International Journal of Dental Sciences and Research, 2015, Vol. 3, No. 4, 102-106
Available online at http://pubs.sciepub.com/ijdsr/3/4/5

DOI:10.12691/ijdsr-3-4-5

Effect of Addition of Novel Chlorhexidine Nanoparticles to a Type II GIC on Its Microshear Bond Strength to Dentin

Tanvi Satpute*, Sanjyot Mulay

Department of Conservative Dentistry and Endodontics, Dr. D.Y. Patil Dental College and Hospital, Pune, India
*Corresponding author: tanvisatpute@gmail.com

Received May 21, 2015; Revised July 21, 2015; Accepted August 07, 2015

Abstract Background: Glass Ionomer cements (GIC) are being used routinely in Restorative Dentistry. Adding Chlorhexidine (CHX) to GIC may enhance their antibacterial property, which may affect their bond to dentin. Aim: To evaluate the influence of incorporating chlorhexidine hexametaphosphate nanoparticles {CHX-HMP} to Type II GIC on the microshear bond strength to dentin at 24 hours and 7 days. Methodology: Cylindrical moulds, placed on flat dentin surfaces of human molar teeth were filled with Type II GIC containing nanoparticles of Chlorhexidine hexametaphosphate (CHX-HMP). Cylindrical molds filled with Type II GIC served as control. The samples were kept at 37 °C and 100% humidity for 24 hours and subjected to microshear testing. Microshear bond strength was determined using Universal Testing machine at 24 hours and 7 days. Results: Microshear bond strength in Conventional GIC increased from a mean of 2.19 Mpa at 24 hours to 3.11 Mpa on 7th day. Microshear bond strength of GIC with Chlorhexidine did not increase significantly ie. 3.28 Mpa at 24 hours to 3.35 Mpa at 7th day. There was a significant difference in microshear bond strength at 24 hours between GIC and GIC-CHXHMP. Conclusion: The addition of CHX in the concentration of 2% did not negatively influence the bond strength of Type II GIC to dentin.

Keywords: *GIC, Chlorhexidine, nanoparticles, microshear bond strength*

Cite This Article: Tanvi Satpute, and Sanjyot Mulay, "Effect of Addition of Novel Chlorhexidine Nanoparticles to a Type II GIC on Its Microshear Bond Strength to Dentin." *International Journal of Dental Sciences and Research*, vol. 3, no. 4 (2015): 102-106. doi: 10.12691/ijdsr-3-4-5.

1. Introduction

In modern dentistry, Glass ionomer cements (GICs) are a class of biomaterials having widespread application especially in Minimally intervention dentistry (MID) for the treatment of dental caries [1,2].

The therapeutic procedures used in the treatment of caries may not always eliminate all the microorganisms in the residual tissues. The persisting cariogenic bacteria, with the lack of hermetic seal, can cause recurrent caries, leading to failure of restoration [3].

Also changing concepts using Atraumatic Restorative technique involves removal of infected, disorganized dentin and preservation of the affected less organized dentin that has the potential to remineralize.

The use of GIC is extremely popular in dental practice, due to its properties of chemical bonding to mineralized tooth structures, coefficient of heat expansion similar to that of dentin, adequate biocompatibility and most importantly fluoride ion release, which contribute to the remineralization process [4,5].

GIC which offers an additional antimicrobial and antibiofilm efficacy would be of considerable clinical benefit. Such a material could protect the pulp from bacterial ingress by providing an antibacterial seal under other materials [6].

CHX is an appealing scientific option for the development of a dental cement which reduces the incidence of recurrent tooth decay; it is not surprising that there have been earlier attempts to incorporate CHX into GICs.

CHX diacetate added to a resin-modified GIC resulted in CHX release, only sustained at significant levels for one week [7] thus offering limited scope for lasting anti-caries effects. Incorporating CHX diacetate into a conventional (not resin-modified) GIC also yielded a CHX-releasing material, but again the CHX release sustained for only around a week with all except the highest substitutions, and these high substitutions resulted in a deterioration of the mechanical properties of the material [8].

Another study by Turkun LS et al describes a longer-term effect of incorporating CHX – as ground CHX diacetate powder or as CHX digluconate solution – into GICs.

It was shown that an antimicrobial effect persisted for between 40 and 90 days. The peak of efficacy was the first 24 h for all GIC specimens, suggesting that CHX may have been released during this initial period, and most specimens showed no antimicrobial behavior after 60–90

days. For some formulations, a limited deterioration in mechanical properties was observed [9].

CHX digluconate solution has also been incorporated into GICs in combination with another antimicrobial agent, cetrimide, and this too had an antimicrobial effect on oral bacteria persisting for up to 180 days [10].

A recent publication in 2014 describes surface functionalization of materials using Chlorhexidine-Hexametaphosphate (CHX-HMP) nanoparticles and it was found that they acted as slow release devices for soluble chlorhexidine (CHX) which is a potent antimicrobial agent in widespread use in medicine and dentistry [11].

Thus the aim of this study was to evaluate the influence of the addition of novel chlorhexidine nanoparticles to Type II GIC on its microshear bond strength to dentin.

2. Materials and Method

2.1. Selection and Preparation of Teeth

Fourty sound extracted human teeth were obtained from the Department of Oral surgery at Dr. D.Y Patil Dental college and Hospital. After the removal of tissue remainders, prophylaxis and washing, only teeth without anatomical and structural defects were selected. These were stored in saline. Teeth were randomly divided into two groups (n=20).Group I specimens were of Type II GIC (XtraCem, Medicept UK Ltd, United Kingdom) and Group II specimens were of GIC functionalized with chlorhexidine.

2.2. Test Specimen Fabrication

The teeth were sectioned in the transverse direction in the occlusal third of the crown with a diamond saw so as to produce a flat surface in dentin (Figure 1).

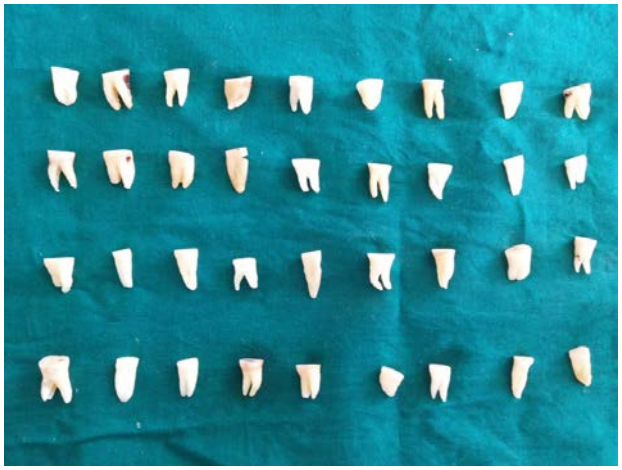


Figure 1. Samples with flat dentin surfaces

2.3. Preparation of Nanoparticles

Chlorhexidine digluconate (Dentochlor, Ammdent, Mohali, India) was mixed with Sodium Hexametaphosphate (Thomas Baker Chemicals Pvt. Ltd, Mumbai, India) in deionized water to obtain a final concentration of 4 mM CHX and 5 mM HMP. The resulting colloidal suspension of CHX-HMP nanoparticles was mixed thoroughly and then centrifuged at 10000 rpm for 60 min (Figure 2 and Figure 3).

Figure 2. Dentachlor (Ammdent, Mohali, India) And Sodium Hexametaphosphate (Thomas Baker Chemicals Pvt. Ltd, Mumbai, India)

Figure 3. High speed centrifuge (Remi Elektrotechnik Ltd,Vasai,India)

The supernatant was removed and discarded. The Nanoparticle sediment was dried in an incubator for 48 h at 40°C.

The sediment composed of nanoparticle aggregates was removed from the centrifuge tubes (Figure 4) and ground to a fine white powder using an agate mortar and pestle.

Figure 4. Centrifuge tubes with nanoparticle sediment

The nanoparticle powder created by grinding the nanoparticle pellet was used to substitute for the GIC powder at fraction of 2 % by mass.

2.4. Preparation of Samples

Square moulds of putty were made (Figure 5a). Cold cure acrylic was filled in the moulds and root portion of

teeth with flat dentin surfaces were embedded in the acrylic till the level of CEJ. (Figure 5b)

Cylindrical GIC specimens with dimensions of 5 mm diameter and 4 mm height were formed by holding mould on flat dentin surface, mixing the GIC according to the manufacturers' instructions and packing into plastic molds lubricated with petroleum jelly to aid removal. (Figure 5c and Figure 5d).

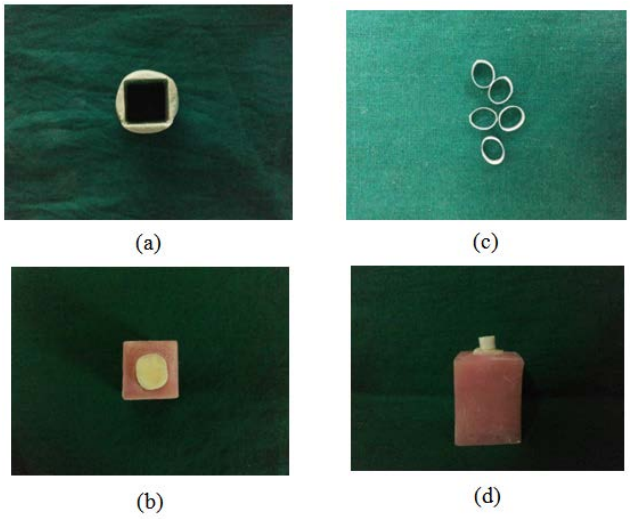


Figure 5. Preparation of Samples (a) putty mould (b) tooth embedded in putty mould (c) cylindrical moulds for material (d) test specimen

2.5. Testing

The mechanical microshear testing was performed in a Universal Testing Machine (Model no: STS 248, Star testing systems, India) at the speed of 3mm/min. In both the groups half the samples (n=10) were tested at 24 hours and the other half (n=10) were tested at 7th day. (Area of the sample =19.64square mm)

Statistical analysis was done by applying Student's Unpaired 't' test for comparison of microshear bond strength (MPa).

3. Results

The mean microshear bond strength values at 24 hours and 7 hours for Group I and II are presented in Table 1.

Table 1. Mean microshear bond strength of Group I and II samples at 24 hours and 7 days

	Mean microshear bond strength value(Mpa)	
	At 24 hours	At 7 days
GIC (Group I)	2.19	3.11
GIC CHXHMP (Group II)	3.28	3.35

Table 2 shows the comparison of microshear bond strength from 24 hours to 7 days done for Group I and II respectively. A percentage increase of 41.84% was observed for microshear bond strength in Group I (GIC). The percentage increase of 0.99% was observed for microshear bond strength in Group II (GIC-CHXHMP).

Table 3 gives comparison of microshear bond strength between Group I (GIC) and Group II (GIC-CHXHMP) at 24 hours and at 7 days.

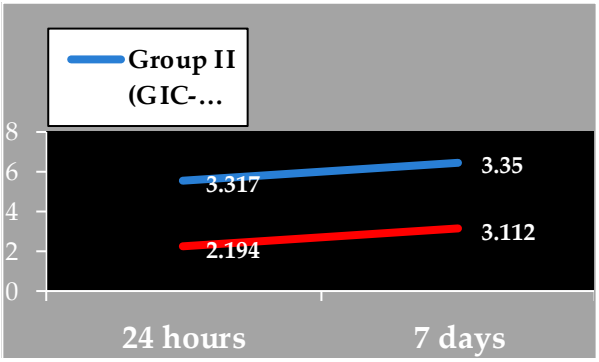
Table 2. Comparison of microshear bond strength from 24 hours to 7 days for Group I (GIC) and Group II (GIC- CHXHMP)

	Difference from 24 hours to 7 days			
	Difference Mean ± SD	Student's Paired 't' test value	'p' value	Result
Microshear Bond Strength (MPa) Group I (n=10)	0.91±1.36	2.12	p<0.05	Significant
Microshear Bond Strength (MPa) Group II (n=10)	0.03±5.57	0.018	0.39	Not Significant

Table 3. Comparison of microshear bond strength between Group I (GIC) and Group II (GIC-CHXHMP) from 24 hours to 7 days

	Group I (GIC)	Group II (GIC-CHX HMP)	Student's Unpaired 't' test value	'p' value	Result
	Mean ± SD	Mean ± SD			
24 hours	2.19±1.02	3.31±0.+97	2.51	p<0.05	Significant
7th day	3.11±1.13	3.35±1.07	0.48	0.31	Not significant

There was a significant difference between mean microshear bond strength at 24 hours (p<0.05) and it was not significant at 7th day when Group I (GIC) was compared with Group II (GIC-CHX HMP).



Graph 1. Graph depicting comparison of microshear bond strength between Group I (GIC) and Group II (GIC-CHXHMP) at 24 hours and at 7 days

4. Discussion

Bonding is a property by which two surfaces are attached by chemical or chemical-physical means. One of the important property of GIC is to bond to tooth structure without any pre-treatment of the substrate [12].

Previous studies have shown that the shear bond strength values of GICs to dentin are usually low, between 1 and 3 MPa and they rarely exceed 5 MPa.

The values tend to be higher in microtensile tests due to the differences in stress distribution and reduction in bonding area when compared with microshear tests [13,14].

Low values of bond strength may have occurred because the plastic matrix used for fabrication was removed with the aid of a scalpel blade, and the pressure exerted on the test specimens for cutting may have been transferred to the material, causing some level of stress at the bond interface.

Various chemicals like cetylpyridinium chloride, cetrimide and benzalkonium chloride have been added to GIC previously for added anti-bacterial action. [15] In this study, CHX was preferred because of following

advantages: 1) It has a broad spectrum anti- microbial efficacy with rapid onset of action. 2) It disrupts the bacterial cell membrane and results in the loss of intracellular components. Thus the evolution of bacterial resistance to CHX is also considered unlikely. 3) CHX is a cationic molecule exhibiting inhibitory effect on activity of dentinal proteolytic enzymes. 4) Its effectiveness due to the property of substantivity [16].

The mixing of solutions of CHX and HMP under the conditions described above results in the immediate formation of a colloid consisting of particles with maximum average diameter of 150 nm and an average zeta potential of −45 to −50 mV. The Atomic force microscopy (AFM) and SEM images showed individual nanoparticles and porous nanoparticle (NP) aggregates. Presumably owing the charge of the particles, it resulted in a good colloidal stability at least in part, to the high zeta potential. The charge is also thought to be the mechanism by which the NPs adhered to the material surfaces.

The different material specimens investigated in a study by Barbour et al (2013) were successfully functionalized with the CHX-HMP antimicrobial NPs, and all materials exhibited a gradual leaching of soluble CHX over atleast a period of 50 days [11].

Insoluble sodium metaphosphate was the form chlorhexidine preferred since it almost completely binds chlorhexidine in aqueous preparations, thus producing nanoparticles which would have sustained release [17].

For clinical success of any restorative technique, it is important that the modified material must present with adequate physical properties, and its property of bonding to tooth substrate must not be altered. The nanoparticle formulation prepared for this study was not at the expense of other properties [18].

There are no documented studies evaluating the bond strength of this novel preparation of GIC composed of Chlorhexidine nanoparticles.

Barbour et al (2014) first reported the development of these novel antimicrobial nanoparticles (NPs) based on a hexametaphosphate salt of CHX. They found that the NPs adhered rapidly to specimens of glass, titanium, and an elastomeric wound dressing, in a dose-dependent manner. The functionalized materials exhibited a gradual leaching of soluble CHX over a period of at least 50 days. The NP colloid was efficacious against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* in both planktonic and biofilm conditions [11].

Hook et al (2014) subjected the formulation to certain tests and characterized the nanoparticle size, morphology and charge and the release of chlorhexidine and fluoride, tensile strength and morphology of the GIC. They formulated the GIC containing novel antimicrobial nanoparticles composed of chlorhexidine hexametaphosphate at 1, 2, 5, 10 and 20% powder substitution by mass. They found that the GICs released chlorhexidine, against a wide range of oral bacteria, over the duration of the experiment in a dose-dependent manner. It was not at the expense of other properties. Fluoride release was not significantly affected by the substitution of antimicrobial nanoparticles in most formulations and internal structure appeared unaffected up to and including 10% substitution. Diametral tensile strength decreased numerically with substitutions of 10 and 20% nanoparticles but the difference was not statistically significant [18].

In this study the mean bond strength at 24 hours for Group I was 2.19 Mpa and for Group II was 3.28Mpa. The mean bond strength at 7th day increased both the groups. For Group I it was 3.11 Mpa and for Group II it was 3.35 Mpa.

Comparison of microshear bond strength was done between Group I (GIC) and Group II (GIC-CHXHMP) at 24 hours and at 7 days. It was seen that there was a significant difference between mean microshear bond strength at 24 hours ($p<0.05$) and it was not significant at 7th day when Group I (GIC) was compared with Group II (GIC-CHX HMP).

The mean bond strength of Group II (GIC-CHXHMP) at both intervals was greater than Group I (GIC). It appears that the addition of Chlorhexidine to GIC does not affect its bond strength. The formulation of chlorhexidine nanoparticles rather increased the bond strength to dentin.

The reason for higher bond strength with CHX nanoparticles incorporation could be due to:

1) The mechanism of bonding of GIC involve an ionic interaction between positively charged Calcium and/or Phosphate ions from the surface of the enamel or dentine and the negatively charged carboxylic groups of polyalkenoic acid. Zeta potential measurements indicated that the nanoparticles formed in the GIC CHXHMP formulation had a mean surface charge of −55 mV, indicating a net negative charge. [18] This may have led to ionic interaction between the nanoparticles and the positively charged calcium ions of tooth structure contributing to better adhesion.

2) Atomic force microscopy indicated that the individual nanoparticles were regularly shaped, globular and had typical diameters of 80–90 nm. These particles would have made the mix denser resulting in better adhesion. 3) Matrix Metalloproteinases (MMP's) are indicated as the active proteases that breakdown the collagen fibrils in the hybrid layer. There is evidence of the ability of Chlorhexidine to prevent bond deterioration by inhibiting MMP action. Therefore the incorporation of Chlorhexidine to GIC could have prevented the bond strength degradation [19].

Although low microshear bond strength values have been observed GIC modified by the addition of CHX, these results cannot yet be considered conclusive, and further studies are necessary, using other tests.

5. Conclusions

Within the limitations of this study the following conclusions were made:

1) Microshear bond strength in Conventional GIC increased significantly from 24 hours to 7 days.

2) There was a significant difference in microshear bond strength values at 24 hours between GIC and GIC with chlorhexidine nanoparticles.

3) Addition of CHX in the concentrations of 2% did not adversely influence the bond strength of Type II GIC to dentin.

Acknowledgements

I am grateful to Dr Khushbu Ajmera and Dr. Kunal Galankar for their valuable support.

Conflict of Interest

I report no conflicts of interest in this work.

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