Original Article

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Dentistry Section

Evaluation of Shear Bond Strength of Incremental Layer of Self-etch Self-adhesive Novel Flowable Composite after Salivary Contamination: An In-vitro Study

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ABSTRACT

Introduction: Contamination of composite restoration during incremental placement leads to decrease in the incremental bond strength. Adhesive application on freshly contaminated resin surface increases the bond strength but is a complex and time-consuming procedure. Recently composite systems combining etchant, bonding agent and flowable composite into a single component have been introduced to simplify bonding and save time. In this study incremental layer shear bond strength is utilised to assess bond stability at resin-resin interface after salivary contamination.

Aim: To evaluate shear bond strength of incremental layer of self-etch self-adhesive novel flowable composite after salivary contamination.

Materials and Methods: The present in-vitro experimental study included 55 acrylic resin cylinders (2×2.5 cm) with square shaped cavity (5×5 mm, thickness 2 mm) restored with DMGTM Constic flowable composite that were randomly divided into five groups with eleven specimens per group. Group I: No salivary contamination, Group II: Salivary contamination followed by air drying, Group III: Salivary contamination followed by rinsing and air drying. Group II and III were subdivided into subgroup a: application and brushing of 0.5 mm of Constic followed by light curing and filling of rest of mold by Constic, subgroup b: direct application of 2 mm of Constic. Shear bond strength between increments of composite was determined by universal testing machine. Data were analysed using One-way ANOVA test and Independent t-test. Level of significance was kept at 5%.

Results: Incremental shear bond strength (MPa) was highest for group I (12.09±1.99) followed by group IIIa (10.21±3.49), group IIa (10.08±3.21), group IIb (7.59±2.31) and lowest for group IIIb (7.35±3.06).

Conclusion: Active application of self-etch self-adhesive flowable composite successfully restores the incremental shear bond strength after salivary contamination.

Keywords: Adhesive, Bond stability, Resin-resin interface, Saliva

INTRODUCTION

Adhesive restorative dentistry is an area of great significance for research as well as clinical practice. New materials and clinical strategies are continuously evolving to restore the form, function, aesthetics and structural integrity of the damaged teeth [1].

The popularity of dental composites is increasing day by day that has led to the importance of moisture and contamination control, as composites do not 'pardon' contamination. Technique sensitivity and the difficulty in achieving contamination and moisture control is a common problem experienced by the clinicians in restorative dentistry [2,3].

Blood, gingival sulcular fluid, or hand piece lubricant contribute in failure of adhesion and retention of composite resin to enamel and dentin [4]. Salivary contamination or contamination with blood has been cited in literature as one of the main issues encountered during direct adhesive restorative procedures [5].

Contamination acts like a barrier and compromises the adhesion of composite to the tooth structure resulting in formation of micro gap between restoration and tooth, postoperative hypersensitivity, discolourations, occurrence of secondary caries all of which will lead to failure of the restoration [4].

In order to warrant complete polymerisation of composite restorations for supreme physical properties, the clinicians are encouraged to place resin composite restorations in increments [3,6-10].

Several studies reported that salivary contamination of enamel and dentin results in decreased bond strength between composite restoration and enamel or dentin [3,4]. It has been reported in discrete studies that contamination of composite with biological fluids like saliva reduces the bond strength at the composite-composite interface decreasing the incremental bond strength [4,11-15].

Previous study has reported that reapplication of self-etching primer after salivary contamination restores the bond strength between self-etch primer and dentinal surface [16]. Application of adhesive on recently contaminated surface has also demonstrated good results [6].

Using etchant and adhesive between each increment is a complex and time-consuming procedure. In recent years self-etching self-adhesive flowable composite systems have been introduced to simplify bonding. These composite systems combine an etchant, bonding agent and flowable composite into a single component example DMGTM Constic, VertiseTM- flow [17,18].

Instead of using separate etchant and bonding agent after contamination of composite with saliva, this novel self-etching self-adhesive flowable composite can be used to restore bond strength at resin-resin interface. None of the studies have been conducted to study the shear bond strength of incremental layer of saliva contaminated composite after application of novel self-etching self-adhesive flowable composite. Thus, the purpose of this study was to evaluate shear bond strength of incremental layer of self-etch self-adhesive novel flowable composite after salivary contamination.

The null hypothesis tested was that salivary contamination causes no detrimental effect on shear bond strength of incremental layer of self-etch self-adhesive novel flowable composite.

MATERIALS AND METHODS

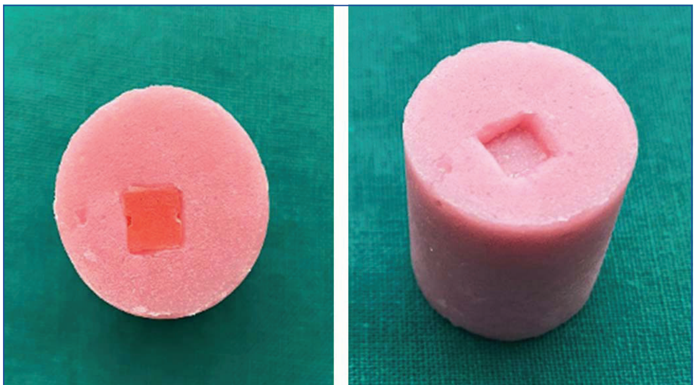
This in-vitro experimental study was conducted in the Department of Conservative Dentistry and Endodontics, Bharati Vidyapeeth Deemed to be University, Dental College and Hospital, Sangli, Maharashtra, India. The duration of study was about six months in the calendar year September 2021- February 2022. The study was approved by the Institutional Ethical Committee on 13th December 2019 (Letter number-BVDUMC&H/IEC/Dissertation 2019-20/D-29). Informed consent was collected from volunteer prior to collection of saliva. Procedure was carried in accordance with the ethical standards of the Institute.

Sample size calculation: The sample size was determined by GPower software. Effect size was calculated from the data obtained from a previous study conducted by Furuse AY et al., [11].

Input:	Tail(s)	=	Two
	Effect size d	=	1.2674108
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Non centrality parameter δ	=	2.9723418
	Critical t	=	2.0859634
	Df	=	20
	Sample size per group	=	11
	Actual power	=	0.8070629
	Total Sample size	=	(11×5) 55

Preparation of Specimens

Fifty-five acrylic resin cylinders (2 cm diameter, 2.5 cm height) with a square shaped modelling wax (5×5 mm, thickness 2 mm) embedded on the surface were prepared [Table/Fig-1], wax was eliminated using boiling water to obtain a square shaped standardised cavity [Table/Fig-2]. DMGTM Constic flowable composite resin indicated for direct restorations was used as per the manufacturer's instructions for the study.



[Table/Fig-1]: Acrylic resin cylinder. **[Table/Fig-2]:** Acrylic resin cylinder after removal of modelling wax. (Images from left to right)

Constic was inserted into the prepared cavities using composite packing instruments in single increment, glass cover slip was placed on top of the mold and gently pressed to produce a flat surface and remove excess. Constic was cured using LED curing unit for 20 seconds [Table/Fig-3]. Oxygen inhibition layer was retained to replicate clinical circumstances of incremental filling technique. Any sample that shows adhesive failure than cohesive was replaced by new one.

Samples were randomly categorised into five equal study groups- eleven samples per group.

Group I (Control group): No salivary contamination was carried; second increment was directly placed and light cured for 20 seconds using LED curing unit.

Group II: Samples were contaminated with saliva, dried with oil free compressed air for 20 seconds from a distance of 10 cm.

It was further divided into two subgroups: -

Group IIa: 0.5 mm of Constic was applied, brushed for 25 seconds [19] and was cured for 20 seconds using LED curing unit. Rest of the Teflon mold was filled with Constic and light cured using LED curing unit for 20 seconds.

Group IIb: 2 mm of second increment of constic was applied directly without brushing and was cured for 20 seconds using LED curing unit.

Group III: Samples were contaminated with saliva, rinsed with water for 20 seconds and dried with oil free compressed air for 20 seconds from a distance of 10 cm.

It was further divided into two subgroups:-

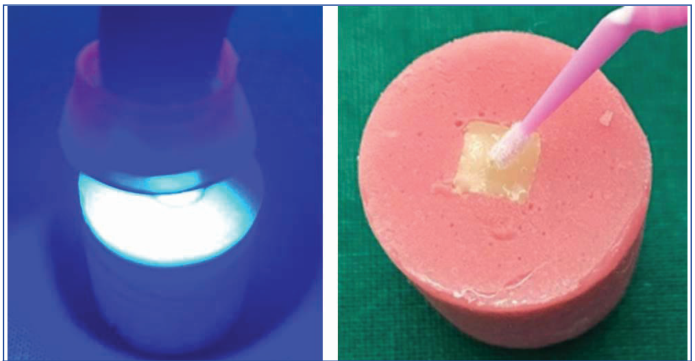
Group IIIa: 0.5 mm increment of Constic was applied, brushed for 25 seconds and was cured for 20 seconds using LED curing unit. Rest of the Teflon mold was filled with Constic and light cured using LED curing unit for 20 seconds.

Group IIIb: 2 mm of second increment of Constic was applied directly without brushing and was cured for 20 seconds using LED curing unit.

Study Procedure

Unstimulated whole saliva was collected from a single healthy individual donor in a sterile test tube and was used within one hour [20,21]. Fresh saliva is considered as an acceptable material to be used in saliva contamination testing [3].

Donor saliva was actively spread on the surface of specimen for 10 seconds using a microbrush on all samples except group I [Table/Fig-4].



[Table/Fig-3]: Curing Constic using LED curing unit. **[Table/Fig-4]:** Contamination of surface of constic with saliva. (Images from left to right)

After contamination and treatments according to the respective groups, a Teflon mold (diameter 4 mm, thickness 2 mm) was placed on first increment and second increment was applied according to the respective groups [Table/Fig-5].

Bond strength measurement is essential for studying the bonding stability [22]. Hence to assess the bonding between resin-resin increment, shear bond strength was assessed.

All the samples were stored in distilled water at 37°C for 24 hours. After 24 hours, shear bond strength between increments of composite was determined by Universal Testing Machine.

Shear bond strength assessment: Samples were mounted and stressed in shear at a rate of 0.5 mm/min using Universal Testing Machine (ACME, India) using chisel knife edge until failure of the bonding occurred [Table/Fig-6].

The maximum load at failure was recorded in Newtons (N) and converted to MegaPascals (MPa) [6].

Shear Bond Strength (MPa)=F(N)/A=F(N)/πr^2

Where π=3.1416, r=radius of composite build-up, N=Load.

[Table/Fig-5]: Placement of second increment.
[Table/Fig-6]: Testing of Sample stressed in shear at a rate of 0.5 mm/min using universal testing machine. (Images from left to right)

Samples which show failure at any other interface apart from composite-composite were replaced by new samples and tested again.

STATISTICAL ANALYSIS

Descriptive statistics were employed to measure mean and Standard Deviation (SD) for shear bond strength. One-way ANOVA test was applied to compare the overall difference among five groups. Pairwise comparisons between different subgroups were performed using Independent t-test. Statistical significance was fixed at ≤0.05. Analysis was done using Statistical Package for Social Sciences (SPSS) software version 23.0.

RESULTS

Incremental shear bond strength (MPa) was highest for group I (12.09±1.99) followed by group IIIa (10.21±3.49), group IIa (10.08±3.21), group IIb (7.59±2.31) and lowest for group IIIb (7.35±3.06). The difference between the groups were statistically significant (p=0.001) [Table/Fig-7]. The difference between mean incremental layer shear bond strength of control group from mean result of group IIa (p=0.092) and group IIIa (p=0.136) was non significant [Table/Fig-8].

Groups	Mean	SD	F-value	p-value
Control	12.09	1.99	5.286	0.001*
Group IIa	10.08	3.21		
Group IIb	7.59	2.31		
Group IIIa	10.21	3.49		
Group IIIb	7.35	3.06		

[Table/Fig-7]: Overall comparison of shear bond strength in MPa of incremental layer amongst different study groups.
One-way ANOVA test; *indicates significant difference at p≤0.05

Interval	Mean	SD	Difference	p-value
Control	12.09	1.99	2.01	0.092 (NS)
Group IIa	10.08	3.21		
Control	12.09	1.99	4.50	0.001*
Group IIb	7.59	2.31		
Control	12.09	1.99	1.88	0.136 (NS)
Group IIIa	10.21	3.49		
Control	12.09	1.99	4.74	0.001*
Group IIIb	7.35	3.06		

[Table/Fig-8]: Comparison of incremental layer shear bond strength in MPa of control group with other groups.
Independent t test; *indicates significant difference at p≤0.05; NS: Non significant difference

The difference between mean incremental layer shear bond strength of control group had a significant difference from mean result of group IIb (p=0.001) and group IIIb (p=0.001).

The mean difference between the incremental layer shear bond strength of group IIa and group IIb was 2.49 MPa. The incremental layer shear bond strength of group IIa was significantly more than that of group IIb (p≤0.05) [Table/Fig-9]. The mean difference between the incremental layer shear bond strength of group IIIa and group IIIb was 2.86. The incremental layer shear bond strength of group IIIa was significantly greater than that of group IIIb (p≤0.05).

Interval	Mean	SD	Difference	p-value
Group IIa	10.08	3.21	2.49	0.050*
Group IIb	7.59	2.31		
Group IIIa	10.21	3.49	2.86	0.050*
Group IIIb	7.35	3.06		

[Table/Fig-9]: Comparison of incremental layer shear bond strength in MPa within group II and within group III.
Independent t test; *indicates significant difference at p≤0.05

Non significant difference was found between mean incremental layer shear bond strength of group IIa and group IIIa, also between group IIb and group IIIb [Table/Fig-10]. Significant difference (p≤0.05) was found between mean incremental layer shear bond strength of group IIa and group IIIb, also between group IIb and group IIIa.

Interval	Mean	SD	Difference	p-value
Group IIa	10.08	3.21	-0.13	0.929 (NS)
Group IIIa	10.21	4.39		
Group IIb	7.59	2.31	0.24	0.837 (NS)
Group IIIb	7.35	3.06		
Group IIa	10.08	3.21	2.73	0.050*
Group IIIb	7.35	3.06		
Group IIb	7.59	2.31	-2.62	0.050*
Group IIIa	10.21	4.39		

[Table/Fig-10]: Comparison of incremental layer shear bond strength in MPa between group II and group III.
Independent t test; *indicates significant difference at p≤0.05; NS: Non significant difference

Thus, subgroup a had an overall improved bond strength as compared to subgroup b. The treatments carried out in groups IIa and IIIa successfully restored the bond strength comparable to that of the control group.

DISCUSSION

Dental composites have unquestionably acquired a prominent place among the filling materials employed in direct techniques [23]. Evolution of self-adhesive composites over the past years has led to establishment of novel self-adhesive composites that are composed of monomers that have self-etching and/or self-adhesive properties. They etch the tooth surfaces and chemically bond to the hydroxyapatite crystals [24].

Constic is composed of 10-Methacryloyloxydecyl Dihydrogen Phosphate (MDP) monomer which holds longer and greater number of hydrophobic spacer chains. MDP forms stable 10-MDP-Calcium salts without leading to major decalcification, resulting in formation of a sturdy chemical bonding with hydroxyapatite crystals of tooth structure [25,26]. Constic etches enamel and dentin, bonds with tooth structure similar to glass ionomer, and it has ability to co-polymerise with the composite resin [19].

Composite resins being a multi-step procedure routinely require discrete conditioning steps with the aid of an adhesive system to enable bonding of composite resin on tooth structure. Contamination by saliva, blood, gingival sulcular fluid, and handpiece oil leads to decrease in the bond strength between the restoration and the tooth substrate, hence they are important determinants that influence adhesion of composite resin [27]. Saliva possess a great risk of contaminating the surface to be restored [27,28].

The clinical performance and longevity of dental restorations can be determined by adhesive bond strength [29]. Adhesion tests measure either tensile bond strength or shear bond strength. Furuse AY et al., [11] assessed the shear bond strength at resin-resin interface using a universal testing machine, similar method was adapted in the present study.

According to the results of this study, the mean incremental shear bond strength value of all groups contaminated with saliva was found to be less than that of control group. This is in accordance with the results published by Eiriksson SO et al., [3], Furuse AY et al., [11] and Jaberi AZ and Mohammadpour A [4]. Thus, salivary contamination lowers the adhesive strength between resin increments and the most anticipated reason behind it is the formation of a film of glycoprotein sugars on the surface of composite resin coming in contact with saliva [3].

Yazici AR et al., [27] studied the effect of saliva contamination on microleakage of an etch-and-rinse and a self-etching adhesive. They attributed the detrimental effects of saliva contamination on the cured adhesive layer to the adsorption of glycoproteins onto the poorly polymerised adhesive surface, similar results were observed in the present study.

In 2004 Eiriksson SO et al., [3] assessed the saliva contaminated resin surface of specimen under a scanning electron microscope, it displayed a flat surface on the specimens. They concluded that this might be the probable reason behind decrease in the incremental layer shear bond strength as it leads to lack of contact of composite resin increment with the contaminated surface.

Comparison of present study with previous studies on effect of salivary contamination on bond strength and various methods employed to regain the bond strength is summarised in [Table/Fig-11] [3,4,11,27,28,30].

Furuse AY et al., [11] concluded that if salivary contamination of resin surface occurs during the procedure of composite layering it decreases the bond strength and hence, requires a plausible decontamination method to restore the bond strength. This is in agreement with the results obtained from the present study.

Group IIb and Group IIIb resulted in significantly lower incremental bond strength than control group. The mean incremental shear bond strength value of group IIb was more than mean incremental layer shear bond strength value of group IIIb. However, the difference was statistically insignificant ($p \leq 0.05$) suggesting that neither of the above methods are reliable to decontaminate the surface of resin after salivary contact. This is in accordance with the study conducted by Jaberi AZ and Mohammadpour A [4] where they assessed the microshear bond strength of composite-composite after salivary contamination. They concluded that air drying of resin surface contaminated with saliva decreases the bond strength considerably.

Eiriksson SO et al., [3] published that bond strength between resin increments after salivary contamination decreases even if saliva is in contact with resin for a short time or is rinsed away with water. They also assessed the saliva contaminated resin surface of specimen that was rinsed and air dried under a scanning electron microscope, it displayed few craters or blisters suggesting that water, air or saliva might still be trapped on the surface of the specimens and might have led to decreased bond strength. Same reason might have resulted in significantly lower bond strength values of group IIb and group IIIb to that of control, group IIa and group IIIa. Furuse AY et al., [11] reported that the lowest incremental shear bond strength was found when the rinsing and drying of the contaminated surface was performed, which is in accordance with the present study.

Shear bond strength values within both the groups that is group II and group III significantly improved by active application of ~ 0.5 mm layer of Constic for 25 seconds on saliva contaminated surface of composite as compared to not brushing and directly applying composite. Thus, suggesting that active application of Constic by brushing aids in restoring the bond strength comparable to that of control group. Probable reason being that active application of Constic leads to better surface wetting and improved penetration of functional monomers (MDP) producing more stable bond. This is in accordance with a systematic review published by Carrilho E et al., [31], where they published that in order to get the best of the adhesive solutions containing 10-MDP, a scrubbing technique must be employed to apply the adhesive system on dental substrates.

S. No.	Author's name and year	Place of study	Sample size	Objective of study	Conclusion
1	Eiriksson SO et al., [3] 2004	USA	96	To evaluate the effects of saliva contamination on microtensile bond strength (mTBS) between resin interfaces and to determine which decontamination methods best re-established the original resin- resin bond strength.	Saliva contamination significantly reduced bond strengths between resin composite surfaces regard less of the materials evaluated. Application of a dentin/enamel adhesive is necessary whenever saliva contamination exists on composite increments to ensure better interfacial bonding.
2	Yazici AR et al., [27] 2006	Turkey	40	To evaluate the effect of saliva contamination on the microleakage of an etch-and-rinse adhesive and a self-etching adhesive.	Contamination of adhesives with saliva before and after adhesive curing did not worsen the microleakage of the two-step etch-and-rinse adhesive single bond or the one-step self-etching adhesive futura bond NR.
3	Furuse AY et al., [11] 2007	Brazil	150	To investigate the effect of different surface treatments on shear bond strength of saliva contaminated resin-resin interfaces.	Either the abrasion of the saliva contaminated surface followed by application of the adhesive system, or the application of silane and adhesive, resulted in more stable resin-resin bonding.
4	Jaberi AZ and Mohammadpour A, [4] 2010	Iran	105	To evaluate the effect of saliva contamination on the microshear bond strength between composite increments and to determine which method best decontaminates saliva from the resin surface and re-establishes the original resin-resin bond strength.	Air drying of the surface after saliva contamination decreases the microshear bond strength significantly.
5	Kholief EA et al., [30]	Egypt	160	Shear bond strength for immediate and delayed repair of composite with microhybrid and nanohybrid resins using different bonding agents.	Time of repair, bonding agent and repair material affected repair bond strength of composite.
6	Nair P and Ilie N, [28] 2019	Germany	1120	To analyse the bond quality in dentine postageing after salivary contamination and decontamination at different stages of dental adhesive application.	Saliva contamination is detrimental after primer application in SE but, decontamination regained the SBS and maintained it over time. In U adhesive, SBS deteriorated over time irrespective of the contamination.
7.	Present study	India	55	To evaluate shear bond strength of incremental layer of self-etch self-adhesive novel flowable composite after salivary contamination.	Active application of self-etch self-adhesive flowable composite successfully restores the incremental shear bond strength after salivary contamination.

[Table/Fig-11]: Comparison of present study with previous studies on effect of salivary contamination on bond strength [3,4,11,27,28,30].

This results in better infiltration of monomers at the same time leads to formation of a much stable bond. Eiriksson SO et al., [3] published that application of an adhesive on saliva contaminated surface increases the bond strength similar to control group. The adhesive used in their study was composed of MDP, which is the functional monomer found in Constic, it might have played a vital role in increasing the bond strength. Carrilho E et al., [31] reported that use of MDP containing bonding agents successfully improved the immediate resin repair bond strength. Furuse AY et al., [11] concluded from their study that application of adhesive on contaminated resin surface increases the shear bond strength similar to that of control group.

Jaberi AZ and Mohammadpour A [4] evaluated the micro-shear bond strength of composite-composite after salivary contamination, and investigated which decontamination method best re-establishes the original resin-resin bond strength. They found that shear bond strength after rinsing, air drying followed by acid etching as well as rinsing, air drying followed by acid etching and application of bonding agent on contaminated surface were almost similar and had no significant difference with that of control group.

Nair P and Ilie N [28] conducted a study to evaluate the long-term consequence of salivary contamination at various stages of adhesive application and clinically feasible remedies to decontaminate, they concluded that the acidity of self-etch adhesives modifies and penetrates the smear layer and also breaks through the mucopolysaccharides in the saliva and develops bond strengths comparable with those obtained on noncontaminated dentine surfaces. Constic has the ability to etch enamel [19], this might have contributed in modification of smear layer and mucopolysaccharides in saliva thus, restoring the bond strength of saliva contaminated surface.

The method adapted in the present study maintained the oxygen inhibited layer to mimic in-vivo incremental filling technique, also the manufacturer's instructions for Constic recommends retaining oxygen inhibition layer [19].

From the results obtained in the study the incremental layer shear bond strength value was highest with control group, followed by subgroups IIIa, IIa, IIIb and minimum bond strength was observed with in IIb. The present study suggests that immediate active application of Constic (self-etch self-adhesive flowable composite) seems to play a vital role in restoring the incremental layer bond strength after salivary contamination, hence rejecting the null hypothesis.

Salivary contamination of resin surface during incremental placement of composite resin is observed frequently in clinical situations and restoring bond strength in such clinical scenario with ease of application and less time consumption enhances the quality and life of treatment and improves public health in a community [4].

Limitation(s)

The specimens made for in-vitro studies are relatively flat, uniform and untextured as compared to intraoral restorations impacting the results considerably. Secondly, in the oral cavity, the additive effects of temperature, area or location, accessibility, distance from tip of light curing unit may influence the results, they were not accounted in this in-vitro study.

CONCLUSION(S)

Within the limitations of the study, it can be concluded that salivary contamination of composite during incremental placement decreases the shear bond strength at resin-resin interface. Air drying or rinsing followed by air drying the contaminated surface did not increase the incremental shear bond strength and thus, are not reliable methods to restore the bond strength. Air drying alone or rinsing followed by air drying the contaminated surface along with active application of Constic by brushing it resulted in shear bond strength values comparable to that of control group. Active application of self-etch self-adhesive flowable composite successfully restores the incremental shear bond strength after salivary contamination.

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