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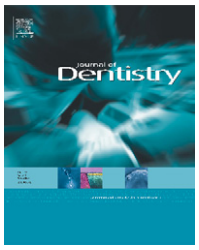

Quantification of monomer elution and carbon–carbon double bonds in dental adhesive systems using HPLC and micro-Raman spectroscopy

Vesna Miletic [a,*], *Ario Santini* [a], *Ivan Trkulja* [b]

[a] *Edinburgh Postgraduate Dental Institute, The University of Edinburgh, Lauriston Building (4th floor), Lauriston Place, Edinburgh EH3 9HA, United Kingdom*

[b] *School of Chemistry, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, United Kingdom*

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ABSTRACT

Objectives: To quantify monomer elution from different adhesive systems using reverse-phase high-performance liquid chromatography (HPLC) and correlate this elution with the ratio of carbon–carbon double bonds from monomer to polymer (RDB) obtained using micro-Raman spectroscopy.

Methods: Thirty dentine discs were cut from 30 human, intact, third molars and randomly allocated to five groups according to the adhesive applied: total-etch, Excite (Ivoclar Vivadent), two-bottle self-etch, Clearfil SE (Kuraray), one-bottle self-etch, Clearfil 3S (Kuraray), ormocer-based, Admira (Voco) and Filtek Silorane adhesive system (FS) (3M ESPE). Monomer elution was studied 1 h, 6 h, 24 h, 96 h and 7 days after immersion in 75% ethanol/water. The RDB was calculated immediately after light-curing and thereafter at 24 h and 7 days. The data were statistically analysed using one-way ANOVA and Pearson's correlation coefficient ($p < 0.05$).

Results: More than 90% of the whole elution occurred during the first 1 h, except for BisGMA in FS, with the highest absolute amount from Clearfil SE and the highest wt% from Admira. Initial RDB was in the ascending order FS < Admira < Excite < Clearfil SE < Clearfil 3S. In all groups, the RDB was significantly higher after 24 h and 7 days than immediately after light-curing ($p < 0.05$). Negative correlation was found only for the elution of HEMA and the RDB of Clearfil 3S.

Conclusions: Different adhesive systems showed different monomer elution kinetics. In all systems, the RDB increased after monomer elution. Overall, no direct correlation exists between the RDB of adhesives and the elution of unreacted monomers.

1. Introduction

Dental adhesives contain resin monomers similar to those found in resin-based composites (RBCs) in order to obtain a covalent bond between the adhesive and the RBC. The cured resin in the adhesive system functions as a backbone providing structural continuity and physical properties, such as strength. Monomer conversion to polymer is an important determinant of the physico-mechanical strength of the resultant polymer.[1]

Conversion is seldom complete and is generally accepted to be low in both adhesives [2,3] and RBCs.[4,5] In the literature, the

* *Corresponding author*. Tel.: +44 7748572850; fax: +44 1315364971.
E-mail address: vesna.miletic@gmail.com (V. Miletic).

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ratio of double-bond content of monomer to polymer is commonly defined as the degree of conversion (DC) and is conventionally calculated as the ratio of C=C double bonds in cured and uncured materials related to an internal standard. In this paper, we consider that it is more accurate to use the term ''the ratio of double-bond content of monomer to polymer (RDB)''.

Low RDB results in higher permeability[2], more water sorption[6] and more leaching of residual uncured monomers. The biocompatibility of RBCs and adhesives has been studied and it has been shown that, after curing, residual monomers may elute into the oral environment.[7] Dimethacrylates, especially, have been shown to exert cytotoxic[8,9] and endocrine disruptive effects.[10,11]

In a clinical situation, unbound components of adhesives and RBCs may diffuse through dentine into the pulp or elute from the restoration into the oral cavity. Dental adhesives may be the prime issue of concern since they consist of monomers that are more hydrophilic and of lower molecular weight than monomers typically found in RBCs.

Several studies have used high-performance liquid chromatography (HPLC) to study elution of leachable monomers from RBCs[12–16], resin mixtures[17–20] and orthodontic adhesives.[21,22] No published data have been traced on monomer elution from dental adhesives applied to dental tissues and correlated with the RDB before and after elution.

A few studies on dental adhesives used HPLC to investigate the hydrolytic stability of self-etch adhesives[23] and the correlation between retention times and partition coefficient values.[24] Kaga et al. studied the cytotoxic effect of monomers eluted from dental adhesives into the cell culture medium on L929 cells in vitro.[25]

The aim of the present study was to quantify monomer elution from different adhesive systems using reverse-phase HPLC and correlate this elution with the RDB obtained using micro-Raman spectroscopy.

The null hypothesis is: (1) that adhesives with different chemistry do not have different monomer elution kinetics (the amount of eluted monomers per time) or a different RDB and (2) that there is no correlation between these two parameters.

2. Materials and methods

2.1. *Preparation of specimens*

Thirty intact human third molars of similar size, extracted for orthodontic reasons, were cleaned of organic debris and stored in 0.02% thymol. Informed consent was obtained from patients for the use of these teeth for research purposes. Ethical approval was granted by the Ethics Committee, Lothian NHS Board, Edinburgh, Scotland, to use such teeth in this study.

Each tooth was embedded in cold acrylic up to the cemento-enamel junction and sectioned perpendicular to the long axis in the mid-coronal zone using an Isomet saw (Buehler, Lake Bluff, IL, USA) to expose flat dentine. From each tooth, one, 1-mm-thick dentine disc was prepared. A smear layer was produced by grinding the flat surface with a 600-grit silicon-carbide disc (Buehler, Lake Bluff, IL, USA) under water for 30 s.

Adhesive systems used in this study are listed in Table 1. Two of the adhesives (Excite and Admira) require dentine to be conditioned with phosphoric acid prior to application. The other three are self-etch materials.

The dentine discs were randomly allocated to five groups with six discs per group: Group I, Excite; Group II, Admira; Group III, Clearfil SE; Group IV, Filtek Silorane and Group V, Clearfil 3S.

In Groups I and II, dentine was first conditioned by etching with 35% phosphoric acid for 15 s, rinsing with water for 10 s and blot-drying in accordance with the conventional wet bonding technique. Then, prior to applying adhesives, each disc was weighed (m_0) using a METTLER TOLEDO balance (AB104; d = 0.1 mg; Mettler-Toledo Inc, Columbus, OH, USA).

In Group I, Excite was applied to the dentine surface, gently agitated for 10 s and immediately dried by a dry stream of air for 3 s. In Group II, Admira was applied to the dentine surface, left for 30 s and gently air-dried.

In groups III, IV and V pre-conditioning was not required. Dentine discs were removed from storage, blot-dried and immediately weighed (m_0).

In Group III, Clearfil SE Primer was applied to the dentine surface and left in place for 20 s. It was then dried using a mild air-stream. Clearfil SE Bond was then applied to the dentine surface and air-dried with a mild stream. In group IV, Filtek

Table 1 – Adhesive systems used in the present study.

Adhesive	Manufacturer	Type	Composition
Excite	Ivoclar Vivadent AG	1-bottle, total-etch	Phosphonic acid acrylate, HEMA, BisGMA, dimethacrylate, silica, ethanol, catalysts, stabilizers
Admira	Voco GmbH	1-bottle, total-etch	HEMA, HPMA, BisGMA, ormocers, acetone, catalysts, additives
Clearfil SE	Kuraray Europe GmbH	2-bottle, self-etch	Primer: MDP, HEMA, dimethacrylate hydrophilic, camphorquinone, N,N-diethanol p-toluidine, water. Adhesive: MDP, BisGMA, HEMA, dimethacrylate hydrophobic, camphorquinone, N,N-diethanol p-toludine, silica
Filtek Silorane	3M ESPE	2-bottle, self-etch	Primer: phosphorylated methacrylates, Vitrebond copolymer, bisGMA, HEMA, water, ethanol, silane-treated silica filler, initiators, stabilizers. Bond: hydrophobic dimethacrylate, phosphorylated methacrylates, TEGDMA, silane-treated silica filler, initiators, stabilizers
Clearfil 3S	Kuraray Europe GmbH	1-bottle, self-etch	Methacryloyoxydecyl dihydrogen phosphate (MDP), BisGMA, HEMA, initiator, stabilizer, ethanol, water, filler

Ivoclar Vivadent AG, Schaan, Liechtenstein, Kuraray Europe GmbH, Frankfurt/Main, Germany, Voco GmbH, Cuxhaven, Germany, 3M Espe, St. Paul, MN, USA.