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Original Article

Mechanical and antimicrobial property of different surface treated glass ionomer cements under desiccated condition

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ABSTRACT

Background: The purpose of this *in vitro* study was to evaluate the effect of five different surface treatments on the mechanical property and antimicrobial effect of three desiccated glass ionomer cements.

Materials and Methods: In this *in vitro* experimental study, 300 rectangular blocks of three different restorative materials were fabricated using an aluminum mold, Group I (n = 100) Micron bioactive, Group II (n = 100) GC Fuji IX GP Extra, and Group III (n = 100) bioglass R. These blocks were stored in 100% humidity for 24 h and then placed in air to desiccate for another 24 h. These groups were further divided into two major groups (n = 50) for both mechanical (Flexural) and antimicrobial testing. The blocks of mechanical and antimicrobial groups were further divided into five subgroups (n = 10) based on the medias used for surface treatment (senquelNaF, MI varnish, chlorhex plus, kedodent mouthwash, and 100% humidity [control]). Flexural strength (FS) was measured using the universal testing machine. Fracture strength of groups was compared using the one-way analysis of variance and Tukey's *post hoc* test with $P \leq 0.05$ considered statistically significant. Antimicrobial effect was carried out by covering the specimens in a suspension of *Streptococcus mutans* followed by incubation for 24 h. The blocks were later washed, vortex mixed, serially diluted, and plated. Ccolony-forming unit/ml was calculated after 3 days of incubation. Data were then analyzed using the Kruskal–Wallis and Mann–Whitney *U* nonparametric test, with $P \leq 0.05$ considered statistically significant.

Results: Micron bioactive with the surface treatment of MI varnish significantly exhibited highest FS. Surface treatment of desiccated restorative materials with chlorhex plus exhibited no growth of *S. mutans*. GC Fuji IX GP Extra with surface treatment of MI varnish exhibited highest reduction in *S. mutans* growth compared to other experimental group.

Conclusion: Surface treatment of restorative material with MI varnish improved their mechanical and antimicrobial property while among three restorative materials Micron bioactive showed better mechanical property, whereas GC Fuji IX GP Extra exhibited better antimicrobial property.

Key Words: Desiccation, flexural strength, glass-ionomer cements

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INTRODUCTION

Xerostomia is a condition of dry mouth due to reduced or complete loss of saliva. It has a deleterious effect on the patient's quality of life.[1] About 30% of 65 years and older patients present with this condition. Oral cancer is more common in patients with the age of 65 years or more. About 12% of the elderly population consume medication which may lead to this condition.[2,3] Since the prevalence of xerostomia is high, it poses a challenge to the clinician to select the right restorative material which would exhibit excellent mechanical and antimicrobial effect and prevent the occurrence of secondary caries.[4] Xerostomia affects patient's quality of life by causing oral discomfort, rampant caries, secondary caries, increased candidal infection, and desiccation of restorative material.[3] There is reduced flushing and buffering action of saliva in xerostomic patients as a result they harbor large amount of microbes and are considered as high caries risk patient.[3]

Selection of restorative material in such patients is challenge in itself as restorative materials are more likely to fail due to microleakage and shrinkage in desiccated condition. Clinician should be cautious to select a restorative material which is biologically, physically, and chemically suitable for xerostomic patient.[5,6] Fluoride-releasing materials are the material of choice in such conditions to prevent secondary caries as they inhibit demineralization and promote remineralization of tooth structure.[7-9]

Glass-ionomer cements (GICs) are fluoride releasing, tooth colored restorative material and are primarily the material of choice for xerostomic patients. Their unique potential is to absorb and leach recharge ions such as fluoride, calcium, and phosphates. These materials then act as a reservoir and inhibit demineralization and promote remineralization of tooth structure.[3] This ability of GIC depends on the composition, frequency of fluoride exposure, and the type and concentration of fluoridating agent which is being used.[4]

However, GICs have the limitation of moisture and desiccation sensitivity. Desiccation may lead to surface crazing and cause failure of restoration. These surface crazing can be rejuvenated by the incorporation of various surface treatment in the restorative material. Various *in vitro* studies have demonstrated that GICs when treated with different surface treatment agents such as Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), chlorhexidine (CHX), sodium fluoride (NaF), and cetylpyridinium chloride (CPC) reduce plaque formation, inhibits demineralization, promote mineralization, and prevent secondary caries. These agents enhance bioactive functioning and increases the survival rate of restorative material.[4]

The purpose of this study was to determine whether different surface-treating agents would offer antimicrobial property to restorative materials without deteriorating or perhaps even improve mechanical property of GIC. Effect of senquelNaF mouthwash, kedodent mouthwash, MI varnish, and chlorhex plus mouthwash on GIC has not been reported in earlier literature; hence, the present study should provide an evidence for the use of such surface treated GICs in high-caries risk conditions such as in xerostomic patients. Therefore, the vitro study was aimed to evaluate the effect of five different surface treatments on the mechanical property and antimicrobial effect of three desiccated GICs. The null hypotheses considered were as follows:

1. No difference in the mechanical property of three different resin-modified GICs
2. No difference in the caries preventive effect of three different resin-modified GICs.

MATERIALS AND METHODS

In this *in vitro* experimental study, three GICs: Micron bioactive (n = 100) (Prevestdenpro, Jammu), GC Fuji IX GP Extra (n = 100) (GC CO. Ltd. Tokyo), and bioglass R (n = 100) (Biodenāmica, Ibipora) were used [Table 1].

A total of 100 rectangular blocks of each restorative material were fabricated using an aluminum mold (25 mm × 2 mm × 2 mm) for testing mechanical (Flexural) and antimicrobial property. The molds were lubricated for the easy removal of blocks. Restorative material was filled inside the mold and to achieve a flat and parallel surface a Mylar strip was placed over it. The blocks were then removed from the mold and lightly gritted with the sand paper to remove the lubricant from the surface.

Rectangular blocks were then stored in 100% humidity at 37°C for 24 h, so that majority of acid base reaction could occur. After that they were kept at the room temperature for another 24 h for surface desiccation and to allow the formation of craze lines. One hundred blocks of each restorative material were

Table 1: Restorative materials used in this study

Material	Type	Manufacture	Filler	Liquid
Bioglass R	Resin-modified glass-ionomer cement	Biodenāmica, Ibipora	Calcium barium aluminum fluorosilicate and inorganic filler	Polyacrylic acid
GC Fuji IX GP Extra	Resin-modified glass-ionomer cement	GC Co. Ltd., Tokyo	Smartglass	Polyacrylic acid
Micron bioactive	Resin-modified glass ionomer cement	Prevestdenpro, Jammu	Fluoro alumino silicate glass powder and hydroxyapatite powder	Polyacrylic acid

divided into two major groups (n = 50) for mechanical and antimicrobial testing. These blocks were further divided into five subgroups (four experimental and one control group) depending on different surface treatment [Figure 1]. After desiccation, 10 blocks of each restorative material were dipped in 4 ml of five different medias (senquelNaF mouthwash, kedodent mouthwash, MI varnish, chlorhex plus and 100% humidity (control) at 37°C for 1 week [Table 2].

Mechanical property evaluated was flexural strength (FS). Each restorative block was then tested using the universal testing machine (Dutt. ×100, India) [Figure 2] at the crosshead speed of 0.25 mm/min. The blocks were placed in the 3-point bending test with span length of 24 mm supported by two supporting rods and central load was applied by 2 mm diameter round knob.

FS was then calculated using the expression $FS = 3Fl/2bd^2$, where F is loading force at fracture point, l is span length, b is width, and d is depth. Mean FS and standard deviation were calculated for each restorative material dipped with different surface treatments. Data were then analyzed using the Statistical Package for the Social Sciences (SPSS, IBM version 20.0, India) by applying the one-way analysis of variance (ANOVA) and Tukey's *post hoc*. The level of significance was set at 5%, and $P \leq 0.05$ was considered statistically significant.

For antimicrobial testing, the number and type of groups prepared were same as that of mechanical testing. Streptococcus mutan bacteria were isolated and were cultured on blood agar plate, incubated at 35°C ± 2°C in an aerobic environment for 24 h. Then, an inoculation suspension was made by harvesting the organism from blood agar plate and suspending it into saliva to turbidity equal to 0.5 Mcfarland (approximately 1.5×10^8 colony-forming unit (CFU/ml). Then, 1:1000 dilution of bacterial suspension was made using brain–heart infusion broth. Each restorative block was placed in 2 ml of bacterial suspension with the sterile forcep into

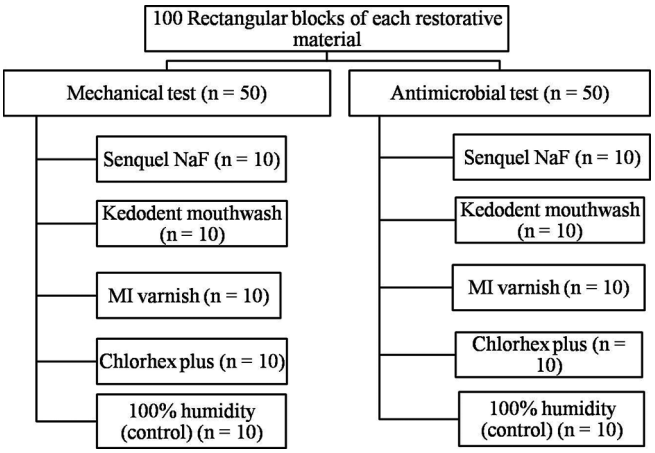


Figure 1: Flow chart of study design.

a test tube and incubated for 24 h at 35°C ± 2°C in an aerobic environment. After that blocks were removed and washed with 2 ml of sterile water for 2 times. They were then placed in 2 ml of saline using sterile forcep into a test tube and votex mixed for 2 min. Saline solution was then plated on a blood agar plate and incubated for 3 days at 35°C ± 2°C in an aerobic environment. Later CFU/ml was calculated. Mean and standard deviation were determined. These data were analyzed with the Kruskal–Wallis and Mann–Whitney U nonparametric test using (SPSS, IBM version 20.0). The level of significance was set at 5%, and $P \leq 0.05$ was considered statistically significant. In the calculation of statistical significance of different surface treatments, the extreme values of controls were not taken into consideration.

RESULTS

A statistically significant difference was observed in the mechanical testing as well as in the antimicrobial testing ($P < 0.001$) when surface treated with five different medias. The mean and standard deviation of FS of three restorative materials were calculated, and the data were analyzed using the one-way ANOVA, and significance among the restorative materials was calculated using Tukey's *post hoc* [Table 3].

Micron bioactive showed significantly higher increase in FS ($P < 0.001$) followed by GC Fuji IX GP Extra and Bioglass R ($P < 0.001$). On the basis of surface treatment with different medias, FS significantly increased in MI varnish ($P < 0.001$) [Graph 1].

To ascertain the effect of different surface treatment on antibacterial efficacy of GICs, a positive control (chlorhex plus) and negative control (100% Humidity) was used. While the negative control using 100% humidity showed affluent growth of *S. mutans* (>100,000 CFU/ml) on selective media, the positive control chlorhex plus was seen with no growth.

Statistical significant difference was found between three different restorative materials ($P < 0.001$) when surface treated with five medias. The mean and standard deviation of CFU/ml of three restorative materials were calculated. Due to nonnormal distribution and large variability, the data were analyzed using the Kruskal–Wallis test and Mann–Whitney *U*-test [Table 4]. MI varnish exhibited significantly lower CFU/ml ($P < 0.001$) followed by kedodent and senquelNaF ($P < 0.001$) [Graph 2].

Table 2: Surface treatment medias

Media	Content
SenquelNaF Mouthwash (Dr. Reddy's, India)	0.02% sodium fluoride 3% potassium nitrate
Kedodent Mouthwash (Indoco PVT Ltd., India)	0.05% sodium fluoride 5% xylitol 0.03% tricolsan
MI Varnish (GC, America)	5% sodium fluoride With 2% recaldent (CPP-ACP)
Chlorhex plus Mouthwash (Dr. Reddy's, India)	0.2% chlorhexidine Gluconate 0.09% zinc chloride

CPP: Casein phosphopeptide; ACP: Amorphous calcium phosphate

DISCUSSION

On dehydration as in xerostomic condition GICs show some limitation such as microleakage which leads to failure of dentin interface and in turn causes secondary caries around the restoration.[10,11] In xerostomic patients, buffering and flushing capacity of saliva is lost and causes reduced pH of the mouth which ultimately leads to decreased mechanical and antimicrobial property of GICs.[4] GICs upon rehydration show the property of self-repair.[11] They exhibit recharging ability as they can take up the fluoride ion from the environment and replace the fluoride which has been lost.[10]

Various studies have stated that, in xerostomic patient, topical fluoride can reduce the incidence of recurrent caries.[2,3,12,13] Simmons *et al.* used five different surface medias (CPP-ACP, CHX, NaF, and CPC) for the treatment of desiccated conventional and resin-modified GIC and found that 5% NaF provided greatest improvement in both mechanical and antimicrobial property of respective restorative materials.[4] The two objectives of this study were to assess the ability of different medias to reduce the incidence of secondary caries around restoration in simulated xerostomic conditions and also to assess whether the surface treatments affected the mechanical property of restorative materials.

In the present study, among all experimental groups, Micron bioactive (Prevestdenpro, Jammu, India) exhibited highest FS when surface treated

Table 3: Comparison of mean and standard deviation of flexural strength of three restorative materials with different surface treatments one-way analysis of variance with *post hoc* analysis (MPa)

Group	Mean±SD				
	SenquelNaF	Kedodent	MI Varnish	Chlorhexidine	Control
GC fuji IX GP Extra	25.31±0.45	26.45±0.12	27.74±0.29	21.37±0.36	21.35±0.36
Micron bioactive	27.54±0.40	28.55±0.33	30.79±0.61	25.18±0.46	25.20±0.48
Bioglass R	16.55±0.27	17.52±0.33	18.66±0.26	13.60±0.22	13.61±0.22
One-way ANOVA					
F	2325.332	4451.014	2279.490	2699.248	2549.351
Significant (*P*)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)
***Post hoc* analysis comparison of flexural strength among experimental restorative material**					
GC_IX versus Micron (*P*)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)
GC_IX versus Bioglass (*P*)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)
Micron versus Bioglass (*P*)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)

HS: Highly significant (*P*<0.001); S: Significant (*P*<0.01); MPa: Megapascals. ANOVA: Analysis of variance; SD: Standard deviation

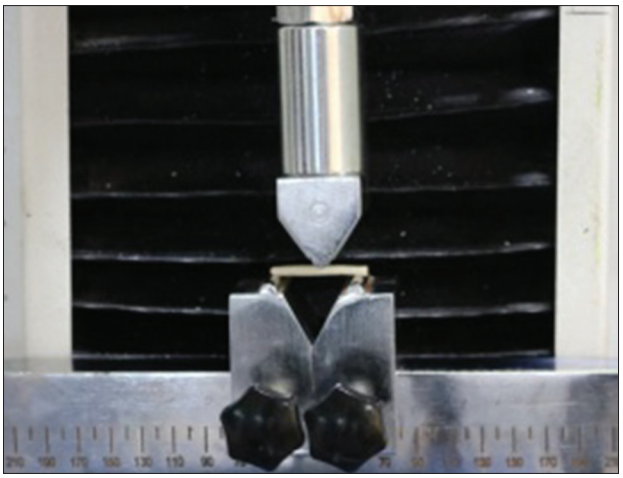
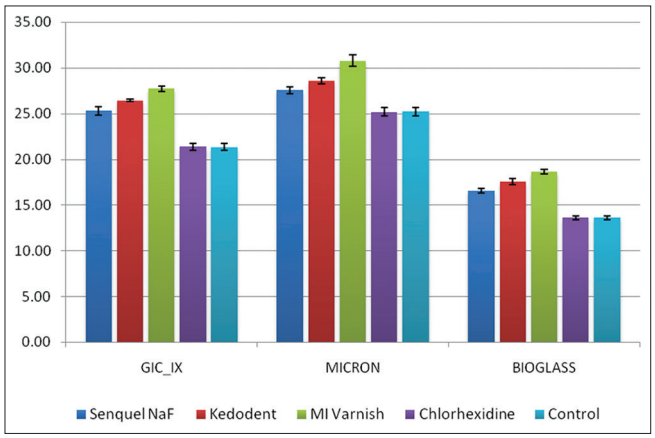
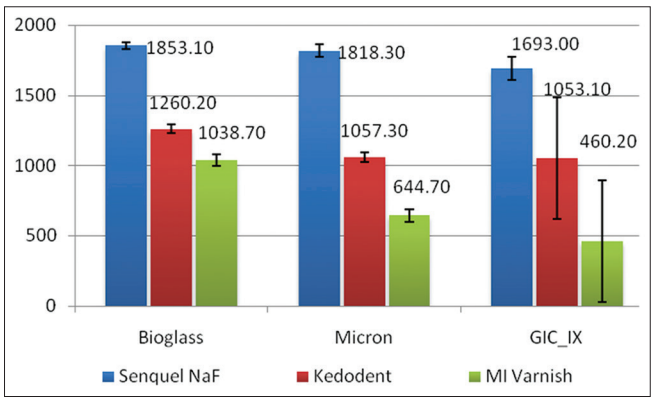


Figure 2: Determining the flexural strength of restorative material using universal testing machine.



Graph 1: Flexural strength (MPa) of restorative materials with different surface treatments.



Graph 2: Microbial growth (CFU/ml) of restorative materials with different surface treatments.

with MI Varnish followed by GC Fuji IX GP Extra and Bioglass R, respectively. Micron bioactive (Prevestdenpro, Jammu, India) is GIC incorporated with hydroxyapatite crystals. Moshaverinia *et al*. stated that hydroxyapatite powder on mixing with acidic solution that is polyacid liquid causes higher degree of acid–base reaction as a result of which calcium ion is extracted from the surface of hydroxyapetite. Hydroxyapatite reacts with inorganic/organic component of GICs through these phosphate and calcium ion and hence improves the mechanical property of GICs.[14] Lucas *et al*. stated that due to the presence of phosphate, hydroxyl and fluoride ions between the GICs and the tooth structure, there is a formation of ionic and hydroxyl bonds between them, which increases the bond strength of hydroxyapetite incorporated GICs.[15] GC Fuji IX GP Extra (GC CO. Ltd., Tokyo, Japan) has smaller glass particle size and sets faster. It exhibits superior physio-mechanical property and good wear resistance thus gives it sufficient strength to resist masticatory stress.[16] These small mean particles size increases the surface area for polymeric acid and glass interaction which leads to faster maturation and higher hardness.[17] Bioglass R (Biodenāmica, Ibipora, Brasil) has better anti-cariogenic properties due to the release of fluoride, thermal compatibility with tooth enamel, biocompatibility, and low toxicity. However, limitations in their applications may result from the low mechanical strength and toughness.[18] Failure mechanisms such as void nucleation, crack propagation, and detachment of particles or sudden, subcritical failure are the common features of the low fracture resistance of bioglass R.[19]

Among the medias used for the surface treatments of experimental groups, MI Varnish showed greatest increase in FS followed by kedodent mouthwash, senquelNaF mouthwash and chlorhex plus. This may be due to high fluoride content which helps in enhancing the mechanical property of restorative material.[4] Control group in which no surface treatment was done exhibited the least FS when compared to other experimental groups. Control group and the experimental group with chlorhex plus showed no statistical significant difference in FS.

In the present *in vitro* study, surface treatment of restorative materials with CHX exhibited no growth of *Streptococcus mutans* and was considered as a positive control. This may be due to its effectiveness against both Gram-positive and Gram-negative bacteria. It increases the cell membrane permeability and leads to its rupture.[20] This result is in consensus with different studies stating that GICs incorporated with CHX shows long-term antimicrobial effect against *S. mutans*.[4,21,22] The control group which

Table 4: Comparison of mean and standard deviation of colony-forming unit/ml of restorative materials with different surface treatments using Krusal-Wallis test

Group	Mean±SD		
	SenquelNaF	Kedodent	MI VarnishMI Varnish
Bioglass R			
Micron bioactive	1818.30±47.37	1057.30±35.26	644.70±44.49
Gc Fuji IX GP Extra	1693.00±82.65	1053.10±432.97	460.20±435.07
Kruskal-Wallis test			
Value	14.417	13.473	13.488
Significant (*P*)	<0.01 (S)	<0.01 (S)	<0.01 (S)
Mann-Whitney U analysis comparison of CFU/ml of experimental restorative materials			
Bioglass versus Micron (*P*)	0.067 (NS)	<0.001 (HS)	<0.001 (HS)
Bioglass versus GC_IX (*P*)	<0.01 (S)	<0.05 (S)	<0.05 (S)
Micron versus GC_IX (*P*)	<0.01 (S)	<0.05 (S)	<0.05 (S)

HS: Highly significant (*P*<0.001); S: Significant (*P*<0.01). CFU: Colony-forming unit; ANOVA: Analysis of variance; SD: Standard deviation

was not subjected to any surface treatments exhibited the maximum number of colony-forming units of *S. mutans* and considered as negative control. To avoid the effect of no growth or affluent growth values on calculation both positive and negative control group were excluded while determining statistical significance. Hence, the statistical tests on antibacterial testing were performed only with SenquelNaF, Kedodent, and MI varnish.

MI varnish consists of 5% NaF and 2% CPP-ACP and exhibited significantly higher degree of reduction of *S. mutans* when compared to other experimental groups. CPP-ACP is a bioactive additive which can be incorporated in GICs as ACP is a precursor of hydroxyapatite.[23] Under acidic conditions, bond between CPP and ACP decreases leading to dissociation of calcium and phosphate ions, thereby counteracting mineral loss from tooth structure. It promotes remineralization and inhibits demineralization.[24] CPP can also decrease the count of *S. mutans* as it has got the ability to integrate in the pellicle. The CPP-ACP and fluoride have additive effects in reducing caries. The fluoride ion incorporates into an ACP phase (Ca,[P0,]5F × H, O) which is stabilized by the CPP, suggest that the CPPs are an excellent delivery vehicle for the co-localization of Ca, F, and phosphate ions at the tooth surface in a slow-release amorphous form, producing superior anticaries efficacy.[25]

NaF causes the inhibition of growth rate of *S. mutans* with glucose as the primary energy and carbon source. Metabolism of glucose or lactose requires enolase enzyme. It is the most fluoride-sensitive enzyme in the glycolytic pathway. Fermentative growth which is dependent upon glycolysis is inhibited by fluoride and thus blocks phosphoenolpyruvate (PEP) synthesis. Since PEP is used for both energy and transport in *S. mutans*, NaF it is detrimental to growth for *S. mutans*.[21] Kedodent mouthwash which consist of 0.05% of NaF showed better reduction in *S. mutans* when compared to senquelNaF (0.02% NaF) but was statistically not significant when compared to MI varnish.

In this study, among the restorative materials, GC Fuji IX GP Extra exhibited significantly less number of colony-forming units of *S. mutans* followed by micron bioactive and bioglass R. GC Fuji IX GP Extra exhibits excellent tendency to absorb fluoride ion and its absorption increases with decrease in pH, thus they have the potential to prevent the caries development.[17] Hence, in the present study, this material proved to be better in antimicrobial property when compared to other experimental groups.

However, since the study conducted was an *in vitro* study, it is difficult to predict whether results will be same as in an *in vivo* setup since replication of the intraoral temperature and humidity of xerostomic patient were difficult to achieve in the laboratory.

CONCLUSION

Within the limitation of this *in vitro* study, it can be concluded that, Micron bioactive with surface treatment of MI varnish exhibited enhanced mechanical properties. Hence, it can be recommended as a material of choice in the area where high mechanical strength is required. Similarly, GC Fuji IX GP Extra with the surface treatment of MI varnish

provides better antimicrobial property hence can be recommended in a patient with moderate-to-high risk of caries.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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