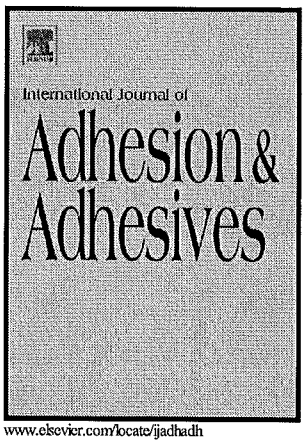

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Angelina Shuhua Wang, Michael G. Botelho, James K.H. Tsoi, Jukka P. Matinlinna

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Effects of silver diammine fluoride on microtensile bond strength of GIC to dentine

Angelina Shuhua Wang[1,3], Michael G. Botelho[2*], James K. H. Tsoi[1], Jukka P. Matinlinna[1]

[1]Dental Materials Science, Faculty of Dentistry, University of Hong Kong, P.R. China
[2]Oral Rehabilitation, Faculty of Dentistry, University of Hong Kong, P.R. China
[3]School of Stomatology, Zhejiang Chinese Medical University, Hangzhou, P.R. China
*Corresponding author: Michael G. Botelho, Oral Rehabilitation, Faculty of Dentistry, University of Hong Kong, 4/F, Prince Philip Dental Hospital, 34 Hospital Road, Sai Ying Pun, Hong Kong, P.R. China, Tel: (852) 28590412. Fax: (852) 28586114. Email: botelho@hku.hk

Abstract

Objectives

The objective of this study was to investigate the effect on the microtensile bond strength of a glass-ionomer cement (GIC), which is used for Atraumatic Restorative Treatment (ART), to normal and artificial carious dentine surface that had been treated with silver diammine fluoride (SDF) and without treatment.

Methods

Twenty-four recent-extracted human molars were sectioned by low-speed blade to expose the dentine surface. Each four surfaces of them were treated by one of the following procedures: a) immersing in artificial demineralized solution for three days; b) demineralization, followed by application of SDF drops, then washing off the precipitate and air drying; c) demineralization, followed by application of SDF drops, light-cured for 60 seconds, then washing off and air drying; d) DI water storage for

three days; e) DI water storage, followed by application of SDF drops, then washing off the precipitate and air drying; and f) DI water storage, followed by application of SDF drops, light-cured for 60 seconds, then washing off and air drying. Then, GIC (Fuji IX, GC) was bonded onto samples and stored in the DI water for 24 hours and 7 days respectively. All the samples were sectioned perpendicularly into sticks with cross-sectioned area of ~1 mm^2. Microtensile bond strength was evaluated. SEM/EDX was performed at the interface.

Results

Generally, the SDF or SDF light-cured pretreatments have no significant influence on the bond strength between GIC and dentine. Among the 24 hours carious dentine groups, the SDF light-cured hold the significant highest bond strength value, compared with that of no treatment and SDF treated only. The bond strength value after 7 days DI water storage is significantly higher than that of 24 hours DI water storage. The artificial carious dentine hold a significant higher bond strength value, compared to that of normal dentine.

Conclusion:

This study concluded the ART technique combined with the application of SDF on the dentine surface does not influence the dentine bond strength.

Keywords: Silver diammine fluoride; Atraumatic restorative treatment; Microtensile bond strength; dentine bonding

Introduction

Since the 1970s, silver diammine fluoride (SDF) has been used as a therapeutic agent for arresting caries for deciduous teeth [1] or in permanent teeth in the elderly [2]. One of the key modes of action is thought to be due to antibacterial effect [1, 3, 4].

In the recent years, SDF has gained popularity due to its economic benefits and ease of use for children or the elderly in disadvantaged or communities with difficult access to dental care [5]. SDF or, $Ag[NH_3]_2F$ is, commercially available from 10 wt% to 38 wt% solution form, among which, the 38 wt% SDF has been shown to be clinically effective in arresting caries [6] and in inhibiting bacterial enzymes in *in vitro* reports [1, 7]. The caries arresting effects of SDF has been attributed to silver ions' ability of bacterial growth inhibition and collagen degradation prevention [1, 4], as well as fluoride ions' feasibility to remineralize carious tissue [8, 9].

Atraumatic Restorative Treatment (ART) approach, was developed in the 1980s, and involves the removal of carious tooth tissue using dental hand instruments and restoration of the cavity with a glass ionomer cement [10]. In a meta-analysis, ART has been shown to be effective as a restorative treatment, which can be used safely in single-surface cavities in both primary and permanent posterior teeth [11]. The survival rate of single-surface ART restorations in primary teeth and permanent teeth ranges from 80% to 93% over 2 to 5 years [12]. However, ART is not recommended

to be routinely used in multiple-surface cavities due to a high percentage of secondary caries [12]. This is supported in another study that the observed failures of ART restorations to be attributed to the remaining minute amount of soft caries after hand excavation, *i.e.* an operative factor, that might lead to mechanical failure of ART fillings and bonding failure at the dentine-GIC interface [13]. The adhesive ability of carious dentine to GIC was decreased in one study [14] compared to sound dentine, which may accelerate the failure of restorations, while others have reported no significant difference when dentine was demineralized [15, 16]. ~~The experimental conditions, as well as the teeth used (*e.g.* Chinese *vs* Caucasian), might raise the variety.~~

In response to failure from secondary caries, an application of 10% AgF on the surface of dentine was suggested to guard against recurrent caries in ART restorations [17]. The *in vitro* study tested the concentration of released fluoride from GIC discs in solution at varying pH. Unsurprisingly, more fluoride was released, however this would presumably not occur at the GIC dentine surface where secondary caries may occur. Thus, it is suggested an application of AgF on the surface of dentine before restoration to prevent secondary caries. However, a reduced shear bond strength between GIC with sound dentine has been reported [18].

In an attempt to prevent tooth surfaces from staining black after silver application, KI was applied after AgF so as to react with free silver ions to produce silver iodide, AgI,

which is a creamy white reaction product, but the influence of KI on bond strength was not clearly discussed in this study [18]. A very recent study by Hamama et al. [19] using SDF together with the KI also ~~got~~ observed the creamy white AgI, but the study ~~was~~ focused on reducing the amount of residual bacteria in dentinal tubules. Some studies [6, 20] have applied SDF with reducing agents such as stannous fluoride and tannic acid to accelerate the deposition of silver on the tooth substrate. ~~arrested~~ Increased surfaces of caries were reported ~~increased~~ compared to those without any treatment or treated with AgF. However, the use of such chemical solutions may influence the adhesive interface between dentine and GIC. ~~Thus~~, In addition, ~~additional~~ light illumination, which has been found to accelerate the ionic change of silver to metallic silver or silver oxide to change black, producing a slightly rough surface ~~was added as~~ is an additional factor in this study. to explore

The aim of this study is to investigate the bond strength of GIC to sound and demineralized dentine with and without SDF application.

Materials and methods

Specimen preparation

Twenty-four caries free human molar teeth were selected. Institutional Review Board approval (HKW-2013-0371) was obtained for this *in vitro* study. The teeth were prepared by first removing the occlusal enamel and root of the tooth using a

low-speed saw with a diamond blade (ISOMET 1000, Buehler, Lake Bluff, IL, USA) under running deionized water. To ensure it was free of enamel, the dentine surface was then examined under a 40× magnification light microscope, and any residual enamel was removed completely by trimming. The coronal dentine surfaces of the specimen was polished with 220-grit silicon carbide paper on a manual polisher (Lunn Major, Struers, Denmark) under running water for 60 s in order to create a uniform surface.

The schematic flow on sample preparation of this study is shown in Fig. 1, and test groups are shown in Table 1. In brief, a layer of nail varnish (Estte Lauder, France) was applied on the non-coronal dentine surfaces of all the samples to protect from demineralization. Then, 12 teeth (*i.e.* sound dentine group) (Table 1) were stored in deionized water at 37°C for 3 days and orbitally stirred (80 rpm). Another 12 teeth (*i.e.* demineralized dentine group) were immersed in a 40 ml of 2.2 mM each of KH_2PO_4 and $CaCl_2$ (pH 4.5) at 37 °C for 3 days with orbital stirring (80 rpm), after which they were washed with deionized water for 5 min [21]. Each of the above test groups was then subjected to three surface treatments.

Two teeth from each group for sound dentine (groups S-SDF and S-SDF-LC) and demineralized dentine (groups D-SDF and D-SDF-LC) were randomly selected, and 2.0 ml 38 w/v % solution of silver diammine fluoride (SDF, Saforide, Morita Corporation, Osaka, Japan) was applied on the dentine surface for 2 minutes based on

manufacturer guidelines. Groups S-SDF and D-SDF were left dried in the dark environment (wrapped by aluminium foil). Groups S-SDF-LC and D-SDF-LC, were exposed under an 60-second light-illuminated with a halogen light-curing unit (Elipar XL 2500, 3M ESPE, Paul, MN, USA). The intensity of the light was tested to be ~1000 mW/cm^2 by a light radiometer (800-2000, Pujing, Taiwan). For the remaining groups S and D, 2.0 ml of DI water was applied on the dentine surface for 2 min as control. After rinsing all the samples with distilled water for 10 s, each of them was air-dried prior to GIC application.

A stainless steel matrix band was fixed around each tooth to support a GIC "core" of 4 mm height. A hand-mixed conventional glass ionomer cement Fuji IX (Fuji IX, GC Corporation, Tokyo, Japan) was placed on the dentine surface. The GIC was compressed with a mylar strip to ensure good adaption. After 2 mins setting, the band was removed and the GIC-dentine specimens were stored in DI water for either 24 hrs (subscript a) or 7 days (subscript b) before bond strength test.

Microtensile bond strength test

Immediately prior to microtensile bond strength (μTBS) testing, the GIC-tooth specimens were sectioned individually and perpendicular to the bonding interface (Figure 1b) with the same low speed diamond saw (ISOMET 1000, Buehler Ltd., Lake Bluff, IL, USA) under running water [22]. For each specimen, beams were sectioned with ~0.9 × 0.9mm (*i.e.* cross-sectional area of ~0.8 mm^2) and a length of

~9.0 mm. The width and thickness of each beam were measured and recorded before the bond strength test with a vernier caliper (Mitutoyo, Japan) to determine the surface area. Twenty beams were sectioned for each group.

When conducting the bond strength testing, the samples were secured to the jig of a universal testing machine (Model 4444, Instron, USA) with a cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA, USA), and tested to failure with a 100 N load cell at a crosshead speed of 1mm/min. The microtensile bond strength (MPa) was determined by dividing the peak break value of load (N) by the sample bonding area (mm^2).

Failure Mode Analysis

The failure mode was initially examined with a light microscope under 40× magnification. Failures were classified: the cohesive failure within the GIC material; the adhesive failure between dentine and GIC; the mixed failure (combination of cohesive failure and adhesive failure). When the fractured dentine surface was observed under the microscope, the cohesive failure referred to fractures in the GIC with no dentine surface exposed. For adhesive failure, the fracture occurred in the adhesive layer with no GIC left on the dentine surface. The mixed failure fractures were mostly in the GIC part with some materials remained in the fractured dentine surface.

SEM and EDX

The selected fractured specimens in each group were prepared for examination in a Scanning Electron Microscopy (SEM) (Hitachi S-3400N VP-SEM, Hitachi High Technologies Europe GmbH, Krefeld Germany), which is equipped with Electron Dispersive X-ray Spectroscopy (EDX) module (INCAx- sight EDS Detectors with INCA Energy Software; Oxford Instruments-, Oxforshire, UK). After elemental information analysis by EDX, the specimens were gold sputter-coated for 100 s and examined under SEM again in order to get micrographs with higher magnification.

Statistical analysis

A statistical software (SPSS Version 22, IBM Corp., Armonk, NY, USA) was used to compare the microtensile bond strength results with statistical significance set at 0.05 level. First, for each group, the data were analyzed with Kolmogorov-Smirnov and Shapiro-Wilk tests for normality. Then, with the aim of general data comparison, a three-way ANOVA analysis was performed with dependent variables as follows: time interval (24 hours and 7 days), SDF treatments (without SDF, with SDF and SDF with light illumination) and dentine condition (demineralized and non-demineralized). Second, the microtensile bond strength on various treatment methods were further tested by a one-way ANOVA with Tukey HSD post hoc test.

Results

Microtensile bond strength test

First, Kolmogorov-Smirnov and Shapiro–Wilk tests revealed that all the groups had P>0.05. The mean microtensile bond strength results are listed in Table 2 and depicted in Fig. 2. Highest microtensile bond strength (6.69 ± 2.72 MPa) was observed for the group with demineralized dentine with SDF and light curing stored for 7 days (group D-SDF-LC_b), and lowest (3.84 ± 1.47 MPa) for demineralized dentine stored in 24 hrs (group D_a).

Three-way ANOVA (Table 3) revealed a significant influence on the state of dentine substrate (P=0.022), time interval to testing (P<0.001) and SDF Treatment with time (P=0.005). In particular, the bond strength of sound dentine groups were the same, demineralized dentine groups have a higher bond strength than sound dentine groups despite the storage time and different treatments with the exception of GIC groups at 24 hours (i.e. group D_a). For time interval, generally a significantly higher bond strength was observed at 7 days than 24 hours.

With regards over

~~Regarding~~ to SDF treatment with time, a significant difference was found in demineralized dentine at 24 hours (i.e. Groups D_a, D-SDF_a, D-SDF-LC_a) ~~that~~ the demineralized dentine with SDF and light illumination (*i.e.* Group D-SDF-LC_a) showed ~~a~~ the highest average microtensile bond strength (6.25 ± 2.60 MPa) among the three groups,

and significantly higher than only demineralized dentine (Group D_a, 3.84 ± 1.47 MPa). Although after 7 days of water storage these microtensile bond strengths were raised, no statistically significant difference was observed between the three test groups. For sound dentine group, no significant difference was observed to all the microtensile bond strength mean values. We might conclude that light illumination only affect the bond strength for demineralized dentine at initial (24 hrs).

Failure mode analysis

The major failure modes of all specimens are shown in Table 2. 67% of the samples failed cohesively (Fig. 3(a)) within the GIC. 25% were mixed (Fig. 3(b)) and 7.9% of them showed adhesive failure (Fig. 3(c)). Additionally, the cohesive failure of the specimens tested showed a higher microtensile bond strength value, compared to those of the mixture failure and adhesive failure mode, but it is not statistically significant from each other (P>0.05).

SEM and EDX analysis

After three days demineralization, a demineralized pattern of dentine surface was observed under SEM (figure not shown here), showing the dentine tubules were open and clean.

Representative SEM images and EDX analysis for dentine surface after pretreatment with SDF and SDF-LC after 24 h are illustrated in Figs. 4 (a) and (b), respectively.

Fig. 4 (b) showed more intensive particles aggregation on dentine surface than SDF treated as illustrated in Fig. 4 (a), that showed sparsely dispersed particles only. Further analysis under EDX was performed and the element mapping illustrated that both of the samples contained mainly Ag, P, O, C and Ca elements. EDX analysis also revealed from the representative samples that SDF together with light illumination (Group S-SDF-LC) showed a higher weight percentage of silver (15.39%) than the sample with SDF alone (Group S-SDF) which has silver percentage (11.95%).

The micro structure of these silver compounds' crystals on the treated dentine substrates with of SDF was observed under SEM higher magnification and showed in Fig 5. Interestingly, there were three main molecule appearances: dendrite (Fig. 5(a)), nodular (Fig. 5(b)) and amorphous (Fig. 5(c)). ~~They are used to describe on the dentine surface~~. In order to confirm the chemical composition of these particles, EDX analysis (Fig. 5) was performed ~~on each of them~~ individually. However, it only showed high silver and oxygen elements with low phosphorous and calcium contained in the three crystals.

Discussion

In this study, the application of SDF on sound dentine with GIC produced ~~a~~ no significant difference in the bond strength at 24 hours, which is comparable to Quock *et al.* [23] ~~that is similar to~~ SDF application with resin composite. In another

study by Soeno *et al.*, lower tensile bond strength has been found between SDF treated sound bovine dentine surfaces to two resinous luting agents [24]. Soeno *et al.* [24] suggested that the reaction products CaF_2, $AgNO_3$, $AgPO_4$ which were found in SDF reacted with hydroxyapatite and protein [8] essentially limited the monomers' infiltration into dentine for the creation of a resin-infiltrated layer in bonding systems and thus decreased the bond strength value [24]. ~~despite the study used the bovine dentine which might not have the same dentine structure with the human dentine.~~ In the current finding, the silver components were found in both the fractured surface of GIC adhesive layer and the dentine surface according to SEM pictures (Figs. 3-5) and EDX analysis. These fluoride- or silver-containing chemicals was found to be harden the demineralized dentine [25], which is effectively increase the interfacial hardness and roughness at the GIC-dentine joint. Therefore, the resin or cement infiltration might not be crucial in such as SDF application on sound dentine. Further investigation will be beneficial in determining the crystals on the dentine surface with application of SDF under SEM observation after 24 h and 7 days respectively to explain the scientific rationale of bond strength.

Demineralized dentine has shown a lower bond strength, compared to sound dentine, which has also been reported in other studies [25, 26]. The soft demineralized dentine surface was advocated to this phenomena [27], despite no direct evidence has been shown to support this statement. Regarding to the duration of water storage, at

24 hours, a significantly higher bond strength was observed to sound dentine than demineralized dentine; whilst at 7 days, on the contrary, a higher bond strength was observed on demineralized dentine. This might be attributed to the continuous polymerization of the GIC, and the water molecules would prevent the dehydration of the cement [28]. Thus, some of the mechanical properties such as hardness and strengths are the same or increased incrementally after 7 days [29, 30]. Therefore, the mechanical interlocking between the dentine and GIC increased.

The rationale of GIC bonds to dentine was due to be the chemical reaction and mechanical bonding, which has been proved [28]. With conditioner set off demineralization reaction, exposed sufficient microporous collagen, enhanced the micromechanical interlocking and subsequently created infiltrations through hybridization [28]. In addition, the chemical reactions between polyacrylic acid (from GIC) and calcium ions (from GIC or hydroxyapatite) formed mainly the ionomer, calcium polycarboxylate, that is able to create a relatively stable and chemical chelation [28]. Furthermore, hydrogen bonds between various free radicals in collagen and carboxyl radicals in cement would contribute to the bond strength [28, 31]. Hence, with SDF application on the dentine surface, silver and silver oxide created, which possibly contributed to the improved bond strength between glass ionomer to metal in the present study [26].

Another interesting point to note that, the application of SDF on demineralized dentine produced a significantly higher bond strength than sound dentine at 24 hours which has not been reported before. In fact, SDF has the ability to increase the microhardness of demineralized dentine at 24 hours by silver deposits' inhibit dentine's soften progress and fluoride's remineralize soft dentine [25, 32], that means the micromechanical interlocking is improved and thus increased the bond strength. Therefore, applying the SDF might be able to arrest the caries and safely ~~to~~ use with GIC ~~despite the~~ SDF applications to sound or demineralized dentine does not significant affect the bond strength to GIC statistically after 7 days. Even so, the aim of using GIC after SDF application is not to increase the bond strength, but to ~~prove the~~ SDF does not affect the bond strength between dentine-GIC. As a result, the combination of the Atraumatic Restorative Treatment (ART) using a GIC with Arrest Caries Technique (ACT) by SDF ~~is~~ feasible.

The fractured dentine surface of a test beam with cohesive failure mode is shown in Fig. 3(a) under SEM. The dentine surface was completely covered with a thin layer of GIC, ~~measured~~ by EDX ~~with component of~~ high percentage Si suggesting ~~that~~ glass particle was in this GIC layer surface. No underlying dentine could be observed. Fig. 3(b) illustrated the fractured dentine side with mixed failure mode. The agglomerates of remnant GIC materials, which had a relatively smooth surface texture, could be distinguished from exposed dentine, which could be recognized by the presence of dentine tubules [33]. The remained GIC covered around 20% area of

matrix in SEM micrograph. EDX analysis showed the dentine surface contained Ca, P, Ag and some Si. A gradual increase of Si composition percentage can be tested under EDX from dentine surface to GIC layer as the fracture was mixed with the cohesive failure and adhesive failure. The adhesive failure mode of SEM micrograph illustrated that the glass particles' structure could not be found and the dentine surface was recognized by the presence of paralleled polishing grooves ~~in the SEM micrograph~~ Fig. 3(c). However, no dentine tubules could be observed from this SEM micrograph. Besides, the appearance of this adhesive layer was not uniform, the dark area illustrated metallic silver ~~stained~~, and this could be confirmed under EDX analysis which showed much higher silver element percentage compared to that of lighter area.

The darken effects of the SDF due to the silver components are well documented [4]. This study revealed that the dentine surface was further darkened, ~~as well as detected in~~ and EDX (Fig. 4), ~~under the light exposure, *i.e.*~~ much more metallic silver was converted ~~under the exposure than not exposure~~ [21]. Since much more silver was produced, the interfacial hardness would further increase between the GIC-dentine. The existence of various forms of particle silver aggregates (Fig. 5) might also help in the micromechanical retention between GIC and dentine, although the mechanism of the particle formation is still unknown ~~that might~~ need to investigate ~~in the future~~. Thus, high bond strength values in the groups of demineralized dentine with SDF light curing (Group D-SDF-LC) at both 24 hours and 7 days were observed. On the other hand, despite a lower bond strength value in the

groups of sound dentine with SDF light exposure (Group S-SDF-LC) was observed, the bond strength was not statistically different from the non-light exposure group.

While Soeno et al observed the SDF caused a lower bond strength of resin luting cement to sound dentine, the same was not observed in the current study using GIC.

Therefore, in clinical setting, applying SDF might be advantageous for GIC-dentine bonding. Even so, specific chemical reactions and interact products of SDF with GIC Fuji IX need to be confirmed in future study.

Conclusion

This *in vitro* study has illustrated that the demineralized dentine that has been treated with SDF light curing prior to the bonding, showed higher bond strengths at 24 h compared to the demineralized dentine samples with pretreatment of SDF or without any treatments. Additionally, the bond strengths in all groups generally increased within 7 days DI water storage, but the results showed no significant difference within subgroups. That means SDF does not significantly influence the bond strength between ART materials to dentine after 7 days. Thus, this study revealed SDF applied in the caries cavity prior to ART restorations was clinically safe. The SDF's effect on mechanical properties of ART materials and adhesive ability to GIC in clinic needs to be studied further.

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Figure legend:

Figure 1 The schematic of sample preparation

Figure 2 μTBS according to substrates, storage times and surface treatments between GIC to dentine

Figure 3 SEM micrograph of fractured beams with (a) cohesive failure, (b) mixed failure, and (c) adhesive failure

Figure 4 Representative SEM views and EDX analysis of dentine surface treated with (a) SDF and (b) SDF-LC after 24 hours

Figure 5 Representative SEM views and EDX analysis of various shapes of particle aggregation on dentine after 38% SDF treatment for 24 hours: (a) dendrite, (b) nodule, (c) amorphous

Tables

Table 1: Test groupings with respect to the substrates and durations (n = 20)

Substrates	Durations	
	24h	**7d**
Sound dentine	S_a	S_b
Sound dentine with SDF	S-SDF$_a$	S-SDF$_b$
Sound dentine with SDF and light curing	S-SDF-LC$_a$	S-SDF-LC$_b$
Demineralized dentine	D_a	D_b
Demineralized dentine with SDF	D-SDF$_a$	D-SDF$_b$
Demineralized dentine with SDF and light curing	D-SDF-LC$_a$	D-SDF-LC$_b$

Table 2: Microtensile Bond Strengths between GIC and Dentine. Groups identified by different superscripts were significantly different at $P < 0.05$ with Tukey HSD post hoc test.

Group	Microtensile bond strength (SD) (MPa)	Failure modes (n=20)		
		Adhesive	Mixture	Cohesive
S_a	4.00(1.68)$^{A, B}$	2	4	14
S-SDF$_a$	4.25(1.80)$^{A, B}$	3	6	11
S-SDF-LC$_a$	4.80(2.53)$^{A, B, C}$	1	1	18

D_a	$3.84(1.47)^{A}$	2	2	16
$D\text{-}SDF_a$	$4.74(1.41)^{A, B, C}$	2	5	13
$D\text{-}SDF\text{-}LC_a$	$6.25(2.60)^{B, C}$	1	6	13
S_b	$5.83(2.04)^{A, B, C}$	1	6	13
$S\text{-}SDF_b$	$5.19(1.77)^{A, B, C}$	3	7	10
$S\text{-}SDF\text{-}LC_b$	$4.53(2.34)^{A, B, C}$	2	7	11
D_b	$6.04(2.24)^{A, B, C}$	1	4	15
$D\text{-}SDF_b$	$6.21(3.28)^{B, C}$	0	5	15
$D\text{-}SDF\text{-}LC_b$	$6.69(2.72)^{C}$	1	7	12

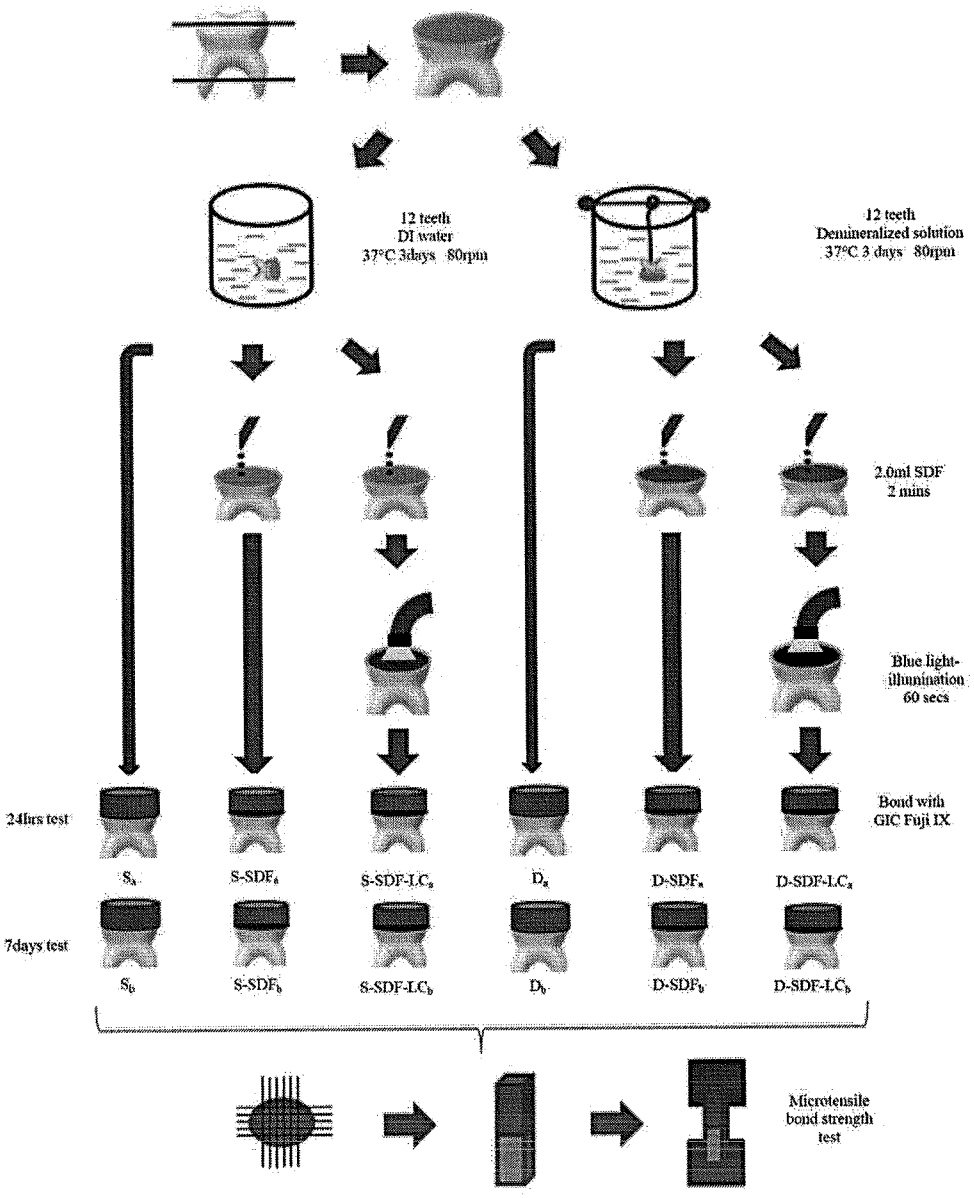
Table 3 Three-way ANOVA test results for the microtensile bond strength (the dependent variable) with respect to dentine condition (demineralized and non-demineralized), SDF treatments (without SDF, with SDF and SDF with light illumination) and time (24h and 7d).

Tests of Between-Subjects Effects

Dependent Variable: MTBS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	145.169[a]	11	13.197	3.718	.000
Intercept	5880.661	1	5880.661	1656.901	.000
Dentine Condition	18.959	1	18.959	5.342	.022
SDF Treatment	4.538	2	2.269	.639	.529
Time	50.455	1	50.455	14.216	.000
Dentine Condition * SDF Treatment	21.598	2	10.799	3.043	.050
Dentine Condition * Time	3.700	1	3.700	1.042	.308
SDF Treatment * Time	38.399	2	19.200	5.410	.005
Dentine Condition * SDF Treatment * Time	6.576	2	3.288	.926	.397
Error	787.921	222	3.549		
Total	6801.403	234			
Corrected Total	933.090	233			

a. R Squared = .156 (Adjusted R Squared = .114)



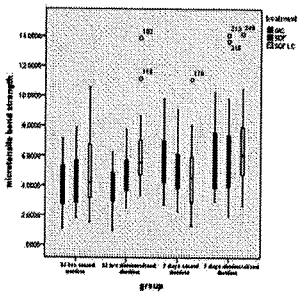


Fig 3

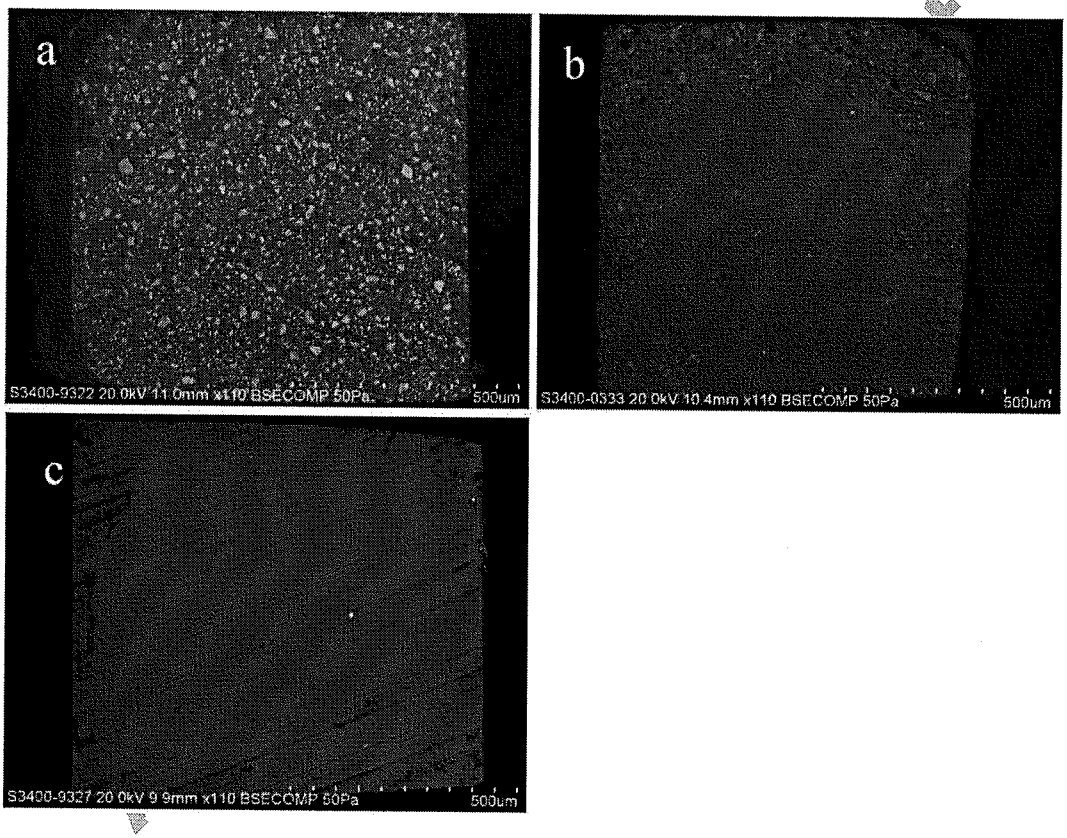
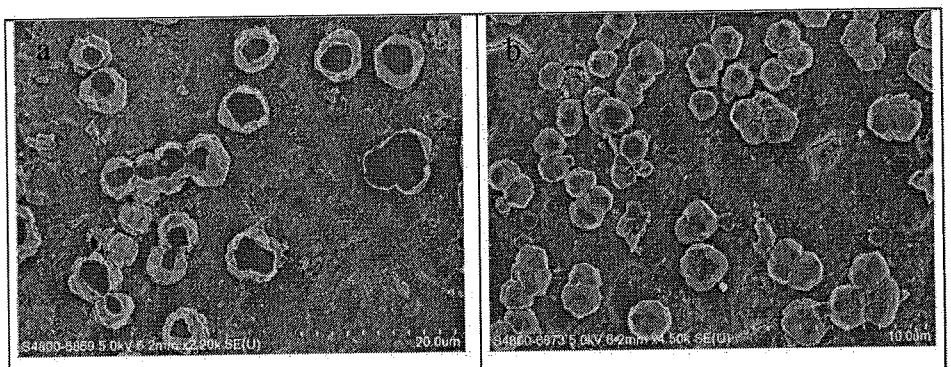
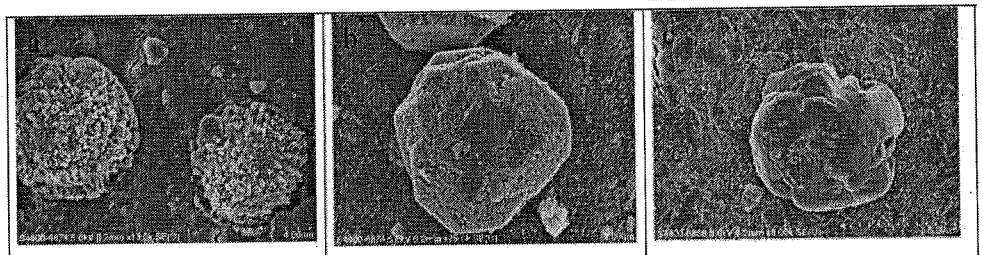


Fig 4.



Element	Weight %	Atomic %	Weight %	Atomic %
C	9.03	17.70	12.18	23.44
O	24.72	36.39	23.77	34.35
F	14.11	17.49	14.31	17.42
P	12.74	9.68	9.85	7.35
Ca	27.45	16.13	14.51	14.14
Ag	11.95	2.61	15.39	3.30

Fig 5



Element	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
C	5.81	17.16	6.04	17.31	5.33	16.04
O	21.67	48.02	22.88	49.21	20.47	46.26
F	1.99	3.72	2.01	3.64	2.36	4.49
P	7.09	8.12	7.24	8.04	7.52	8.78
Ca	3.82	3.38	3.87	3.32	5.05	4.56
Ag	59.62	19.60	57.96	18.49	59.26	19.86