance of mixed failures. ***Conclusions.*** The solvent content may interfere with the dentin bond strengths for the conventional and ethanol bonding techniques.

Key Words: *adhesives, ethanol, sodium hypochlorite, solvents, viscosity*

Introduction

Bonding to dentin via the etch-and-rinse approach relies on hybridization with the demineralized substrate after a conditioner (usually ~ 35% phosphoric acid) dissolves the hydroxyapatite crystals and exposes the collagen network. Organic solvents (commonly ethanol or acetone) are important constituents of dental adhesives; solvents displace water from the demineralized dentin and allow infiltration of the resin monomers [1]. Due to their high volatility, solvents may spontaneously evaporate from the adhesive bottle as a function of repeated use [2–5], high frequency of use and increased storage temperature might accelerate the evaporation. Reduction in the solvent content may interfere with the effectiveness of dentin bond [3,5]. Considering that loss in solvent content is commonly observed, adhesive techniques that reduce the solvent dependency are of clinical interest.

Adhesive techniques alternative to the conventional water-wet bonding technique have been proposed to reduce the moisture dependence of the demineralized dentin for proper bonding. The collagen removal with NaOCl (deproteinization) after acid etching was initially proposed to eliminate the non-encapsulated collagen at the base of the hybrid layer because this unprotected area is susceptible to hydrolytic degradation and may compromise the bond stability [6,7]. A more recent technique advocates treatment of the dentin using solutions with increasing ethanol concentration [8]. This approach involves gradual

Correspondence: Dr André Luis Faria-e-Silva, Departamento de Odontologia, Rua Cláudio Batista s/n, Sanatório, Aracaju, SE, 49060-100, Brazil. Tel: +55 79 2105-1823. Fax: +55 79 2105-1823. E-mail: faria_silva@ufs.br

(Received 5 March 2012; revised 7 May 2012; accepted 15 June 2012)

ISSN 0001-6357 print/ISSN 1502-3850 online © 2013 Informa Healthcare
DOI: 10.3109/00016357.2012.715195

replacement of water by ethanol, maintaining the interfibrilar spaces in the collagen mesh. As the ethanol-saturated dentin is less hydrophilic, the technique permits the use of more hydrophobic resin monomers [9].

Considering that in either the ethanol-wet or deproteinization techniques the adhesive solution is applied to a water-depleted dentin, the bonding might be expected to be less sensitive to the solvent concentration in the adhesive agent. However, the effect of the solvent contained in bonding solutions has not been tested using these alternative bonding approaches. Thus, the aim of this study was to investigate the bond strength to dentin of experimental etch-and-rinse bonding agents with different solvent contents (simulating the gradual loss of solvent content) applied using the water-wet, ethanol-wet or deproteinization bonding techniques. The hypotheses tested were that (i) the reduction in solvent content would decrease the bond strength to dentin using the water-wet bonding technique and that (ii) the alternative bonding techniques (ethanol-wet and deproteinization) would eliminate the solvent-dependency for adhesive agents to bond to the demineralized dentin.

Materials and methods

Formulation of the experimental bonding agents

A model co-monomer blend was obtained by mixing bisphenol-A glycidyl dimethacrylate (Evonik, Essen, Germany) and hydroxyethyl methacrylate (Sigma-Aldrich, St. Louis, MO) at a 3:2 mass ratio. A mass fraction of 0.4% of camphorquinone (Esstech Inc., Essington, PA) and 0.8% of ethyl-4 dimethylamino benzoate (Sigma-Aldrich) were included to make the blend photocurable. Absolute acetone or absolute ethanol was used as solvent. In order obtain bonding agents with low, medium and high solvent contents, different solvent concentrations were added to the co-monomer: 15, 30 or 60% for acetone and 7.5, 15 or 30% for ethanol, respectively. The full solvent content (60% for acetone and 30% for ethanol) was established based on previous studies with commercial solvated bonding agents [5,10,11]; the other contents were used to simulate volatilization of $^1/_2$ and $^3/_4$ of the solvent carrier.

Viscosity measurements

Viscosity measurements (mPa.s) of the adhesive solutions were performed using an oscillatory digital viscometer (LVDV-II+Pro; Brookfield, Middleboro, MA). Defined volume of the materials was dispensed in the equipment operating under the following settings: temperature of 20°C, speed of 7.5 rpm, shear rate of 10 Hz and run time of 60 s. Three specimens were tested for each material. Data did not reach homocedasticity and were submitted ANOVA on Ranks and Student-Newman-Keuls' test ($p < 0.05$).

Bonding procedures

Forty-five sound third molars were used. Each tooth was sectioned to its mesio-distal axis, parallel to the long axis, into two halves, resulting in 90 hemi-sections. Each hemi-section was embedded in acrylic resin with the buccal/lingual face exposed. The surface was wet-ground with 400-grit SiC abrasive papers until a flat surface in medium dentin was obtained. The dentin was wet-polished with 600-grit SiC abrasive papers to standardize the smear layer, then etched with 34% phosphoric acid (3M ESPE, St. Paul, MN) for 15 s, followed by rinsing with water for 10 s. The specimens were randomly allocated into three groups of 30 hemi-sections each, defined by the dentin treatment before the bonding procedures:

- *Water-wet*: Excess dentin moisture was removed using absorbent paper, leaving the dentin wet;
- *Ethanol-wet*: The surface was treated with a series of increasing concentrations of ethanol solutions: 50%, 70%, 80% and 95% for 30 s, followed by three applications of absolute ethanol for 30 s; and
- *Deproteinization*: 5% NaOCl solution was applied for 1 min, followed by rinsing with water for 10 s and air-drying with compressed air for 10 s.

For each bonding procedure–bonding agent combination, five hemi-sections were used. The adhesive was applied with a microbrush and left undisturbed for 20 s; the solvent was gently evaporated for 5 s with direct low-pressure air stream ~ 5 cm away from the dentin surface. Two polyvinyl tubes with a cylinder-shaped orifice (1 mm inner diameter × 2 mm height) were placed onto the surface of each hemi-section and the adhesive light-activated for 10 s using a light-emitting diode unit (Radii Cal; SDI, Bayswater, Victoria, Australia) with 800 mW/cm^2 irradiance. The orifices were filled with a resin composite (Filtek Z350; 3M ESPE) and light-activated for 20 s.

Microshear bond test and failure analysis

After 24 h, a shear bond test was conducted on a mechanical testing machine (DL500; EMIC, São José dos Pinhais, PR, Brazil). A thin steel wire (0.2 mm diameter) was looped around each cylinder and the shear load applied to the base of the cylinder at a crosshead speed of 0.5 mm/min until failure. The average value of the two bonded cylinders for each hemi-section was recorded as the microshear bond strength (MPa) for that specimen. Data from each bonding technique were separately submitted to two-way ANOVA (solvent type × solvent content) and Fisher's LSD method ($p < 0.05$) using the software

SigmaStat 3.5 (Systat Software, Erkrath, Germany). After the bond test, the dentin surfaces were evaluated under optical microscopy at 40 × magnification; failure modes were classified as adhesive, mixed or cohesive failure within dentin. Failure mode data for solvent type, solvent content and dentin bonding technique were individually analyzed using chi-square tests ($p < 0.05$).

Results

Results for viscosity are shown in Table I. All groups showed significant differences between each other ($p < 0.05$), except for the adhesives with medium and high acetone content. Viscosity of ethanol-based agents was remarkably higher than acetone-based solution. Results for bond strength are shown in Table II. The factor 'solvent type' was not significant for any of the bonding techniques ($p \geq 0.473$), whereas the factor 'solvent content' was significant for the water-wet ($p = 0.018$) and ethanol-wet ($p = 0.008$) techniques, but not for the deproteinization technique ($p = 0.264$). The interaction 'solvent type × solvent content' was not significant, irrespective of the bonding technique ($p \geq 0.141$). The power of the performed tests, however, was not always above 0.8.

For the water-wet technique using the ethanol-based adhesive, significantly lower bond strength was observed for the low solvent content compared with the medium and high solvent contents, whereas no significant differences were detected for the acetone-based materials, irrespective of the solvent content. For the ethanol-wet technique, significantly lower bond strengths were observed for the low solvent content compared to the medium content for both solvent types, whereas the high solvent content showed intermediate values. For the deproteinization technique, no significant differences were observed for any of the groups.

Distribution of failure modes is shown in Figure 1. The statistical analysis showed no appreciable differences in failure modes for solvent content ($p = 0.873$) or solvent type ($p = 0.506$), whereas the results were significant for bonding technique ($p = 0.013$). For the water-wet and deproteinization techniques, mixed failures were predominant, whereas adhesive failures were predominant for the ethanol-wet technique. Few cohesive failures were observed for all bonding techniques.

Table I. Means (SD) for viscosity (mPa.s) of the adhesive solutions ($n = 3$).

	Solvent type	
Solvent content	Ethanol	Acetone
Low	267.9 (12.2)^A	32.7 (0.3)^C
Medium	57.3 (0.2)^B	1.9 (0.1)^E
High	18.5 (0.3)^D	1.6 (0.2)^E

Distinct letters indicate significant differences ($p < 0.05$).

Discussion

Reduction in solvent content had a detrimental effect on the bond strength to dentin using the water-wet technique only for the ethanol-based adhesive. Therefore, the first hypothesis tested is partially accepted. The full solvent content used in this study was based on commercially-available adhesives, such as Prime&Bond NT and One-Step (acetone-based agents), and Excite, OptiBond Solo Plus and Single Bond 2 (ethanol-based agents). It is known that acetone evaporates more from the adhesive bottle as a function of its repeated use compared with ethanol [2,3]. Thus, the solvent content in commercial acetone-based adhesives is higher than in ethanol-diluted agents. The low solvent content was set at 15% and 7.5% for acetone and ethanol, respectively. This may explain why only for the ethanol-based agent the reduction in solvent content interfered with the dentin bond strengths. The 15% of acetone combined with its higher vapor pressure than ethanol was probably sufficient to displace water and allow infiltration of the resin monomers into the demineralized dentin, while the ethanol-based agent was not effective in doing the same [12,13]. Solvents increase the hydrophilicity and reduce viscosity of bonding agents [13], as shown by the present findings. It is conceivable that the increased viscosity may compromise their penetration into the collagen mesh [11,14] and that lower hydrophilicity may result in poor dentin wetting, interfering with hybridization [15].

The solvent content also had a significant effect on the dentin bonds when the ethanol-wet technique was tested. Irrespective of the solvent type, adhesives with low solvent content showed lower bond strengths compared with adhesives with medium solvent content, which is likely a result of the remarkable differences in viscosity observed interfering with resin diffusion into the exposed collagen mesh. However, adhesives with high solvent content, either acetone or ethanol, showed intermediate results, which highlights that differences in adhesive viscosity may not completely explain distinct bonding performances. In the ethanol-wet bonding technique, dentin water is slowly replaced by ethanol; the dentin is saturated with ethanol in the end of the dehydration process. When adhesives with high solvent concentration are used, the amount of solvent in the dentin is excessive. This may impair full solvent volatilization [16] and, as evidenced by the bond strength results, interfere with the bonding mechanism. In addition, the results may be also related to the excess of non-volatilized solvent reducing the degree of C=C conversion [16,17] and

Table II. Means (SD) for bond strength (MPa) according to the solvent content and type for the different bonding techniques ($n = 5$).

Bonding technique	Solvent content	Solvent type	
		Ethanol	Acetone
Water-wet	Low	9.1 (6.2)*A*,b	14.3 (5.2)*A*,a
	Medium	18.8 (8.9)*A*,a	17.1 (6.9)*A*,a
	High	20.3 (4.6)*A*,a	18.2 (7.4)*A*,a
Ethanol-wet	Low	10.8 (4.8)*A*,b	11.8 (5.9)*A*,b
	Medium	22.9 (5.5)*A*,a	22.1 (10.8)*A*,a
	High	18.4 (2.3)*A*,ab	13.7 (10.7)*A*,ab
Deproteinization	Low	22.2 (7.5)*A*,a	14.8 (7.8)*A*,a
	Medium	15.0 (2.9)*A*,a	18.2 (5.6)*A*,a
	High	13.3 (5.4)*A*,a	14.5 (6.5)*A*,a

For each bonding technique, capital letters in a same line indicate differences for solvent type, whereas lowercase letters in a same column indicate differences for solvent content ($p < 0.05$).

negatively affecting the mechanical properties of the forming polymer [18,19].

On the other hand, no significant differences in bond strength were observed for either solvent contents or types when the NaOCl technique was tested, which provides evidence that dentin deproteinization before the bonding procedure may eliminate the solvent-dependency of adhesives to bond to

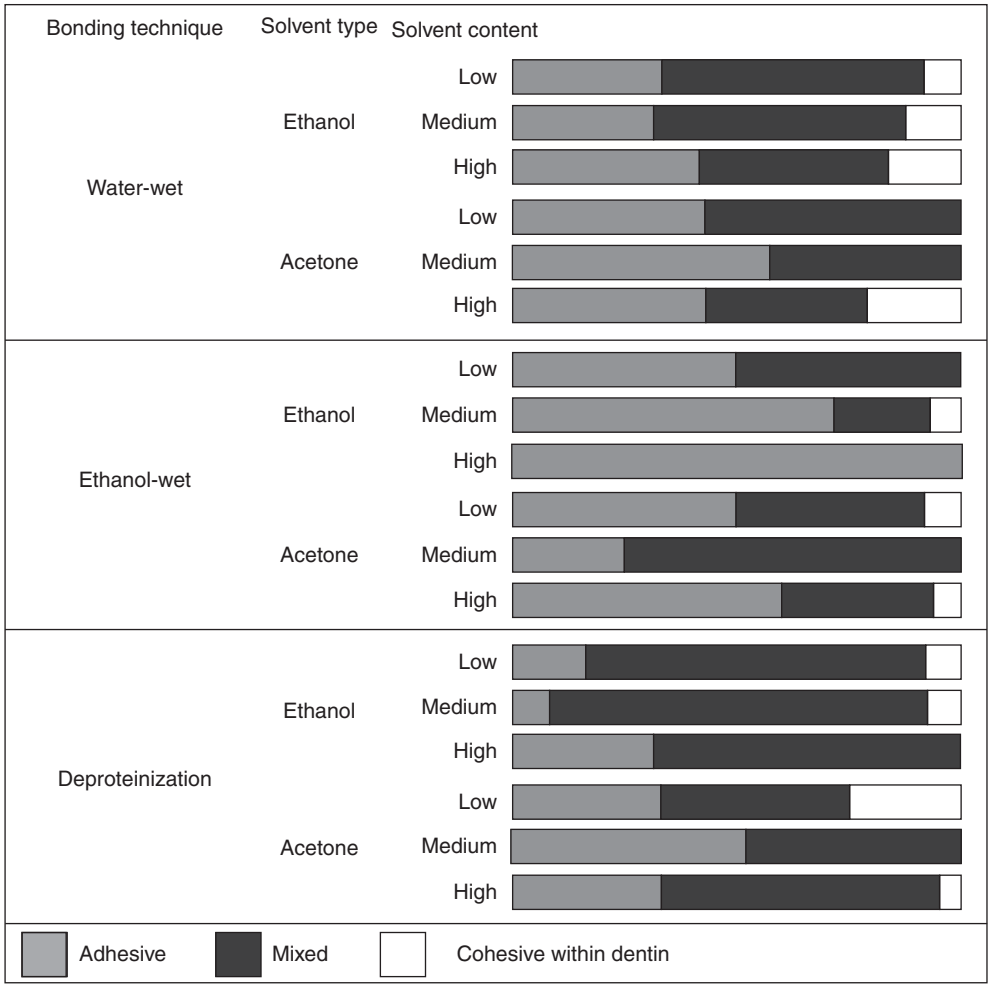

Figure 1. Distribution of failure modes among groups. For the water-wet and deproteinization techniques, mixed failures were predominant, whereas adhesive failures were predominant for the ethanol-wet technique. No appreciable differences in failure modes were observed for solvent content or solvent type.

dentin. Therefore, the second hypothesis tested is also partially accepted. This result can be explained by the absence of the collagen mesh and water, which was eliminated using air stream. In the water-wet technique, it is well-known that drying the etched dentin leads to a collapse of the collagen network, impairing resin penetration [12,20,21]. This collapse results from formation of hydrogen bonds between the collagen fibrils [20]. Dilution of resin monomers in solvents with high solubility parameter, able to break these hydrogen bonds, is necessary in such a situation [22]. As the collagen mesh is removed by NaOCl application, the dentin can be fully dried without interfering with the adhesive penetration [7,23]. Moreover, there is less competition between water and resin monomers for space into the dry dentin, as only fluid accessing the surface from the opened dentin tubules is available.

The increase in viscosity of the adhesive solution as a function of a reduction in solvent content did not have a negative effect on the dentin bonds for the deproteinization technique. The adhesive viscosity seems to have a significant role on the dentin bonds mainly when the interfibrillar collagen spaces are occupied by water. For the NaOCl technique, adhesive penetration into the dentin tubules may occur even for more viscous solutions due to the large diameter of the tubules. Although it is known that resin tags have only a minor contribution to dentin bonding when the collagen network is present, it has been suggested that the formation of larger resin tags and innumerous lateral branches might also account for the bond strengths of adhesives applied to deproteinized dentin [24]. However, it should be acknowledged that bonding agents may behave differently after acid-etching under *in vivo* vs *in vitro* conditions, especially regarding the dentin fluid accessing the surface and its interaction with the adhesives under *in vivo* conditions.

Regarding the solvent types in terms of viscosity, acetone solutions showed significantly lower viscosity than ethanol solutions; this is in line with findings from a recent study [25]. The results also showed no changes in viscosity between the medium and high acetone contents, which indicates that lower acetone content could be used in commercial bonding agents. The reason for that is the high vapor pressure of acetone that causes higher rates of spontaneous evaporation from the adhesive bottle compared with ethanol. In light of that, it should be pointed out that ethanol-based agents would probably need longer periods of repeated clinical usage to reach a low solvent content than acetone-based solutions.

In conclusion, the solvent content may interfere with the dentin bond strengths for the water-wet and ethanol-wet bonding techniques, whereas deproteinization of the etched dentin may eliminate the solvent-dependency to bond to dentin. Clinically, the ideal scenario for a proper dentin bonding involves uniform adhesive penetration into all extension of the demineralized dentin, solvent/water elimination and *in loco* polymerization of the resin monomers. Considering that spontaneous loss of solvent is expected for solvated bonding agents, dentin deproteinization may reduce the technique-sensitivity of dentin adhesive procedures. This, in turn, may be expected to reduce the clinical drawbacks related to improper dentin bonding procedures, such as occurrence of post-operative sensitivity. Furthermore, the collagen removal by NaOCl treatment may reduce the degradation of the adhesive layer, which may have a role on the longevity of restorations [7,26]. However, further studies are necessary to corroborate this assumption.

Acknowledgments

J.E.A. (CNPq/Brazil), E.R.P. (COPES/UFS/Brazil) and A.S.O. (Capes/Brazil) are grateful for their scholarships. The authors thank Esstech Inc. for donation of camphorquinone.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Kanca J 3rd. Improving bond strength through acid etching of dentin and bonding to wet dentin surfaces. J Am Dent Assoc 1992;123:35–43.

[2] Abate PF, Rodriguez VI, Macchi RL. Evaporation of solvent in one-bottle adhesives. J Dent 2000;28:437–40.

[3] Lima FG, Moraes RR, Demarco FF, Del Pino FA, Powers J. One-bottle adhesives: *in vitro* analysis of solvent volatilization and sealing ability. Braz Oral Res 2005; 19:278–83.

[4] Nihi FM, Fabre HS, Garcia G, Fernandes KB, Ferreira FB, Wang L. *In vitro* assessment of solvent evaporation from commercial adhesive systems compared to experimental systems. Braz Dent J 2009;20:396–402.

[5] Reis AF, Oliveira MT, Giannini M, De Goes MF, Rueggeberg FA. The effect of organic solvents on one-bottle adhesives' bond strength to enamel and dentin. Oper Dent 2003;28:700–6.

[6] Abo T, Asmussen E, Uno S, Tagami J. Short- and long-term *in vitro* study of the bonding of eight commercial adhesives to normal and deproteinized dentin. Acta Odontol Scand 2006; 64:237–43.

[7] Goncalves LS, Consani S, Sinhoreti MA, Schneider LF, Saboia VP. Effect of storage and compressive cycles on the bond strength after collagen removal. Oper Dent 2009;34: 681–7.

[8] Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resin using a macromodel of the hybrid layer. Am J Dent 2007;20:7–20.

[9] Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, Tay FR. Ethanol wet-bonding challenges current anti-degradation strategy. J Dent Res 2010;89:1499–504.

[10] Cho BH, Dickens SH. Effects of the acetone content of single solution dentin bonding agents on the adhesive layer thickness and the microtensile bond strength. Dent Mater 2004;20: 107–15.
[11] Wang Y, Spencer P, Yao X, Brenda B. Effect of solvent content on resin hybridization in wet dentin bonding. J Biomed Mater Res A 2007;82:975–83.
[12] Pashley DH, Carvalho RM, Tay FR, Agee KA, Lee KW. Solvation of dried dentin matrix by water and other polar solvents. Am J Dent 2002;15:97–102.
[13] Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials 2007;28:3757–85.
[14] Meyer-Lueckel H, Paris S. Infiltration of natural caries lesions with experimental resins differing in penetration coefficients and ethanol addition. Caries Res 2010;44:408–14.
[15] Guo X, Spencer P, Wang Y, Ye Q, Yao X, Williams K. Effects of a solubility enhancer on penetration of hydrophobic component in model adhesives into wet demineralized dentin. Dent Mater 2007;23:1473–81.
[16] Dickens SH, Cho BH. Interpretation of bond failure through conversion and residual solvent measurements and Weibull analyses of flexural and microtensile bond strengths of bonding agents. Dent Mater 2005;21:354–64.
[17] Cadenaro M, Breschi L, Rueggeberg FA, Suchko M, Grodin E, Agee K, et al. Effects of residual ethanol on the rate and degree of conversion of five experimental resins. Dent Mater 2009;25:621–8.
[18] Cadenaro M, Breschi L, Antoniolli F, Navarra CO, Mazzoni A, Tay FR, et al. Degree of conversion of resin blends in relation to ethanol content and hydrophilicity. Dent Mater 2008;24:1194–200.
[19] Kanehira M, Finger WJ, Hoffmann M, Endo T, Komatsu M. Relationship between degree of polymerization and enamel bonding strength with self-etching adhesives. J Adhes Dent 2006;8:211–16.
[20] Fawzy AS. Variations in collagen fibrils network structure and surface dehydration of acid demineralized intertubular dentin: effect of dentin depth and air-exposure time. Dent Mater 2010;26:35–43.
[21] Tay FR, Gwinnett JA, Wei SH. Micromorphological spectrum from overdrying to overwetting acid-conditioned dentin in water-free acetone-based, single-bottle primer/adhesives. Dent Mater 1996;12:236–44.
[22] Miller RG, Bowles CQ, Chappelow CC, Eick JD. Application of solubility parameter theory to dentin-bonding systems and adhesive strength correlations. J Biomed Mater Res 1998;41: 237–43.
[23] Munksgaard EC. Wet or dry, normal or deproteinized dentin surfaces as substrate for dentin adhesives. Acta Odontol Scand 2002;60:60–4.
[24] Pioch T, Kobaslija S, Schagen B, Gotz H. Interfacial micromorphology and tensile bond strength of dentin bonding systems after NaOCl treatment. J Adhes Dent 1999;1: 135–42.
[25] Moraes RR, Garcia JW, Wilson ND, Lewis SH, Barros MD, Yang B, et al. Improved dental adhesive formulations based on reactive nanogel additives. J Dent Res 2012;91:179–84.
[26] Silva GO, Barcellos DC, Pucci CR, Borges AB, Torres CR. Longitudinal bond strength evaluation using the deproteinized dentin technique. Gen Dent 2009;54:328–33.