Effect of Remineralisation with Nano-hydroxyapatite (nHAp) and Non-collagenous Protein Analogues (PAA and STPP) on the Micro Tensile Bond Strength of the Resin Composites to Dentin – An In vitro Study

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Resin-dentin bonds are less durable than enamel as they rely on organic part for mineralisation. Bond denaturation mainly takes place because of hydrolysis of polymerized resin or degradation of water-rich, resin-sparse collagen by collagenolytic endogenous matrix metalloproteinase and cysteine cathepsins. Hence, this limited stability of dentin bonding tenaciously reduces the longevity of tooth-coloured restorations. One of the strategies adopted to encounter this shortcoming is to induce biomimetic remineralisation.

Aim: To assess the microtensile bond strength of resin composites to dentin following remineralisation using an experimental paste containing nano-hydroxyapatite (nHAp) and biomimetic analogues, Poly Acrylic Acid (PAA) and Sodium Tri Polyphosphate (STPP).

Methodology: Nine intact freshly extracted third molar were used for this study to evaluate the

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microtensile bond strength after treating with three different groups; Group I with paste containing nano hydroxyapatite nHAp Group II paste containing nHAp and non-collagenous protein analogues such as (Polyacrylic acid and Sodium tripolyphosphate) and the control groups containing adhesive and resin composite with no additional treatment. The samples were observed over a period of 24 hours and 1 week the inter group data analysis was done using ANOVA and paired sample t test and the intra-group data analysis over period of 24 hours the post HOC Bonferroni test was used for the statistical analysis.

**Results:** After 24 hours the group with nHAp group showed highest value for microtensile bond strength among other groups, however after one week the group with nHAp +NCP analogues(PAA+STPP) showed highest value for microtensile bond.

**Conclusion:** The treatment with the experimental paste [nHAp+NCP] analogues (PAA+STPP)] can bring about remineralisation to improve bonding to dentin.

**Keywords:** Microtensile bond strength; Nanohydroxy apatite; Poly Acrylic Acid (PAA) and Sodium Tri polyphosphate (STPP).

1. INTRODUCTION

The resin–dentin interface and its limited durability is often a clinical challenge [1]. The resin infiltration is hampered by the inability to thoroughly replace loose water from internal and external compartments of collagen fibrils [2]. This water-rich, resin-sparse collagen fibrils have mechanical properties far inferior to those of resin-infiltrated collagen or mineralized collagen [3].

Another drawback to bonding is the degradation of resin-sparse collagen fibrils that is associated with the activation of MMP’s and cysteine cathepsins due to the acid etching procedure which is responsible for collagenolytic and gelatinolytic activities within hybridised dentin [1]. These collagen related challenges are deleterious and the bond interface is susceptible to creep or cyclic fatigue rupture after prolonged function [4].

One of the recent attempts made to improve resin-dentin bonds is by a novel approach of ‘Biomimetic remineralisation’ which mimics the process of natural bio-mineralisation by replacing demineralised collagen matrix water with apatite crystallites. By replacing the water with minerals at the dentin-resin interface, the mechanical properties could be improved and water related hydrolysis obstacles could be tackled. Resin-sparse regions become remineralised after biomimetic remineralisation as the apatite crystallites penetrate into the intrafibrillar and interfibrillar water compartments of a collagen fibril [1].

In bone, enamel or dentin the crystal formation is determined by combination of collagen and a particular non-collagenous protein (NCP). Several studies have been done in the past to study the remineralising ability of different NCP analogues such as PAA and PVPA, to dentin. Polyaspartic acid (PSPA) and poly acrylic acid (PAA) have shown to have very high ability to chelate calcium ions due to rich carboxyl groups. In addition to phosphate containing biomimetic analogues, sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), sodium ascorbyl phosphate were employed as templates to bring about remineralisation. In a recent study it has been proved that STMP can adsorb on demineralised collagen matrices and form covalent bonds under high alkaline pH. The hydrolysed form of STPP shows better properties to bring about similar results [5].

In comparison to the fluoride technology, nano hydroxyapatite (nHAp) use promises a long lasting solution to remineralisation that is shown to penetrate deeper into the denuded collagen fibrils. Nano-hydroxyapatite (nHAp) is considered one of the most biocompatible and bioactive materials with nano-sized particles 20 nm in size, as well as the capacity to attract calcium from storage solution, thus leading to a remineralisation effect in deeper layers of dentin.

This study is thus aimed at evaluating use of the remineralising paste containing nHAp with biomimetic analogues PAA+STPP on the resin-dentin bond strength. The null hypothesis tested was that use of nano hydroxyapatite and biomimetic analogues had no effect on the microtensile bond strength of resin composites to dentin.
2. METHODOLOGY

2.1 Sample size Determination

A power analysis was established by G*power, version 3.0.1 (Franz Faul universitat, Kiel, Germany). A sample size of 90 subjects (30 in each group) would yield 80% power to detect significant differences, with effect size of 0.6 and significance level at 0.05.

2.2 Sample Preparation

Nine freshly extracted intact third molars were selected for this study. A flat dentin surface was prepared perpendicular to the longitudinal axis of each tooth using a slow-speed diamond saw (Minitom, Struers, Germany) under water-cooling. Each dentin specimen was acid etched for 15 seconds using 37% Phosphoric Acid Meta Etchant (Meta Biomed, Chalfont, Korea) washed for 20 seconds, gently air-dried for 5 seconds. The samples were divided as follows based on remineralizing treatment regimen: Group I- Pre-treatment with nHAp paste (n=10), Group II- Pre-treatment with a paste containing nHAp and NCP analogues PAA and STPP (n=10), Group III- Control group with no pre-treatment (n=10)

2.3 Experimental Remineralising Paste Preparation

10wt% nHAp, 3wt% PAA and 8 wt% STPP were also weighed and blended using mortar and pestle to form a homogenous mixture. This was then stored in an air-tight plastic container. The liquid used in this study was Simulated Body Fluid (SBF)

The powder and liquid were freshly mixed on a glass slab in a ratio of 3:1 with the help of steel spatula.

2.4 Experimental Protocol

The pastes were applied on the dentin surface using microtips for a period of 60 seconds, rinsed off and gently air-dried (Group II and III).

Following the application of the remineralising paste two coats of the bonding agent Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) were applied as per manufacturer’s instructions using a microtip brush, light-cured for 15 seconds using a LED unit, 3M ESPE Elipar 2500 Halogen Curing Light (3M Health Care Service Center, North Oakdale). Composite (Filtek Z350 XT, 3M-ESPE, St. Paul, USA) was built up in two 2mm-increments to achieve a 4mm thickness and light-cured for 40 seconds each.

Dentin stick preparation for microtensile bond strength testing: Resin-bonded specimens were then sectioned into resin-dentin sticks (1 mm x 1 mm x 6 mm) using Slow speed diamond saw (Minitome Strueres, Germany). The sticks from the most peripheral area presenting residual enamel were excluded. Sticks so obtained were stored in SBF prior to microtensile bond strength testing. Ten samples were subjected to micro UTM testing after 24 hours and rest of the samples were tested after one week of storage in SBF.

Microtensile bond strength testing: An aluminium jig was custom made for mounting the dentin stick on the micro UTM. The sticks were attached to the jig using cyanoacrylate adhesive and tested in a micro UTM with a load of 50N at a cross head speed of 1 mm/min. Cross-sectional area of each dentin stick after fracture was measured using a digital vernier calliper. The microtensile values were expressed in Mpa. Fracture pattern analysis was done to access the mode of failure of each fractured stick using a Stereomicroscope (Olympus SZ61, Tokyo Japan) at x100 magnification. The fractures were classified as adhesive (at the interface), cohesive in composite, cohesive in dentin or mixed.

The statistical analysis was done using statistical software namely SPSS version 20.0 was used to calculate descriptive data. Data of microtensile bond strength after 24 hours were analysed for their intragroup variability using POST HOC Bonferroni test. The microtensile values of the three groups were interpreted and compared using ANOVA and paired sample t test at p value = 0.05.

3. RESULTS AND DISCUSSION

3.1 Microtensile Bond Strength Testing: (after 24 hours)

It can be observed that the highest bond strength value for nHAp group (mean = 24.9) followed by NCP group which showed (mean = 23.5) and lastly the control group (23.4).

Distribution of the subjects based on fracture pattern under stereomicroscope after 24 hrs - The mode of fracture analysis showed no
difference in fracture pattern between groups of NCP and control. There was no significant difference that was observed comparing all the three groups. Slight change in frequency of adhesive fracture pattern was noticed in nHAp group after 24 hours.

### 3.2 Microtensile Bond Strength Testing (after One Week)

There was significant change in the bond strength values after one week of storage in SBF. The group of samples treated with NCP (mean=25.6) analogues showed highest value of microtensile bond strength value followed by nHAp (mean=24.9) and control group (mean=23.4). When comparing the value after 24 hours and one week using paired sample t-test the value in the NCP group were statistically significant.

**Distribution of the subjects based on fracture pattern under stereomicroscope after 1 week**

The mode of fracture analysis showed no statistically significant difference in fracture pattern between groups. Slight change in frequency of adhesive fracture pattern was noticed in NCP group after one week followed by nHAp and then the control group.

Comparison of the Groups after 24 Hrs and After 1 Week Using ANOVA

|                  | t value | p value |
|------------------|---------|---------|
| After 24 hrs     | 3.81    | 0.035*  |
| After 1 week     | 2.71    | 0.084   |

No differences were seen in the bond strength values on comparison after 24 hours and one week.

### 3.3 POST-HOC Bonferroni (after 24 hrs)

|                  | Mean Difference | P value |
|------------------|----------------|---------|
| nHAP NCP         | -1.400         | 0.087   |
| nHAP Control     | 0.00           | 1.000   |
| NCP Control      | 1.500          | 0.060   |

After 24 hours the bond strength values increase nHAp (p=0.038) group was statistically significant.

### 3.4 Comparison of the Groups after 24 Hrs and after 1 Week using Paired Sample t-test

**Table 3. Comparison of bond strength values after 24 hours and 1 week**

|         | t value | p value |
|---------|---------|---------|
| nHAP    | -2.16   | 0.059   |
| NCP     | 0.00    | 1.00    |
| Control | 0.00    | 1.00    |

No significant changes (all 3 groups) were observed after comparison of the values using paired sample t-test.

### 4. DISCUSSION

The callous mechanical and chemical environment of the oral cavity always challenges composite–dentin bonds. Clearly, there is an undeniable need to practise alternative methods to preserve resin–dentin bond integrity and extend the longevity of resin-based restorations. Resin–dentin bonds created are susceptible to degradation via water sorption, hydrolysis of resin ester linkages and activation of endogenous matrix metalloproteinases. An attempt was made in this study to use the concept of biomimetic remineralisation and evaluate its effect on the microtensile bond strength of composite resin to dentin.

Biomimetic remineralisation fundamentally involves a polymer–induced liquid precursor to craft nano-scale precipitation for mineralisation [4]. It is a strategy based on the use of polyanionic molecules to imitate the sequestering and templating functions of non-collagenous dentin proteins during the natural biomineralisation [6].

Nano-hydroxyapatite (n-HAp) is known to be one of the most biocompatible and bioactive materials. Nano-sized particles have similarity to the apatite crystals of tooth enamel/dentin. The nano-scale, hydroxyapatite (nHAp) show higher Ca$^{2+}$ ion release rates also shows superior functional properties due to uniform grain size. The remineralising ability of nHAp was proved in a study [7]. Another advantage of nHAp is its ability to induce mineralisation from within the teeth. Principal drawbacks as far as nHAp is concerned are lack of strength, brittleness, high degree of crystallinity and low solubility at neutral pH requiring an acidic pH to dissolve. A wide
range of ions, both cations and anions, that can be used to substitute into the structure of synthetic HAp may bring in alteration in the degree of crystallinity.

Fig. 1. Resin-dentin stick obtained

Fig. 2. Fractured resin-dentin stick on customised jig

Fig. 3. Adhesive fracture of resin-dentin stick

Fig. 4. Cohesive in dentin fracture seen in resin dentin stick
Fig. 5. Cohesive in resin fracture seen in resin dentin stick

Fig. 6. Microtensile values of the groups after 24 hours

Fig. 7. Fracture pattern analysis after 24 hours

Fig. 8. Microtensile bond strength values after 1 week
Polyvinylphosphonic acid, is used as a collagen-binding template to induce the nucleation and growth of apatite from the initial amorphous mineral phase [8]. Sodium trimetaphosphate (STMP, Na₃P₃O₉), which has been frequently employed as a chemical phosphorylating reagent in the food industry, has the potential for phosphorylating type I collagen [9]. Polycrylic acid can be used as an analogue for sequestering calcium ions as they function by acting as surfactants to prevent fluidic ACP nanoparticles from aggregating into larger particles, and to inhibit self-transformation of the ACP nanoparticles into apatite (i.e. apatite nucleation inhibitor) prior to their entry into the intrafibrillar water compartments of the collagen fibril. When only a templating analogue was used, large extrafibrillar mineral spheres were deposited around the collagen matrix [10]. Thus, two biomimetic analogues (sequestration and templating) must be utilized to reproduce the dimension and hierarchy of the apatite crystallites that are found in natural mineralized dentin.

Using polyacrylic acid and sodium tripolyphosphate as dual biomimetic analogues of matrix proteins, intrafibrillar apatite platelets were deposited in an ordered manner within collagen fibril [11].

Hence an experimental paste was formulated using nHAp and NCP analogues PAA and STPP (Group II) in an attempt to facilitate biomimetic remineralisation and in-turn to evaluate the effect of this paste on µTBS of composite-resin to dentin.

According to the results of the study mean µTBS values for Group II was 23.50 MPa at the end of 24 hours and 25.60 at the end of one week. There was an increase in bond strength following one week of storage. This increase was not statistically significant.

The resin-dentin bonded specimens in the control showed mean µTBS value of 23.4MPa after 24 hours which was not affected by one week of storage. This is in accordance with study by Giuseppe, C. et al. [12] in which the use of Ca/P-doped self-etching adhesives applied in combination with analogs of phosphoproteins provides durable resin-dentin bonds.

On comparison of the mean values obtained after 24 hours and 1 week the intergroup analysis by ANOVA showed significant changes in bond strength values in nHAp group after 24 hours. However, the Group II showed higher µTBS value post 1 week. This shows the remineralising potential of the experimental paste but the significant changes in µTBS value could not have been prominent because of the shorter duration of aging.

The results were in accordance with the study of [13] where an increase in bond strength was observed when samples were pretreated with nano-HA 0.15% [14]. Kim et al. [15] also observed an increase in bond strength in samples treated with metastable Ca-P solution as well as CPIC 50.

The etch and rinse and self-etch adhesives are incapable completely replacing water from the extrafibrillar and intrafibrillar collagen compartments with resin monomers leading to leakage and bond failure. Advantages of biomimetic remineralisation is that- 1)Free and loosely bound water in the dentin are replaced by apatite crystals ensuring physiologic dehydration.
mechanism maintaining the internal mineralised collagen fibrils relatively dry to preserve the integrity of entrapped bioactive molecules [2] MMPs and Cathepsins become fossilised within dentin by the apatite crystals thereby protecting the dentin collagen matrix from degradation [5]. This could further validate the effect of the experimental paste as compared in other studies, [13] Thereby Improving the mechanical properties of dentin

Considering these advantages incorporating biomimetic remineralisation step during bonding procedure could enhance the longevity of adhesively bonded restorations.

5. CONCLUSION

Within the limitation of this in-vitro study it can be concluded that remineralisation with nHAp and NCP analogues (PAA+STPP) had a positive influence on the bond strength in the limited time period of one week. Considering the advantages of biomimetic remineralisation, further studies on the characterisation and mechanical properties of remineralised dentin, the most optimum mode of delivery of these remineralising agents for clinical applications, needs to be researched before it could be considered to be incorporated as a step into all routine bonding procedures.

6. LIMITATIONS

- The aging of the specimens for longer duration of 1-3 months to facilitate biomimetic remineralisation could have enhanced the µTBS values
- Varying the weight percentage of the components of the experimental pastes could have been compared to assess the most effective formulation
- A paste formulation was used in this study. Most biomimetic remineralisation studies use a solution form of delivery

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.
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