"COMPARISON OF ORTHODONTIC RESIN ADHESIVES INCORPORATED WITH ARGININE AND CITRULLINE – AN INVITRO STUDY OF PHYSICAL, ADHESIVE AND ANTIMICROBIAL PROPERTIES"

By

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Dissertation

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ABSTRACT

Introduction: The main goal of developing an adhesive that could bond the brackets directly to the tooth surface was to withstand forces from treatment and mastication while allowing complete control of biomechanical needs in orthodontics and prevention of damage to the enamel after removing these brackets. Further research in this aspect of orthodontics has led to achieving the goals of the adhesion of the bracket to the tooth surface. Arginine and Citrulline are semi-essential amino acids. These amino acids increase the cytoplasmic and environmental pH due to the synthesis of ammonia through the arginine deaminase pathway. This leads to an increase in the pH levels and synthesis of ATP. Thereby, there is maintenance of a neutral pH that is less beneficial for cariogenic activity.

The purpose of this in vitro study is to evaluate and compare the effects of the addition of arginine, citrulline and a combination of Arginine and Citrulline to commercial orthodontic resin adhesive on the tensile strength, shear bond strength, and Antimicrobial Properties

This study is focused on incorporating arginine and citrulline without unfavourably affecting the physical properties.

Materials and methods: The two amino acids, Arginine and Citrulline were incorporated with Orthocem adhesive using a Dual Axis Centrifuge. A total of three experimental groups were made; 1, Adhesive and Arginine; 2, Adhesive and Citrulline; 3, Adhesive and a combination of Arginine and Citrulline. The experimental groups were compared with the control group; Orthocem.

Brackets were bonded to the extracted premolars. They were subjected to a shear bond strength test using a Universal testing machine. The teeth specimens were then scored using the Modified Adhesive remnant index. Hour-glass specimens were prepared for each group and subjected to a Tensile strength test using a Universal testing machine. Discs were fabricated for each group and subjected to antimicrobial sensitivity tests by counting the colony-forming units. Similar Discs of each group were imaged using a Scanning electron microscope at 500x and 5000x to study the particle size and surface morphology.

Results: The group with Orthocem and citrulline showed the least bond strength compared to the control group. The group with Orthocem and Arginine showed having the most shear bond strength among the experimental groups. The group with Orthocem showed maximum samples with complete composite retained on the surface with the impression of the bracket base. The group with Orthocem and Arginine and the Group with Orthocem and Citrulline showed maximum samples showing 26% to 50% of adhesives retained on the surface of the tooth. In the group with Orthocem and the combination of arginine and citrulline, maximum samples showed 76% to 99% of adhesives retained on the surface of the tooth. The Group with Orthocem and Citrulline showed the least Tensile strength compared to the Control group with Orthocem. The Group with Orthocem and Arginine showed having the most Tensile strength compared to other experimental groups. The group with only citrulline showed the most promising result compared to other experimental groups for antimicrobial properties. The maximum mean area was seen with Group with Adhesive and Arginine. The minimum mean area was seen with the Group with Adhesive and Citrulline followed by the Group with Adhesive and the combination of

Arginine and Citrulline. The Surface morphology was seen to be rough in the groups with Citrulline

<u>Conclusion:</u> Adding arginine and citrulline to the Orthocem resin adhesive influenced the physical properties and decreased the growth of Streptococcus mutans for all the experimental groups. The addition of citrulline has shown promising results with increasing the anti-microbial properties of the adhesives. Further research on incorporating citrulline without affecting the physical properties can provide an alternate method for controlling the occurrence of White spot lesions in orthodontic treatment.

Keywords: Brackets, Orthodontic treatment, Streptococcus mutans, shear bond strength, tensile strength test, composites.

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INTRODUCTION

[1.1] Description of the Adhesives

[1.1.1] History

The brackets were initially welded to the bands of gold or stainless-steel bands. The placement of these orthodontic bands required space creation in the inter-proximal region using separators. The separators used were wires and later on replaced by elastomerics. This process was time-consuming for the orthodontists and uncomfortable for the patients. The bands also created spaces after removal of them, which proved to be a challenge for the orthodontists to close. In addition, bands also created trauma to the gingiva and decalcification under the bands. Therefore, to deal with such shortcomings, the solution was to attach the brackets directly onto the tooth, eliminating the need for the bands.

The main goal of developing an adhesive that could bond the bracket directly to the tooth surface was to withstand forces from treatment and mastication while allowing complete control of biomechanical needs in orthodontics and prevention of damage to the enamel after removing these brackets.

In 1956, Ray L Bowen published a paper titled “Use of Epoxy Resins in Restorative Materials” and introduced Dental bonding, further research in this field led to the development of a new type of material known as composite resin. Professor Fujio Miura developed a technique for bonding polycarbonate plastic brackets to the etched enamel using a material used in restorative dentistry which was developed by Masuhura et al in the early 1970s [1-4]. Professor Miura found that the bond strength decreased with time as it came into contact with saliva. This concept of adhesion directly to the teeth became popular and further research was conducted to create stronger adhesives and durable brackets. Retief et al partnered with 3 M Unitek to develop a mesh grid on the base of the brackets aimed at increasing mechanical retention, however, the area where the bracket and this base were welded prevented the flowing of the adhesive which resulted in reduced mechanical retention. This led to further development and refinement of designs of the bracket base to achieve the goals of the adhesion of the bracket to the tooth surface.

In 2015, Paul Gange published an article describing the Orthodontic bonding timeline from 1970 to 2015 with the Methyl Methacrylate/ Polymethyl methacrylate with plastic brackets in 1970. Subsequently, the following adhesive systems were launched, An adhesive comprising of two Paste chemical cure systems in 1972, Adhesives using an Ultraviolet light cure system in 1983, multisurface hydrophilic primer as a base in adhesives in 1985, colour change adhesive to facilitate removal of excess adhesive in 1986, dual cure glass ionomer cement, an adhesive with both chemical and light curing in 1995, pre-pasted brackets to reduce chair-side time in 1996, new generation hydrophilic primers which link hydrophilic primer and hydrophobic adhesive in 1998, self-etching primers provides clinician with all in one adhesive system in 2000, non-porous enamel protective sealants to protect the teeth from caries activity in 2003, light cure colour change adhesives in 2004, all surface hydrophilic primers in 2014.[5]

[1.1.2] Modifiers in Adhesive systems

Several additions to the adhesive systems have been made to improve the adhesive system to obtain anti-microbial properties.

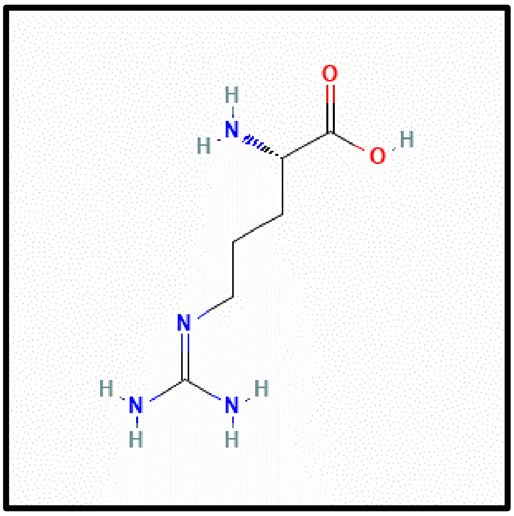
Antimicrobial agents incorporated into Orthodontic Bonding Systems: White spot lesions are frequently observed on the tooth surface of orthodontic patients. The progress of the formation of white spot lesions is mainly due to the prolonged accumulation of plaque over the irregular surfaces of orthodontic appliances and flash around the brackets that are iatrogenically caused by the orthodontist. There have been approaches to prevent white spot lesions such as strict Oral hygiene instructions, fluoride regiments and avoiding certain food types. These preventive measures however are dependent on patient compliance, for this reason, several orthodontic bonding systems have incorporated fluoride in an attempt to reduce demineralisation. With the incorporation of fluoride into the adhesive system, several researchers have suggested the incorporation of different antimicrobial agents into orthodontic bonding systems. The substances that have the potential for bacterial inhibition, methacryloyloxydodecylpyridinium bromide, chlorhexidine triclosan, benzalkonium and glutaraldehyde silver nanoparticles have been shown to have antimicrobial effects. C. M. De Almeida et al in the year 2018 conducted a systematic review and a meta-analysis to study the efficacy of antimicrobial agents incorporated in orthodontic bonding systems. They concluded that the agents incorporated in the adhesive systems inhibited bacterial growth without compromising their physical properties. And interpreted that there is a requirement for long-term studies to confirm the effectiveness of antimicrobial properties in preventing caries.[6]

[1.2] Description of the Amino acids

Arginine is an amino acid which is classified to as an essential amino acid for younger individuals and a semi-essential amino acid for adults. The Molecular formula is $C_6H_{14}N_4O_2$. According to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature, the chemical name of arginine is (2*S*)-2-amino-5-(diaminomethylideneamino)pentanoic acid [Fig 1]. The natural dietary sources for L-Arginine are red meat, poultry, fish and dairy products. Arginine plays an important role in the synthesis of protein, amino acids, its derivatives, and the urea cycle. L-arginine is converted to L-ornithine as a precursor to the urea, which is important in the Urea Cycle [Fig 3]. Arginine is derived from the diet and some from the citrulline. Citrulline is converted to arginosuccinate with the enzyme arginosuccinate synthetase. Arginosuccinate is converted to arginine with the help of the enzyme arginosuccinase. The molecular formula of L-citrulline is $C_6H_{13}N_3O_3$. According to the IUPAC nomenclature, the chemical name of Citrulline is (2*S*)-2-amino-5-(carbamoylamino)pentanoic acid. [Fig 2]

Arginine is secreted in saliva at concentrations of about 50 microlitres. There is an increase in cytoplasmic and environmental pH due to the synthesis of ammonia through the arginine deaminase pathway. This condition benefits the oral bacteria by protecting them from acid-killing and providing the oral cavity with bio-enriching conditions such as an increase in pH levels and synthesis of ATP. Thereby, there is maintenance of a relatively neutral environmental pH that is less beneficial for cariogenic activity.[7]

Nitric oxide is a water-soluble gas with a broad range of physiological and pathological processes in mammals. Nitric oxide is produced by the nitric oxide synthase as a consequence of L-arginine conversion to L-citrulline with the help of nicotinamide dinucleotide phosphate [Fig 3].



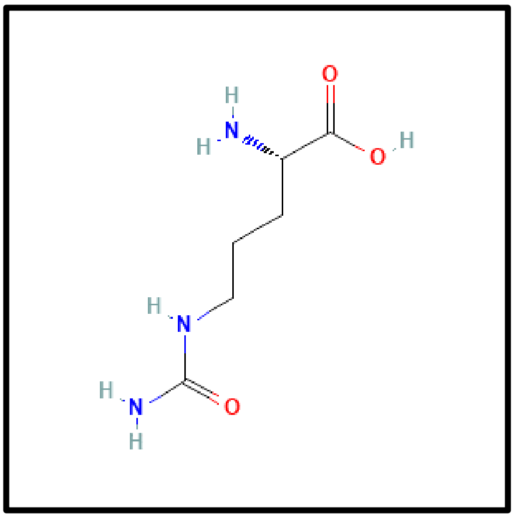


Fig 1. Chemical Structure Depiction of L-Arginine (PubChem CID 6322)

Fig 2. Chemical Structure Depiction of L-Citrulline (PubChem CID 9750)

[1.3] Description of the Role of Nitric oxide

Nitric Oxide (NO) is a lipophilic and hydrophilic gas. NO is produced during the process of conversion of L-arginine to L-citrulline with the action of Nitric oxide synthase (NOS) with the help of nicotinamide dinucleotide phosphate and oxygen [Fig 3]. NO processes every cellular response when mechanical forces are applied to PDL and Bone. During the mechanical loading over a bone, the Wnt/beta-catenin pathway is activated. This mechanical loading acts as a signalling event in PDL fibroblasts osteoblasts and osteocytes.

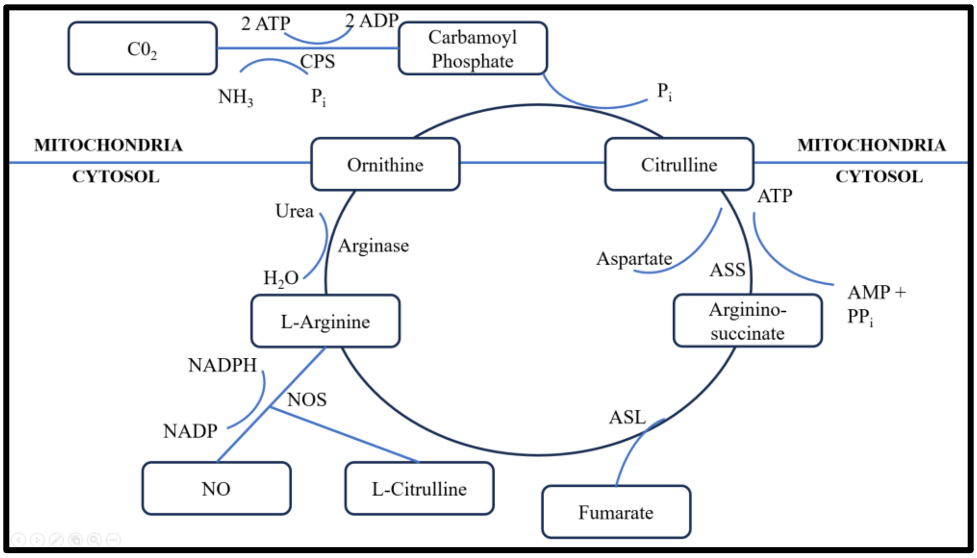


Fig 3. Urea Cycle and Nitric Oxide Synthesis

Several studies have demonstrated the biphasic effect of NO on the formation and function of osteoclasts. This effect on the osteoclasts has a direct implication on bone resorption rate. Studies have revealed that at lower concentrations of NO, there is an inhibitory effect on osteoclasts. [9,10,11,12]

Researchers have shown the role of NO and NOS in initiating immune response and inflammation. The antimicrobial action of NO is due to the chemical alteration of DNA structure. NO damages DNA by three mechanisms: Direct reaction of reactive nitrogen oxide species (RNOS) with DNA structure, inhibiting the repair of DNA and increasing the generation of hydrogen peroxide and alkylating agents. The cytotoxic effects of NO are enhanced by acid, reactive oxygen species, and glutathione which are products of macrophages.[13]

Now, there is an increased microbial resistance against antibiotics. It is important to find alternatives to the conventional, the effects of which can be long-lasting. Therefore, the current research is focused on incorporating arginine and citrulline into adhesives without unfavourably affecting the physical properties and controlling the occurrence of white spot lesions in orthodontic treatment

AIM AND OBJECTIVES

[2.1] Aim

Evaluation and comparison of the effects of addition of arginine, citrulline and combination of arginine and citrulline to commercial orthodontic resin adhesive on the tensile strength, shear bond strength and antimicrobial properties.

[2.2] Objectives

1. To assess the changes in the mechanical properties after the addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive.
2. To assess the changes in the surface morphology after the addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive.
3. To study the anti-microbial effects after the addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive.

[2.3] Hypothesis

[2.3.1] Null Hypothesis

1. The addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive will not influence in the material's ultimate tensile strength and enamel shear bond strength.
2. The addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive will not reduce the growth of streptococcus mutans

[2.3.2] Alternate hypothesis

1. The addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive will influence the material’s ultimate tensile strength and enamel shear bond strength.
2. The addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive will reduce the growth of Streptococcus mutans.

REVIEW OF LITERATURE

- **Geraldeli S et al -** A 2.5% weight of the amino acid, Arginine, was added to the orthodontic adhesive with the help of a centrifugal mixing device. Forty Bovine incisors were freshly extracted and cleaned. Prophylaxis was conducted on these incisors using pumice paste and a polishing rubber cup over the buccal surfaces for 30 seconds. Using a 30% phosphoric acid gel the enamel was conditioned for bonding. The arginine mixed orthodontic adhesives were used to bond brackets over the incisors' etched and primed buccal surfaces. This study concluded that the 2.5% addition of arginine in the Transbond XT and Orthocem adhesives had no influence on the ultimate tensile strength and shear bond strength and it showed a decrease in the growth of S. mutans for only the Orthocem adhesive groups.[13]
- **Nascimento MM et al -** The study aimed at measuring the effect of arginine on oral biofilm. There is an increase in the pH of the oral biofilms which reduces the risk of the development of caries due to the production of alkalis by the oral bacteria through the arginine deaminase system (ADS). In this study, the ADS activity was measured by quantifying the ammonia produced from arginine by the samples at baseline, after the washout period, 4 weeks of ongoing treatment, and 2 weeks after the completion of the treatment. the study concluded by declaring that the plaque microbial profile of caries-active individuals when treated with arginine toothpaste showed a shift in bacterial composition to a healthier community, more similar to that of caries-active individuals. [14]

- **Nascimento et al** - . The study measured the arginine deaminase system (ADS) activity in the oral cavity to understand the relationship between oral arginine and dental caries. This study took 100 samples from children and correlated them to their caries presence. The important role of alkali production shows a reduction in caries this study directly explains the changes in the micro-flora when the caries develop and associates with the change in the production of the alkali by the oral microbiota. This study observed a high degree of variability in the ADS activity. This study concluded that there is a highly significant correlation between ADS activity and caries levels.[15]
- **Huang, X et al -** The study is aimed at understanding the factors associated with alkali production from arginine from oral biofilms. The alkali production by the oral microbiota has an established link to dental caries. This study assessed the parameter which had an association with the ammonia which is produced by the arginine catabolism in the oral biofilms. The experimental model proved valuable to assess the bacterial reactions to assess the overall pH changes. This study concluded that the initiation of dental caries is influenced by the pH levels. [16]
- **Zheng, X et al -** This study was aimed at understanding the ecological effect of arginine on oral microbiota. This study was conducted to study the effect of arginine on the oral microbiota. The authors employed a clinical cohort and saliva-derived biofilm models and these models were subjected to arginine treatment to demonstrate that arginine can favourably modulate the oral microbiota. The study concluded that fluoride and arginine have a potential synergistic effect in maintaining a healthy microbiota which favours caries risk management.[17]

- **Bijle et al -** This study was aimed at understanding the Combined antimicrobial effect of arginine and Fluoride toothpaste. Here, the authors have taken biofilm cultures of S. mutans, S. sanguis and S. gordonii inoculated discs and these discs were then subjected to different toothpaste formulations. This study states that increasing the concentration of arginine does not affect the viability of the microbes and concluded that incorporating 2 % arginine in the sodium fluoride toothpaste showed enrichment of the alkali-producing bacteria and enhanced the counter mechanisms against the cariogenic microbes when compared to plain sodium fluoride toothpaste.[18]
- **Agarwal U et al -** The Animal models (Mice) were fitted with gastric catheters to provide experimental diets for 2 weeks. The control/basal diet contained 2.5 g L-arginine/Kg, whereas the experimental diet contained additional 2.5, 7.5, and 12.5 g/Kg diets of either L-arginine or L-citrulline. This resulted in a linear increase in plasma arginine concentration from 109 mmol/L for the basal diet to 159 and 214 mmol/L for the diets with the highest arginine and citrulline supplementation levels. However, it is of interest that there was a marked increase in plasma arginine levels due to the introduction of supplemental citrulline. The study concluded that citrulline supplementation is more efficient at increasing the availability of arginine than arginine supplementation itself in mice. [19]
- **Suzuki et al -** The study aimed to establish the effects of combined Oral citrulline and arginine supplementation on plasma L-arginine levels. The study was conducted with combining 1g of L-citrulline and 1g of L-arginine as oral supplements. After the supplementation, the human subjects were tested for plasma levels of L-arginine. The study concluded that the combination of both

the amino acids, citrulline and arginine increases arginine levels in humans. Therefore, the combination of the amino acids might have a more beneficial effect than a single dose of L-arginine alone. [20]

- **Ambe et al -** NO is produced by Oral bacteria and is a component of saliva. It is produced and released in the oral cavity by the expression of Nitric oxide synthase in oral mucosae, such as gingiva. The study explains the various physiological and pathological roles of NO, which are involved in vasodilation, platelet aggregation, immune responses, cell migration, neurotransmission, apoptosis, and wound healing. The study concludes that there is an NOS expression induced by the orthodontic treatment in the gingiva and dental pulp and the expression of this in such tissue showed higher than that in normal conditions. [21]
- **Schairer D.O et al -** Nitric oxide is a lipophilic gas, diatomic, and short-lived is shown to play an integral role against pathogens. NO plays a role in the signalling of immune cells and in biochemical reactions by which immune cells defend against bacteria, fungi, viruses, and parasites. NO signalling leads to a broad spectrum of processes such as differentiation, proliferation, and apoptosis of immune cells. When immune cells are activated NO is secreted and diffuses across the cell membranes and causes nitrosative and oxidative damage to the invading pathogen. The study concluded that developing NO delivery systems can harness the antimicrobial properties of this evanescent gas.[12]

MATERIALS AND METHODS

[4.1] Materials and Instruments

1. Orthocem orthodontic resin adhesive
2. 3M Unitek Gemini Metal Brackets
3. Orthodontically Extracted Premolar Teeth
4. 3sec Woodpecker curing light
5. Condac 37 Acid Etchant
6. Bonding agent
7. Arginine and Citrulline
8. Centrifugal mixing device
9. Universal testing machine
10. Stereomicroscope
11. Petri dish
12. Brain heart infusion (BHI) culture medium with 1% sucrose
13. Inoculum of Streptococcus mutans with Incubator set at 37 degrees
14. Scanning Electron Microscope
15. Pumice paste and polishing cup

[4.2] Study Design

An In-vitro study was conducted using one control group and three experimental groups

[4.3] Source of Data and Laboratory Details

1. The study was conducted at BSRC, KLE Academy of Higher Education and Research, KLE VK Institute of Dental Sciences, Belagavi.
2. The Shear strength of the bracket-enamel interface and tensile strength test was assessed at Praj Metallurgy Laboratory, Pune.
3. The Scanning Electron Microscope analysis was conducted at BITS PILANI Goa campus.

[4.4] Sample Size Estimation

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 ({SD_1}^2 + {SD_2}^2)}{(\bar{x}_1 - \bar{x}_2)^2}$$

SD_1 = 3.70

SD_2 = 6.73

$\bar{x}_1$ = 17.52

$\bar{x}_2$ = 20.57

α = 5%

1 – β = 80%

SD_1 : Standard Deviation in the 1st group

SD_2 : Standard Deviation in the 2nd group

$\bar{x}_1$: Mean of 1st group

$\bar{x}_2$: Mean of 2nd group

α : Significant level

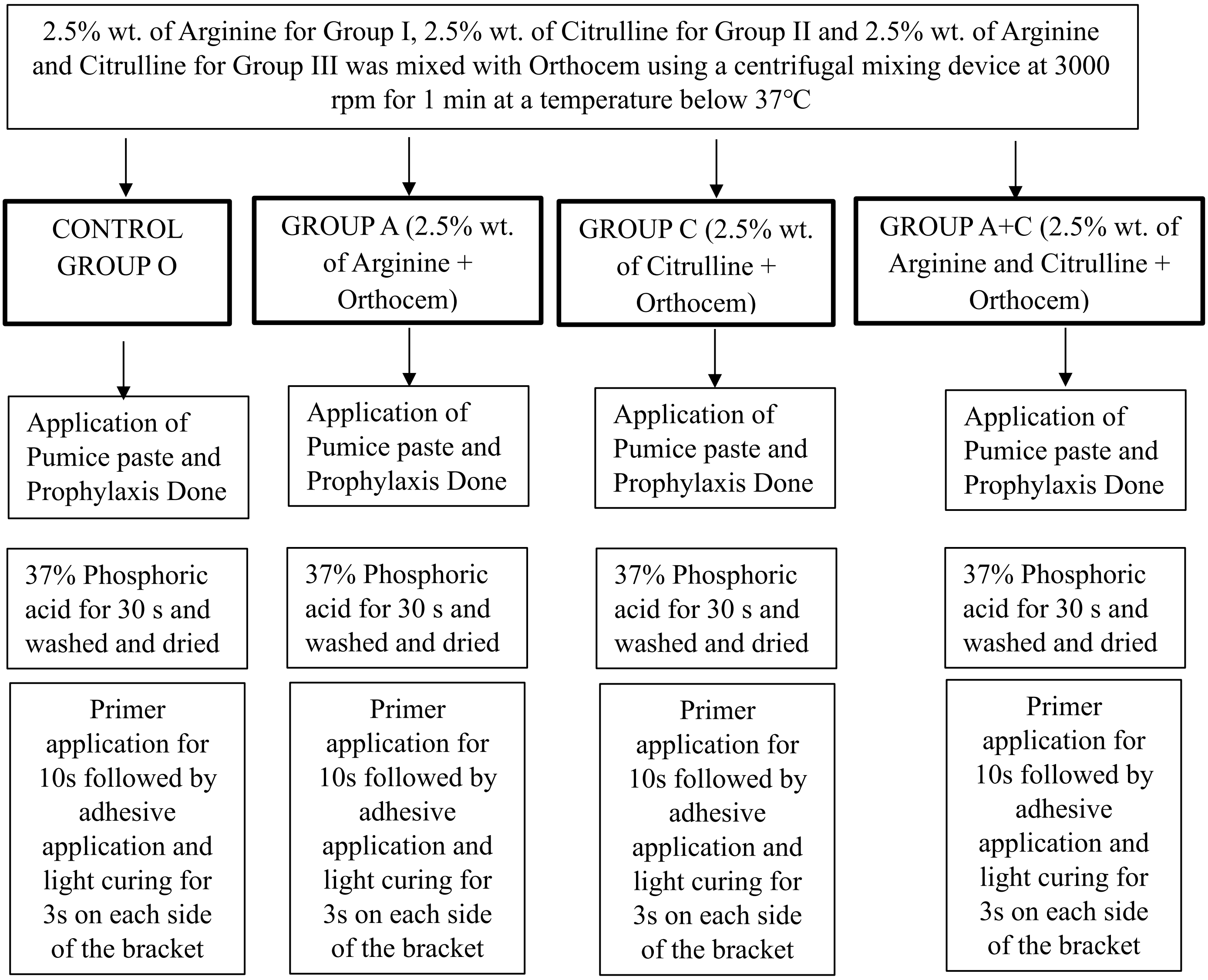
1 – β : Power

Estimated sample size for each group, n = 50

For an α value equal to 0.05 and a power of 80% (β = 0.2), $Z_{1-\alpha/2}$ = 1.96, and $Z_{1-\beta}$ = 0.842, the sample size was calculated to be **50 samples in each group**, accounting to a **total sample size of 200** for the main hypothesis at a power of 0.80 with 0.05 Type I error (Geraldeli S *et al*., 2021).

[4.5] Methodology

[4.5.1] Method to prepare samples of four groups



[4.5.2] Test to study the Shear Bond Strength

The bracket-enamel interface of each specimen was positioned

↓

The SBS test was conducted using a Universal Testing machine

↓

Interpret the results and Conclusion

[4.5.3] Test to study the Adhesive Remanent Index (ARI)

After de-bonding, the enamel surfaces were evaluated for orthodontic resin adhesive remaining using MARI index

↓

Interpret the results

↓

Conclusion

[4.5.4] Test to study the Tensile strength test

Hour glass shaped specimens of each adhesive Group. Photo curing procedures are performed using light curing unit

↓

Tensile Strength test was conducted using a Universal Testing machine

↓

Interpret and Conclusion

[4.5.5] Test to study the Antimicrobial effects

Discs (6mm in diameter X 2mm in height) of each orthodontic resin adhesive (with or without arginine and citrulline)

↓

Six Discs from each adhesive group were placed individually in a 24 well culture plate and immersed in 1.5 ml of brain heart infusion (BHI) culture medium with 1% sucrose

↓

The plates were then incubated at 37°C with 10% CO_2 for 7 days. Every 24 H, the culture media was aspirated and replaced with 1.5 ml of sterile BHI medium with 1% sucrose

↓

After 7 days, the resin discs are immersed in 0.9% saline solution and vortexed. 50µLof saline solution with bacteria is transferred to microtubes containing 450µL of sterile

↓

Three drops of 25µl of the dilution is dispensed in petri dish with BHI medium, they are stored at 37°C with 10% CO2 and kept for 48h

↓

CFU Is performed using Compound Microscope and arrived at Conclusion

[4.5.6] Test to study the Images from Scanning Electron Microscope

One circular Disc (6mm in diameter X 2mm in height)

↓

Digital images at 500 X and 5000 X were obtained

↓

Interpret and Conclusion

Three experimental groups were taken and compared with the control group. Group I - 2.5% wt. of Arginine with Orthocem, Group II – 2.5% wt. of Citrulline with Orthocem, and Group III – 2.5% of Arginine and Citrulline with Orthocem will be selected and evaluated for changes (if any) that can occur in their mechanical properties, surface morphology, and antimicrobial properties. The Orthocem is supplied by the FGM dental group. The Arginine and Citrulline are supplied by Ultra Pure Lab Chem Industries llp, Mumbai, Maharashtra. The materials were mixed in a homogenous state mixing the composite material and amino acid using Hauschild SpeedMixer DAC 150.1 FVZ at 3000 rpm for 1 min [Fig. 4,5]. This centrifuge uses the concept of planetary motion to rotate its container in its axis and the opposing centrifugal axis [Fig 6]. The mixed material was collected in the containers and the containers were made opaque to block out the light. [Fig. 7]

Fig 4. Hauschild SpeedMixer DAC 150.1 FVZ

Fig 5. The dual axis container holder of Hauschild SpeedMixer DAC 150.1 FVZ

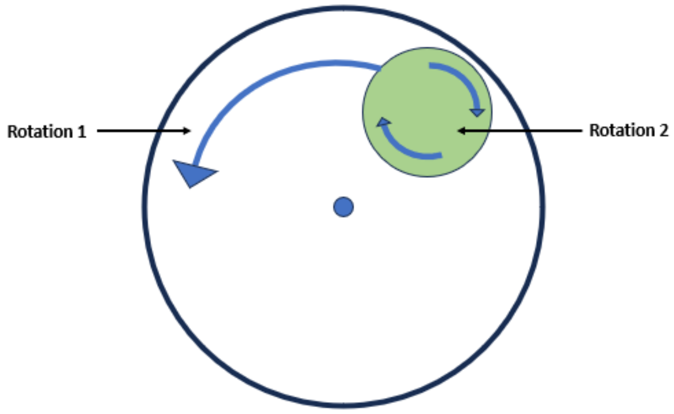


Fig 6. Principle of Dual-Axis Centrifuge. Both the rotations are opposite similar to the motion of planets around the sun

Fig 7. Mixed Adhesives stored in light-opaque containers

The enamel surface of the teeth was applied with pumice paste and polished with a rubber cup. Etching with 35% phosphoric acid for 30 seconds was done followed by washing and thorough drying. Primer was applied and light cured followed. The bracket was then bonded to the tooth surface using the adhesives and light cured for 3 seconds on each side of the bracket.[Fig. 8, 9]

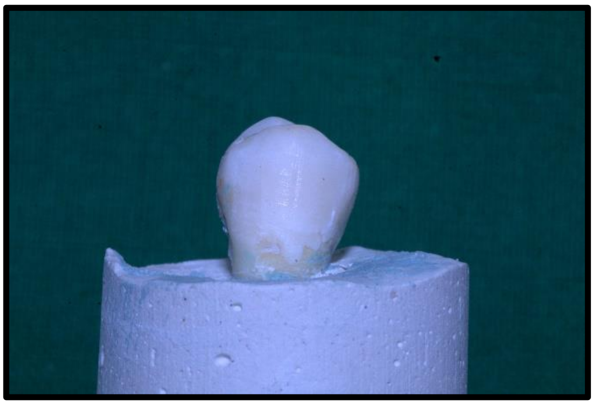


Fig 8. Extracted tooth model mounted on dental plaster after polishing with pumice

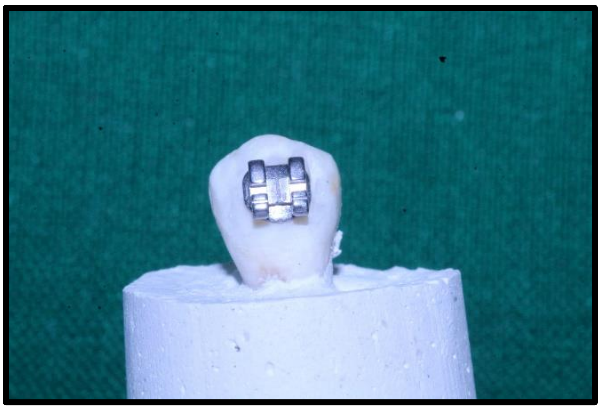


Fig 9. Bracket bonded to the etched and primed enamel surface

The Shear bond Strength test was conducted on the tooth bonded with the bracket of each group. The tooth was positioned and subjected to a crosshead speed of 1mm/min using a Universal testing machine of ACME Engineers, India., Model: UNITEST 10. The area of the bracket base was 10.50 mm^2. The shear bond strength of each sample is recorded in Newton and then divided by the area of the bracket base to obtain MPa [Fig. 10]

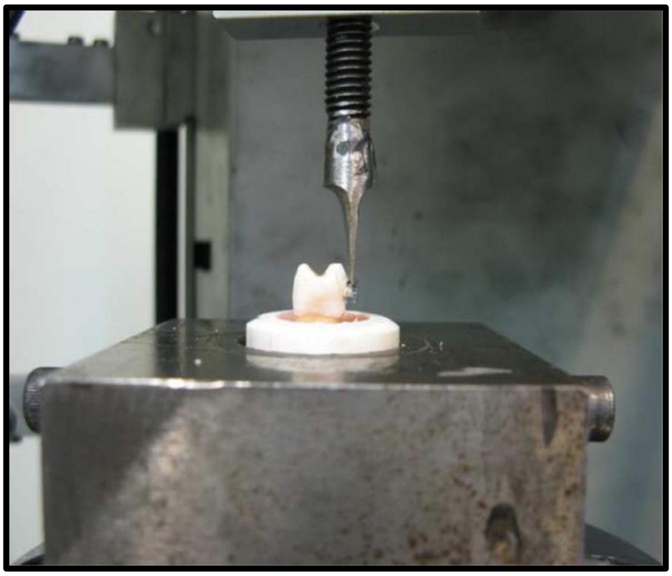


Fig 10. The tooth enamel to bracket interface is tested using the universal testing machine

After shear bond strength test, the debonded samples were subjected to test the Adhesive remnant index. The brackets which were debonded after the shear bond strength test were discarded. The enamel surfaces of the debonded teeth were evaluated and the orthodontic resin adhesive remaining was recorded using the Modified Adhesive Remnant Index (ARI). The criteria for ARI scoring are: 0, no resin adhesive on the tooth surface; 1, 1%- 25% of resin adhesive on the tooth surface; 2, 26%-50% of resin adhesive on the tooth surface; 3, 51%-75% of resin adhesive on the tooth surface; 4, 76%-99%; 5, all resin adhesive remained on the tooth surface with the impression. [Fig.11, 12].

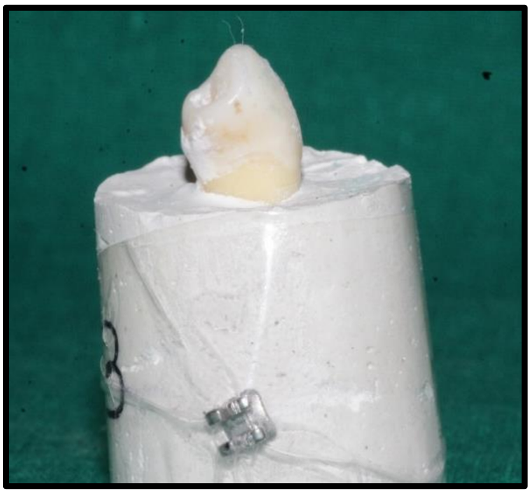


Fig 11. Tooth Sample after Shear bond test

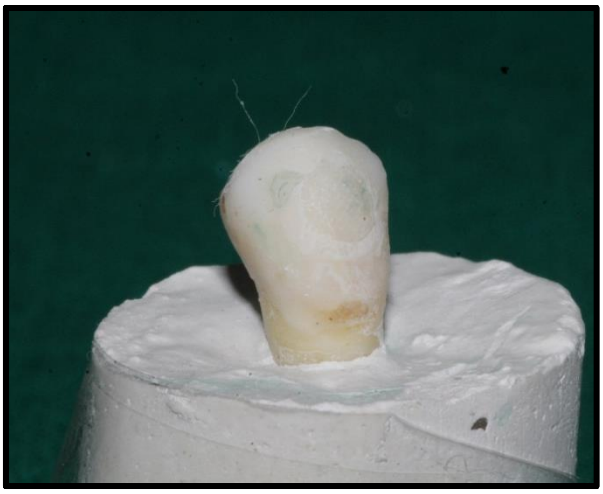


Fig 12. Composite remnant on tooth sample to be tested for ARI

The Hourglass specimens of each adhesive group (n = 10/ group) were prepared [Fig. 12]. The specimens were then submitted to a tensile strength test in a Universal Testing Machine at a 1 mm/min crosshead speed using a Universal Testing Machine manufactured by Star Testing Systems, India, Model: STS 248. The Ultimate Tensile Strength was calculated in MPa using the formula: UTS = F/A, where F is the tensile load (N) and A is the transversal cross-section area (mm^2). [Fig. 13, 14]

Fig 13. Hourglass-shaped adhesive material to be tested for tensile strength test (Set of 4, Total 10 sets)

Fig 14. Universal Testing Machine

To test the antimicrobial effect of the prepared material, discs (6 mm in diameter × 2 mm in height) of each adhesive group were made. After light curing, the specimens were left under a UV light for 15 minutes for decontamination. [Fig 15,16]

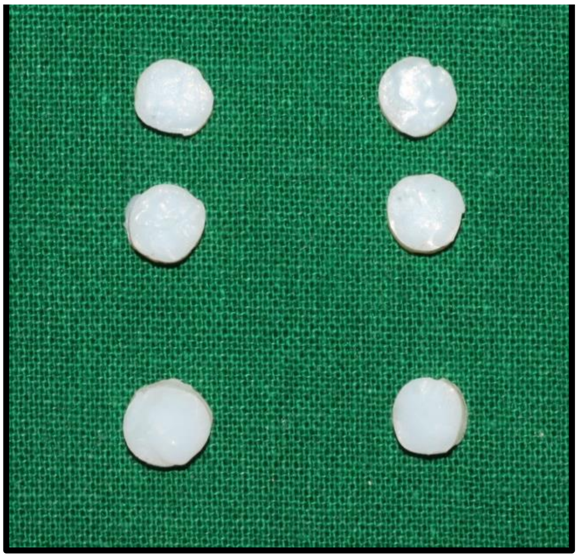


Fig. 15 Six discs of each orthodontic adhesive group were made

Fig. 16 Streptococcus Mutans colony in the culture plate

Six discs from each group were fabricated and sterilised. These discs were then placed individually in a 24-well culture plate. This plate was immersed in 1.5 mL of brain heart infusion (BHI) culture medium with 1% sucrose as the source of energy for the growth of the inoculated *Streptococcus mutans* (live strain procured from HiMedia laboratories). The plates were then incubated at 37 °C with 10% CO_2 for 7 days. Every 24 hours, the culture medium was aspirated and replaced with fresh 1.5 mL of BHI medium and 1% sucrose.

After 7 days, the resin discs were then submerged in 0.9% saline solution and vortexed. After the removal of the cells using vortex, 50 μL of the vortexed saline solution with the bacterial cells was serially diluted with 450 μL of sterile saline. Three drops of 25 μL from the diluted solution were dispensed separately into Petri dishes containing a BHI agar medium and incubated at 37 °C with 10% CO_2 for 48 hours. After 48 hours, the colony formation units (CFU) were performed and the data obtained was expressed in CFU/ml. [Fig. 16]

To Study the images obtained from the Scanning Electron Microscope, one circular sample (6 mm in diameter × 2 mm in height) of each orthodontic resin adhesive group was fabricated. Digital images at 500× and 5000x magnification were obtained, under a voltage acceleration of 20 KV, Z = 25 mm, WD = 9.8 mm, and a spot size 3.0. The images were then processed using ImageJ 1.54i, Wayne Rasband et al, National Institutes of Health, USA [Fig. 17 - 24]

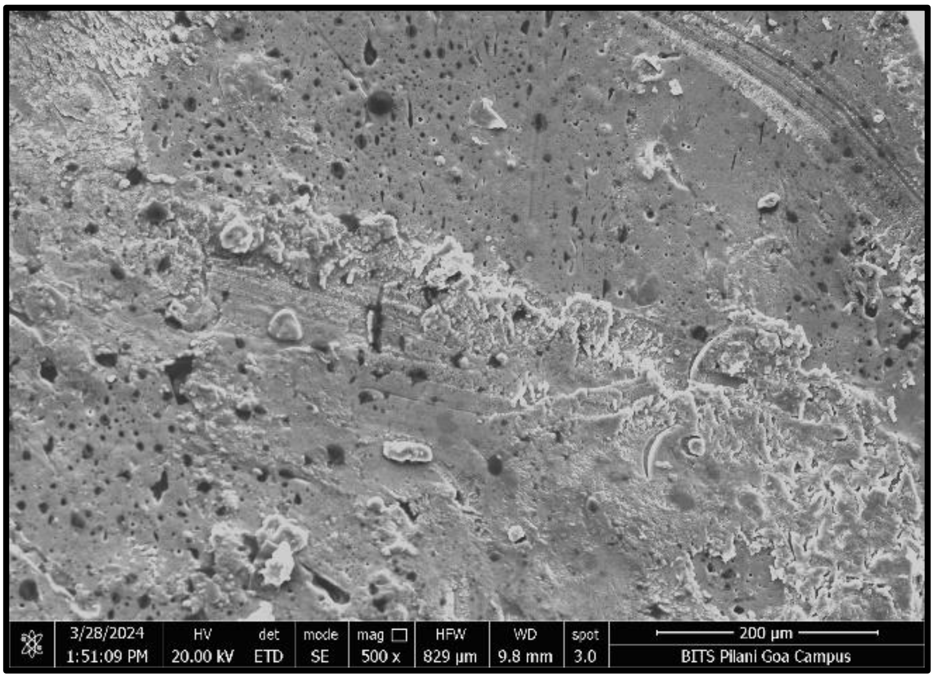


Fig. 17 SEM Image at 500x Magnification of Group with only Orthocem

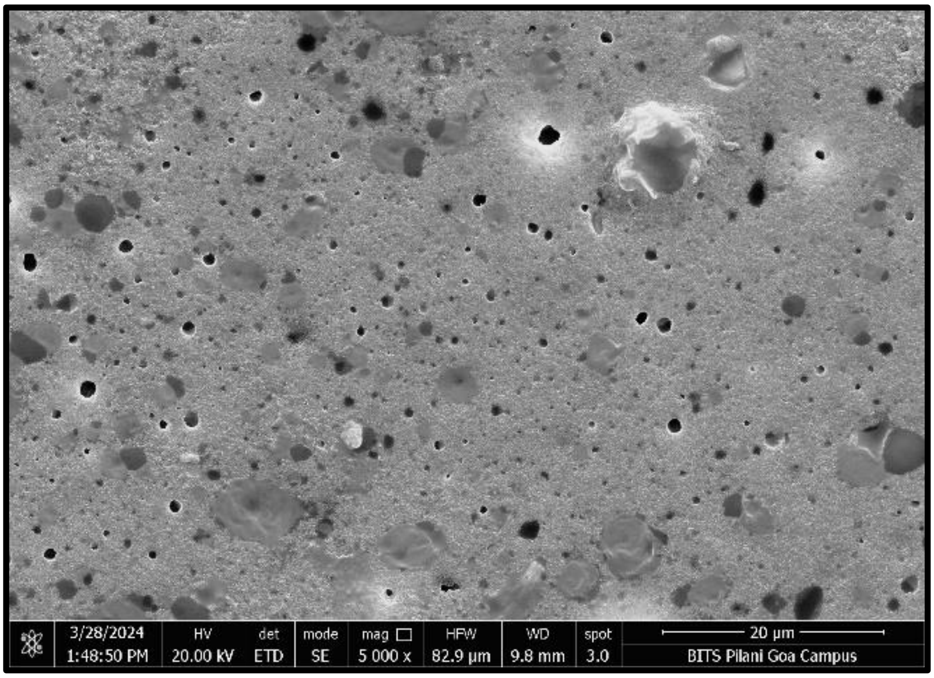


Fig. 18 SEM Image at 5000x Magnification of Group with only Orthocem

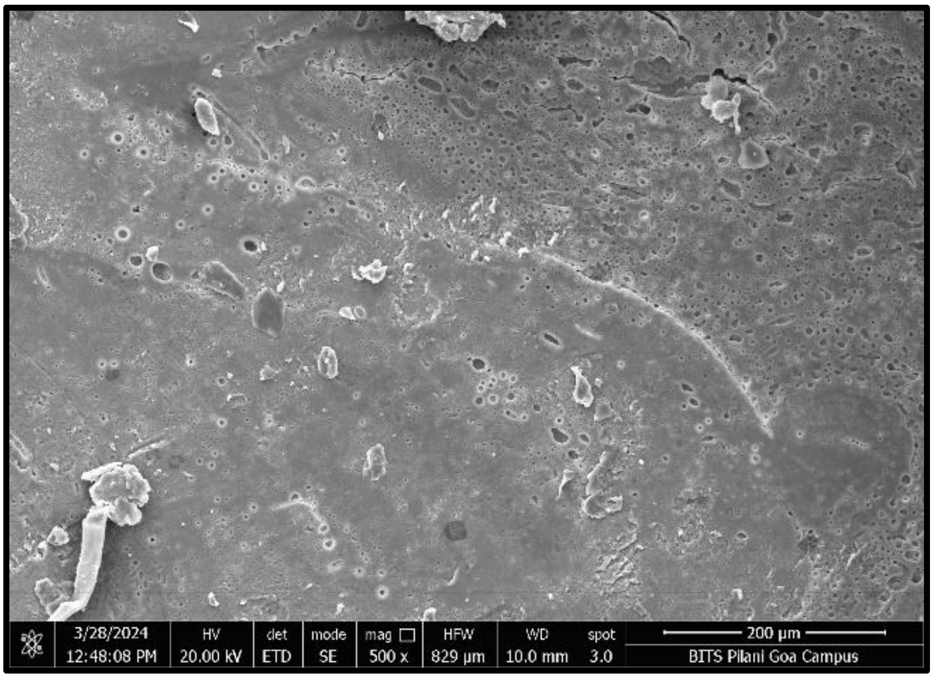


Fig. 19 SEM Image at 500x Magnification of Group with Orthocem and Arginine

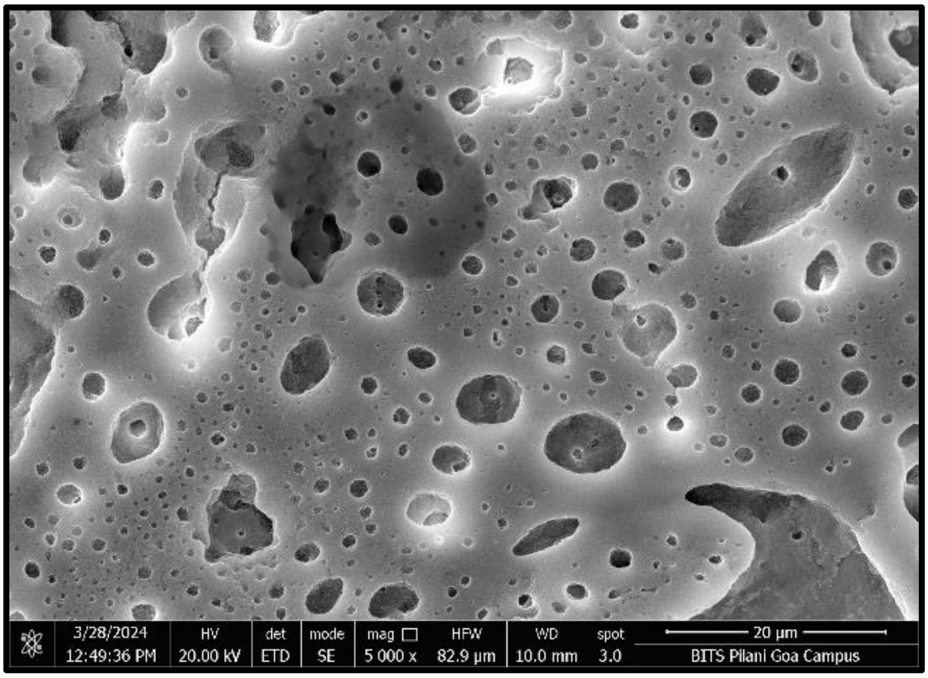


Fig. 20 SEM Image at 5000x Magnification of Group with Orthocem and Arginine

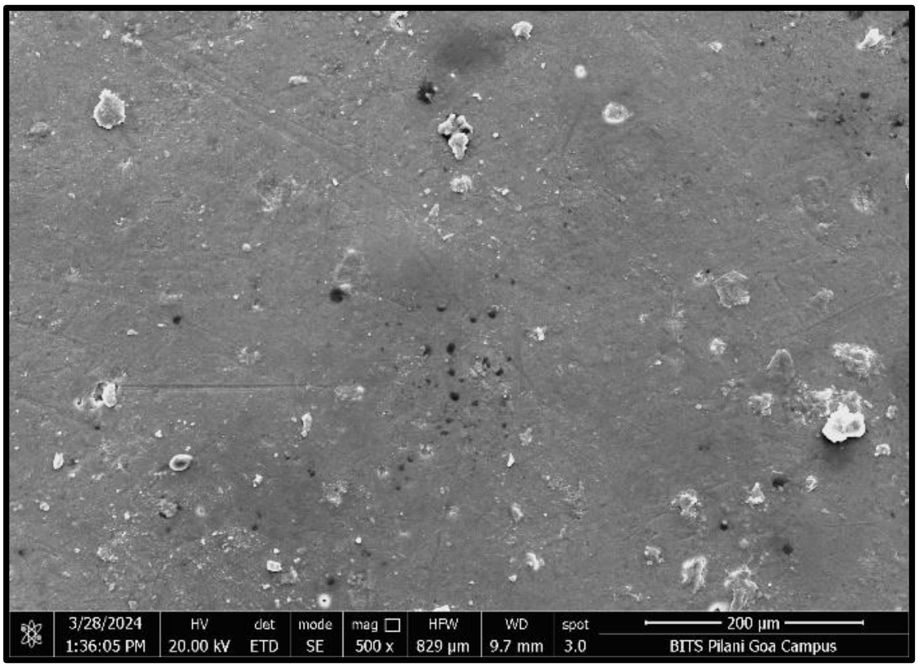


Fig. 21 SEM Image at 500x Magnification of Group with Orthocem and Citrulline

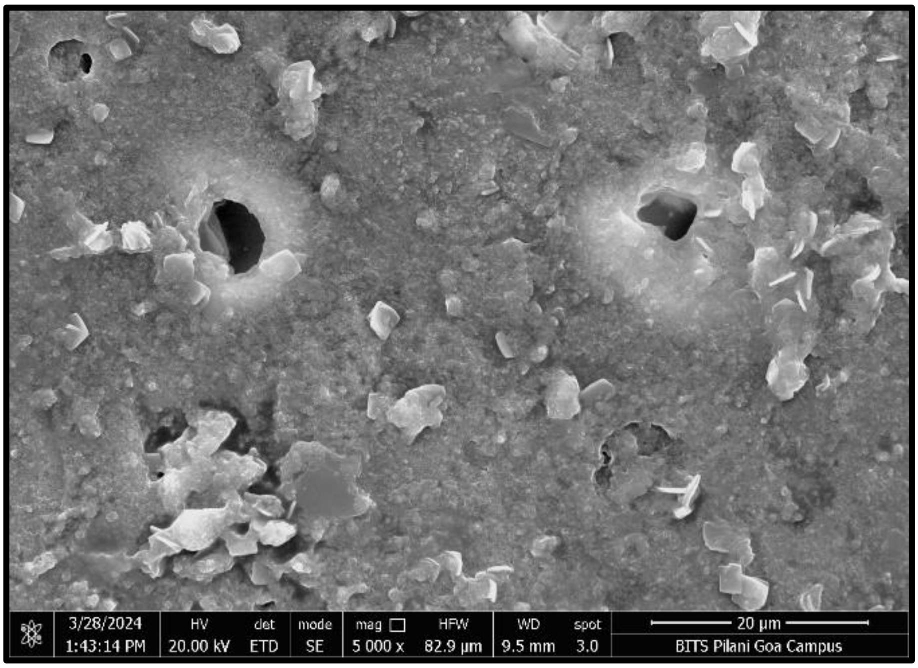


Fig. 22 SEM Image at 5000x Magnification of Group with Orthocem and Citrulline

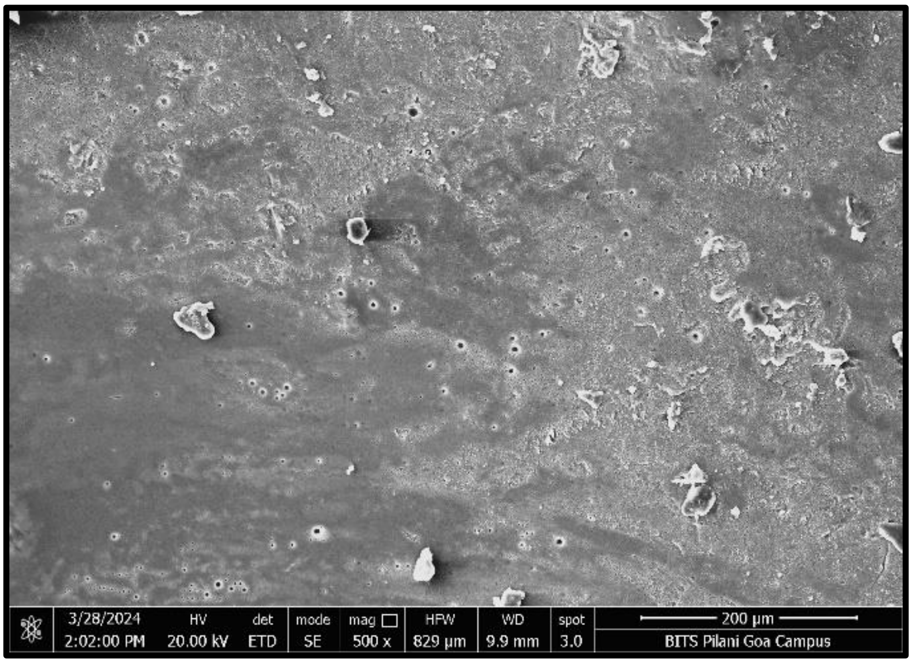


Fig. 23 SEM Image at 500x Magnification of Group with Orthocem, Arginine, and Citrulline

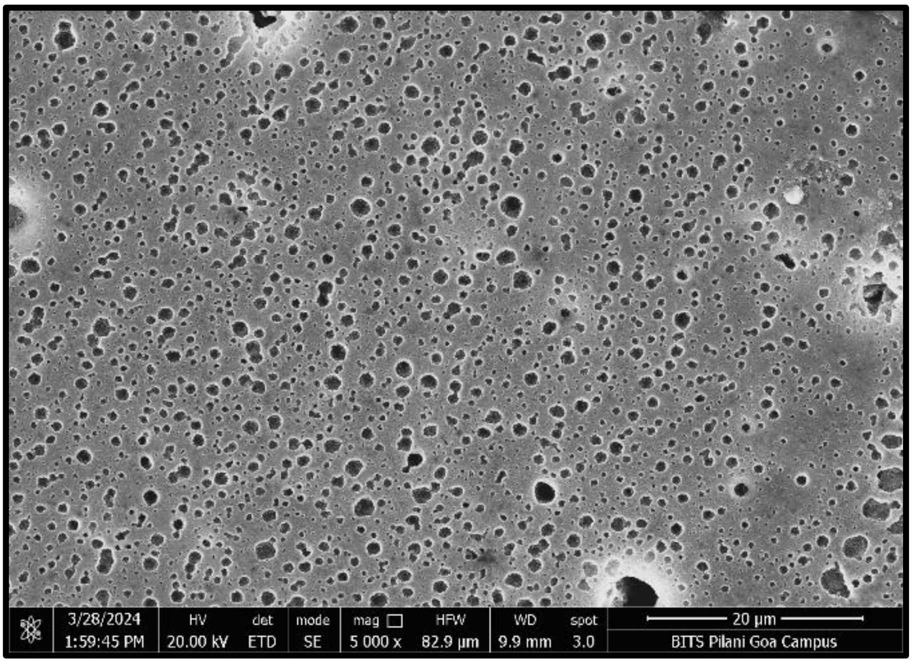


Fig. 24 SEM Image at 5000x Magnification of Group with Orthocem, Arginine, and Citrulline

Group O	Orthocem control
Group A	Orthocem with 2.5% wt. Arginine
Group C	Orthocem with 2.5% wt. Citrulline
Group A+C	Orthocem with 2.5% wt. Arginine and Citrulline

SL NO	**PROPERTIES TO BE MEASURED**	**UNIT OF MEASUREMENT**
1	Mechanical Properties	
	Shear bond strength	Megapascal (MPa)
	Tensile strength	Newton/(millimeter)^2 [N/mm^2]
2	Antibacterial test	Colony formation units (CFU/mL)

[4.6] Statistical Analysis

- Data obtained was entered and sorted in Microsoft Excel (v.2013).
- Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software (IBM Corp) (v.21.0).
- Descriptive and inferential statistics were performed for all the different parameters assessed in the study.
- A data normality test was performed to determine the normal distribution of the data.
- Intergroup comparison was performed using One-way analysis of Variance (ANOVA) to assess significant differences between different groups for continuous variables.
- Intergroup comparison was performed using Chi-square test to assess significant differences between groups for discrete variables.
- All statistical tests were performed at 95% confidence intervals.
- A p-value of less than 0.05 was considered statistically significant in the study.

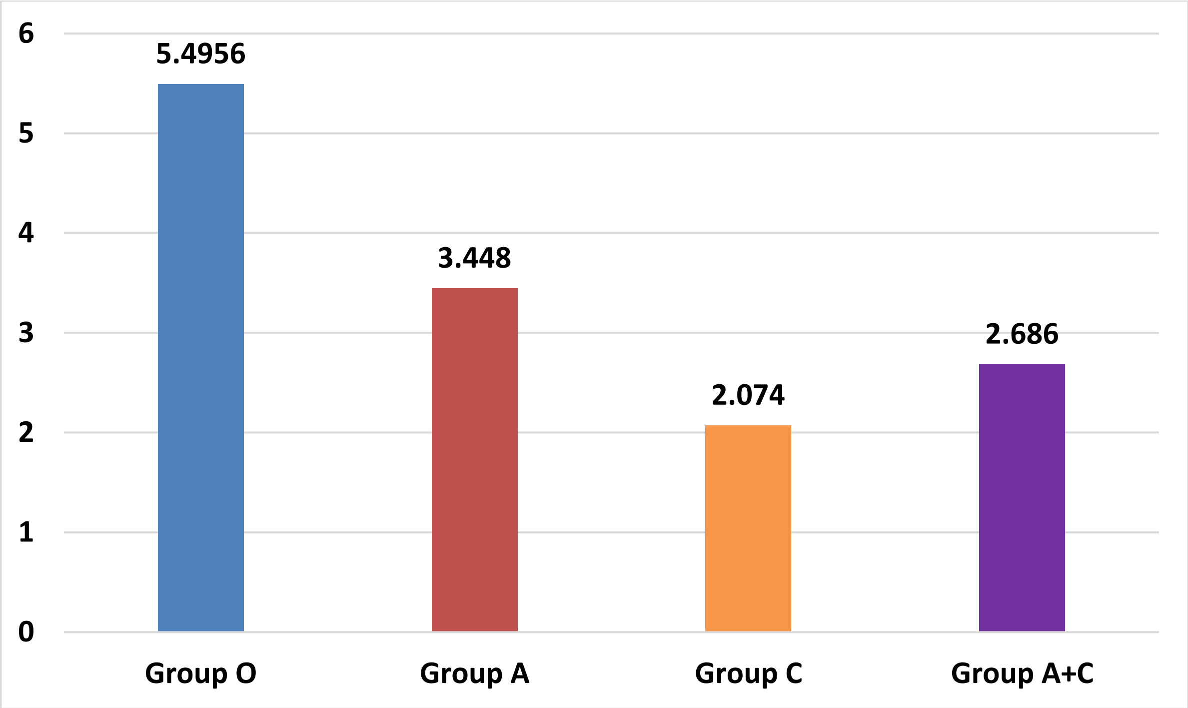
RESULTS

- This study was conducted to evaluate and compare the effects of the addition of arginine, citrulline, and the combination of Arginine and Citrulline to commercial orthodontic resin adhesive on the tensile, shear bond strength, and antimicrobial properties.
- A total of 200 specimens were randomly divided among 4 groups. Conventional metal brackets were bonded using the prepared adhesives with or without arginine, Citrulline, and the combination of Arginine and Citrulline.

Table 1 – Descriptive statistics of Shear Bond Strength (MPa) of the study samples amongst different groups.

Shear Bond Strength (MPa)	N	Minimum	Maximum	Mean	Std. Deviation
Group O	50	3.61	6.98	5.4956	.77122
Group A	50	2.20	4.10	3.4480	.39312
Group C	50	1.60	2.71	2.0740	.27329
Group A+C	50	1.33	4.18	2.6860	.64837

Graph 1: Mean Shear bond strength (MPa) of the samples among different group



- The mean Shear bond strength for the Adhesive with only Orthocem is 5.4956 ± 0.7712 MPa.
- The mean Shear bond strength for the Adhesive with Orthocem and Arginine is 3.448 ± 0.3931 MPa.
- The mean Shear bond strength for the Adhesive with Orthocem and Citrulline is 2.074 ± 0.2732 MPa.
- The mean Shear bond strength for the Adhesive with Orthocem and the combination of Arginine and Citrulline is 2.6860 ± 0.6483 MPa.

It can therefore be concluded that

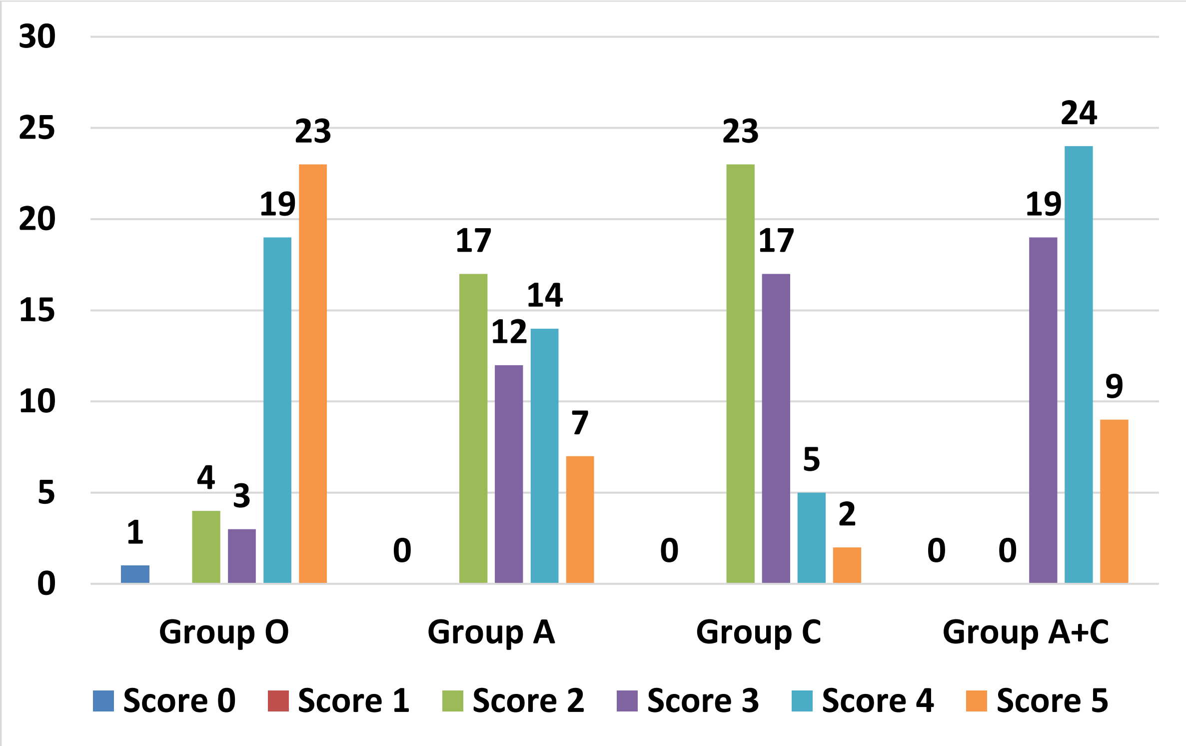
- The Group with Orthocem and Citrulline showed the least shear bond strength compared to the Control group with Orthocem.
- The Group with Orthocem and Arginine showed having the most shear bond strength compared to other experimental groups.

Table 2 shows the descriptive scoring of the ARI based on the Modified Adhesive Remnant Index by Bishara SE and Trulove TS (1990)

Table 2 – Frequency distribution of the study samples according to Adhesive remnant index scores amongst different groups

Group	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5	Total
Group O	1	0	4	3	19	23	50
Group A	0	0	17	12	14	7	50
Group C	0	0	23	17	5	2	50
Group A+C	0	0	0	19	24	9	50

Graph 2: Frequency distribution of the study samples according to Modified Adhesive Remnant index scores amongst different groups



- The frequency of the distribution of the study samples according to Adhesive remnant index scores amongst different groups show
  - The Control Group with Orthocem showed a majority of the samples displaying Score 5 (n=23) followed by Score 4 (n=4)
  - Group with Adhesive and Arginine showed a majority of samples displaying Score 2 (n=17) followed by Score 4 (n=14) and Score 3 (n=12).
  - Group with Adhesive and Citrulline showed a majority of samples displaying Score 2 (n=23) followed by Score 3 (n=17)

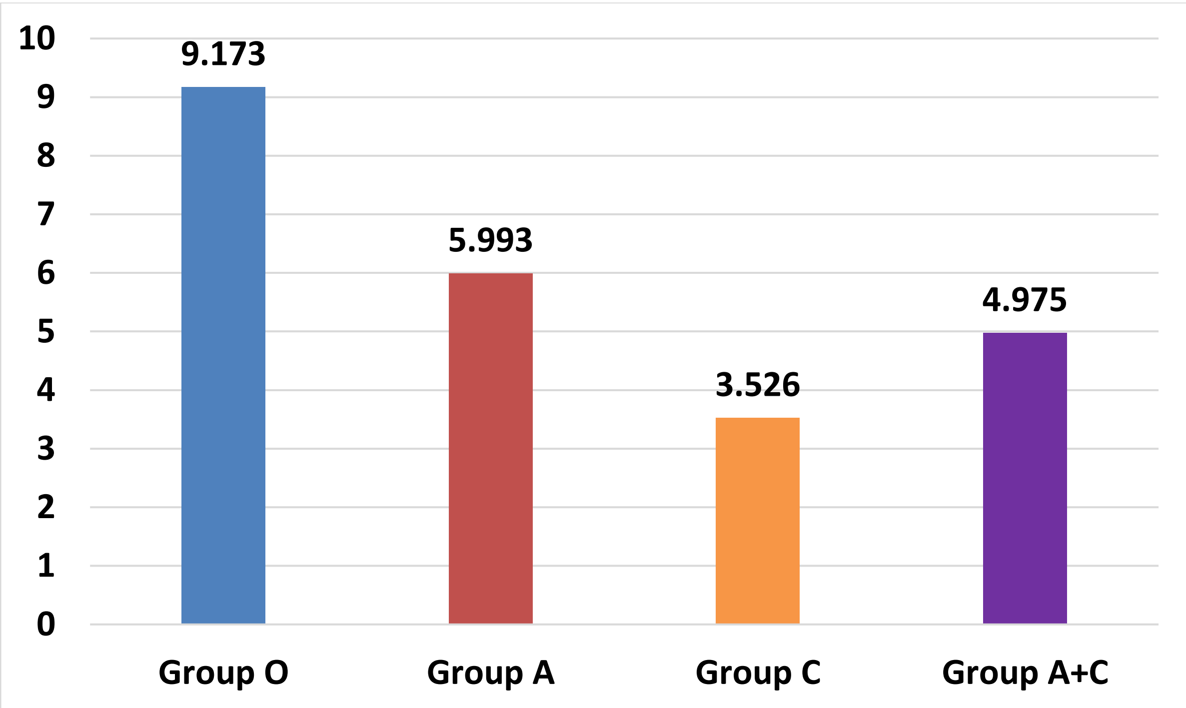
  - Group with Adhesive and the combination of Arginine and Citrulline showed a majority of samples displaying Score 4 (n=24) followed by Score 3 (n=19).

It can therefore be concluded that

- The group with Orthocem showed maximum samples showing complete composite retained on the surface with the impression of the bracket base.
- The group with Orthocem and Arginine and the Group with Orthocem and Citrulline showed maximum samples showing 26% to 50% of adhesives retained on the surface of the tooth
- the Group with Orthocem and the combination of arginine and citrulline, maximum samples showing 76% to 99% of adhesives retained on the surface of the tooth.

Table 3 – Descriptive statistics of Tensile Strength (MPa) of the study samples among different groups					
Tensile Strength (MPa)	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Group O	10	6.01	10.70	9.1730	1.57949
Group A	10	4.60	6.83	5.9930	.81023
Group C	10	2.75	4.75	3.5260	.64054
Group A+C	10	2.21	6.96	4.9750	1.54204

Graph 3: Mean Tensile Strength (MPa) of the study samples among different groups



- The mean Tensile strength for the Adhesive with only Orthocem is 9.1730 ± 1.5794 MPa.
- The mean Tensile strength for the Adhesive with Orthocem and Arginine is 5.9930 ± 0.8102 MPa.
- The mean Tensile strength for the Adhesive with Orthocem and Citrulline is 3.5260 ± 0.6405 MPa.
- The mean Tensile strength for the Adhesive with Orthocem and the combination of Arginine and Citrulline is 4.9750 ± 1.5420 MPa.

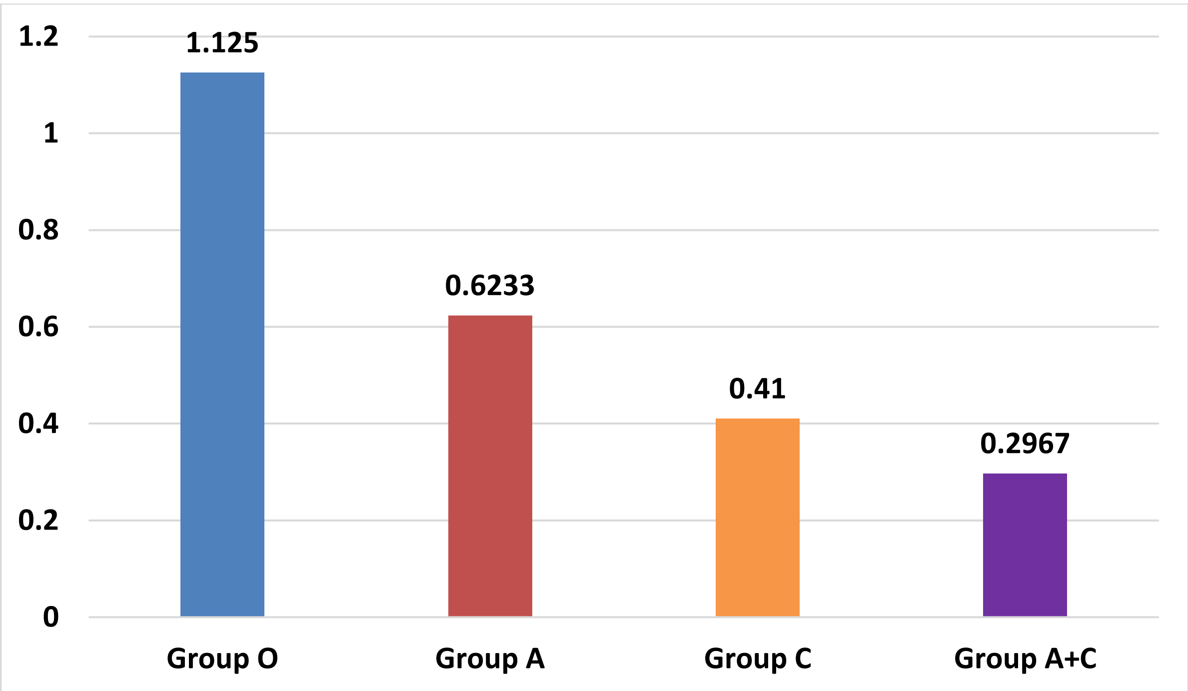
It can therefore be concluded that

- The Group with Orthocem and Citrulline showed the least Tensile strength compared to the Control group with Orthocem.
- The Group with Orthocem and Arginine showed having the most Tensile strength compared to other experimental groups.

Table 4 – Descriptive statistics of antimicrobial test (*10^5 CFU/ml) of the study samples amongst different groups

Antimicrobial test (*105 CFU/ml)	N	Minimum	Maximum	Mean	Std. Deviation
Group O	6	1.02	1.20	1.1250	.06686
Group A	6	0.43	0.72	0.6233	.10985
Group C	6	0.30	0.54	0.4100	.09508
Group A+C	6	0.23	0.38	0.2967	.04967

Graph 4: Mean CFU (*10^5 CFU/ml) of the study samples among different groups



- The mean CFU/ml for the Adhesive with only Orthocem is 1.1250 ± 0.0668 $*10^5$ CFU/ml.
- The mean CFU/ml for the Adhesive with Orthocem and Arginine is 5.9930 ± 0.8102 $*10^5$ CFU/ml.
- The mean CFU/ml for the Adhesive with Orthocem and Citrulline is 3.5260 ± 0.6405 $*10^5$ CFU/ml.
- The mean CFU/ml for the Adhesive with Orthocem and the combination of Arginine and Citrulline is 4.9750 ± 1.5420 $*10^5$ CFU/ml.

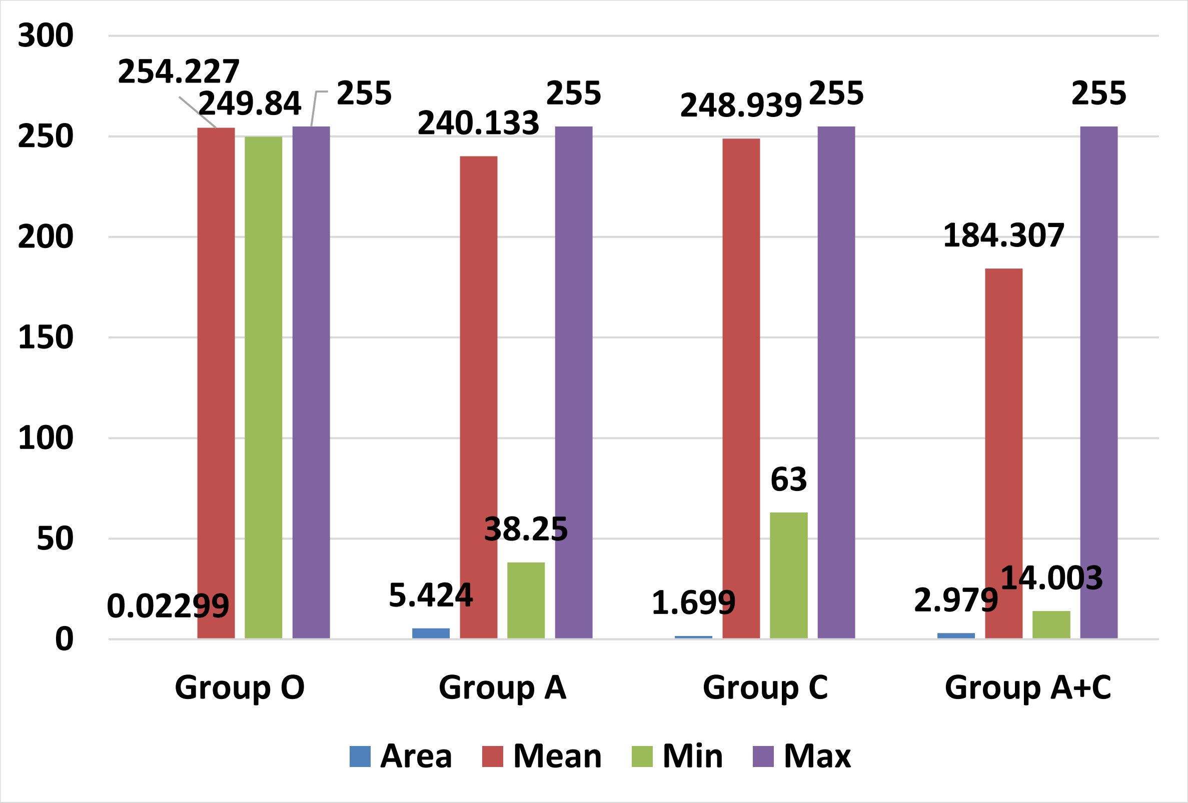
It can therefore be concluded that

- The group with only citrulline showed the most promising result compared to other experimental groups

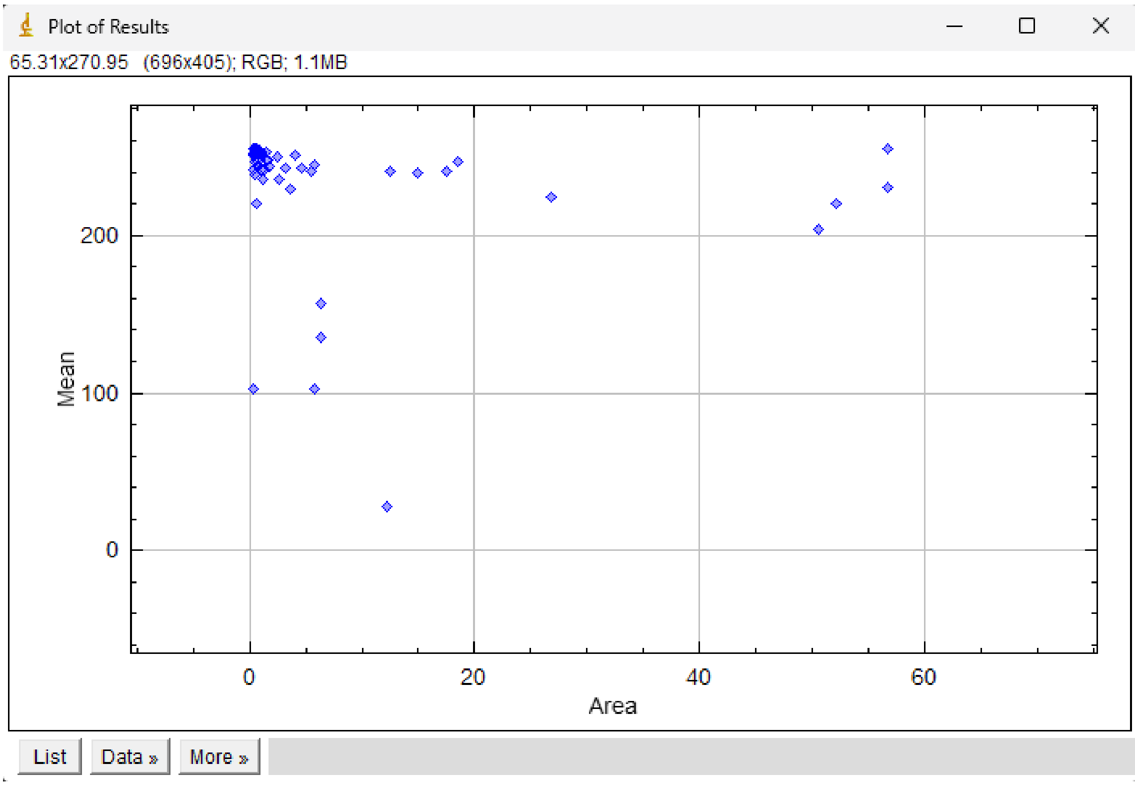
Table 5 – Descriptive statistics of the mean particle size of samples [5000x] among different groups

Groups	Particle size	Area	Mean	Min	Max
Group O	**Mean**	0.02299	254.227	249.8485	255
	SD	11.211	23.672	87.238	0
	Min	0.003	211.73	0	255
	Max	1.774	255	255	255
Group A	**Mean**	5.424	240.133	38.25	255
	SD	12.148	27.535	91.822	0
	Min	0.333	102.966	0	255
	Max	56.634	255	255	255
Group C	**Mean**	1.699	248.939	63	255
	SD	3.81	8.24	110.635	0
	Min	0.194	220.874	0	255
	Max	27.467	255	255	255
Group A+C	**Mean**	2.979	184.307	14.003	255
	SD	8.089	49.834	58.176	0
	Min	0.441	109.149	0	255
	Max	126.208	255	255	255

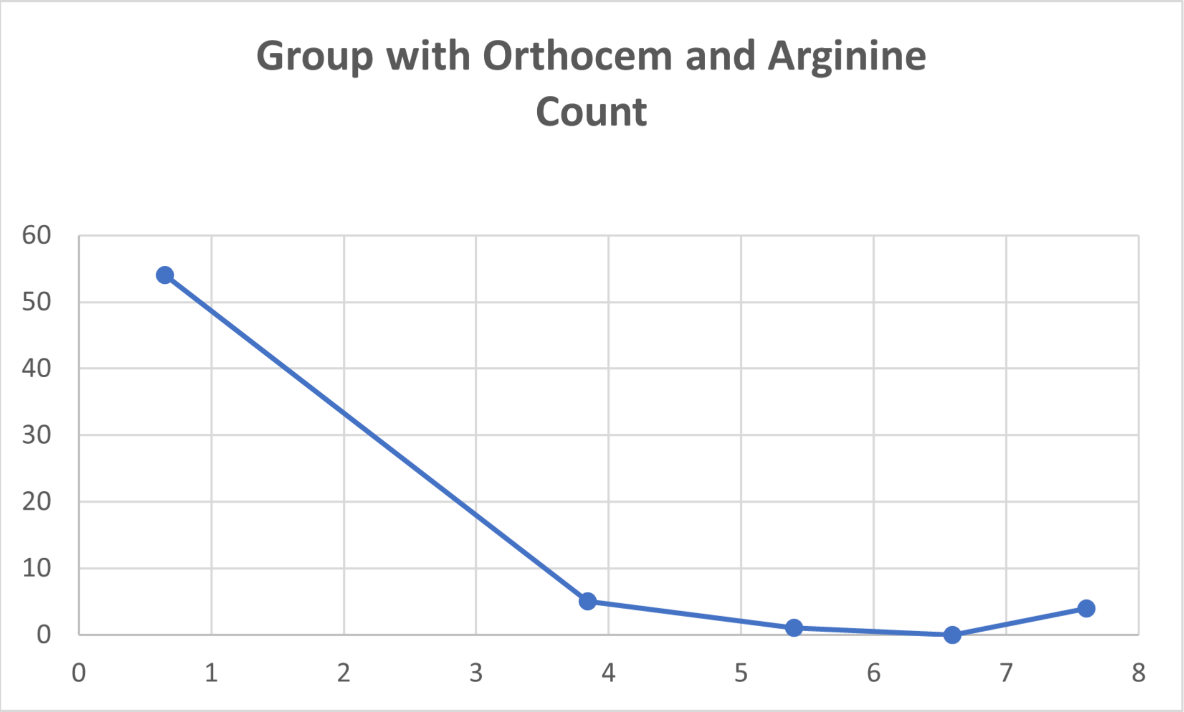
Graph 5 –Mean of the parameters of mean particle size [5000x] among different groups



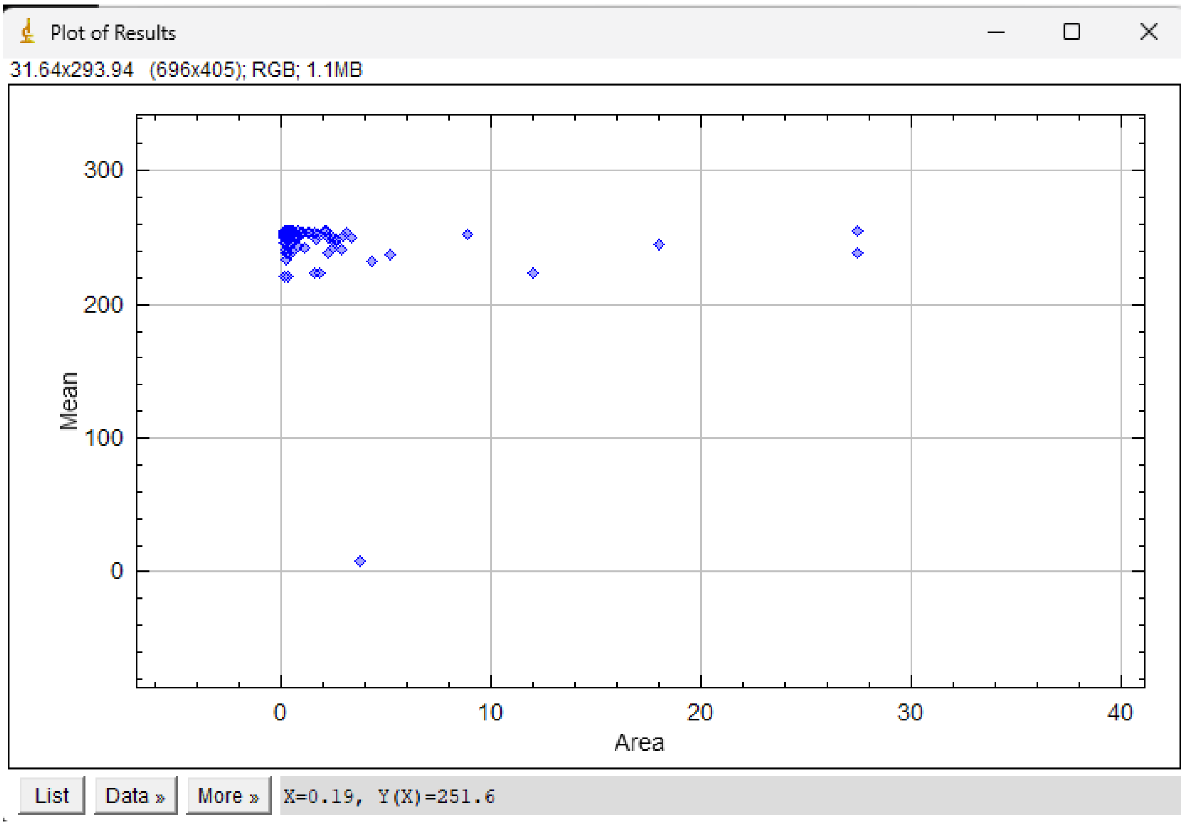
Graph 5.1: Plot of Area of particles obtained from the Scanning Electron Microscope of Group with Orthocem and Arginine (Mean particle size: 5.424 ± 12.148 µm).



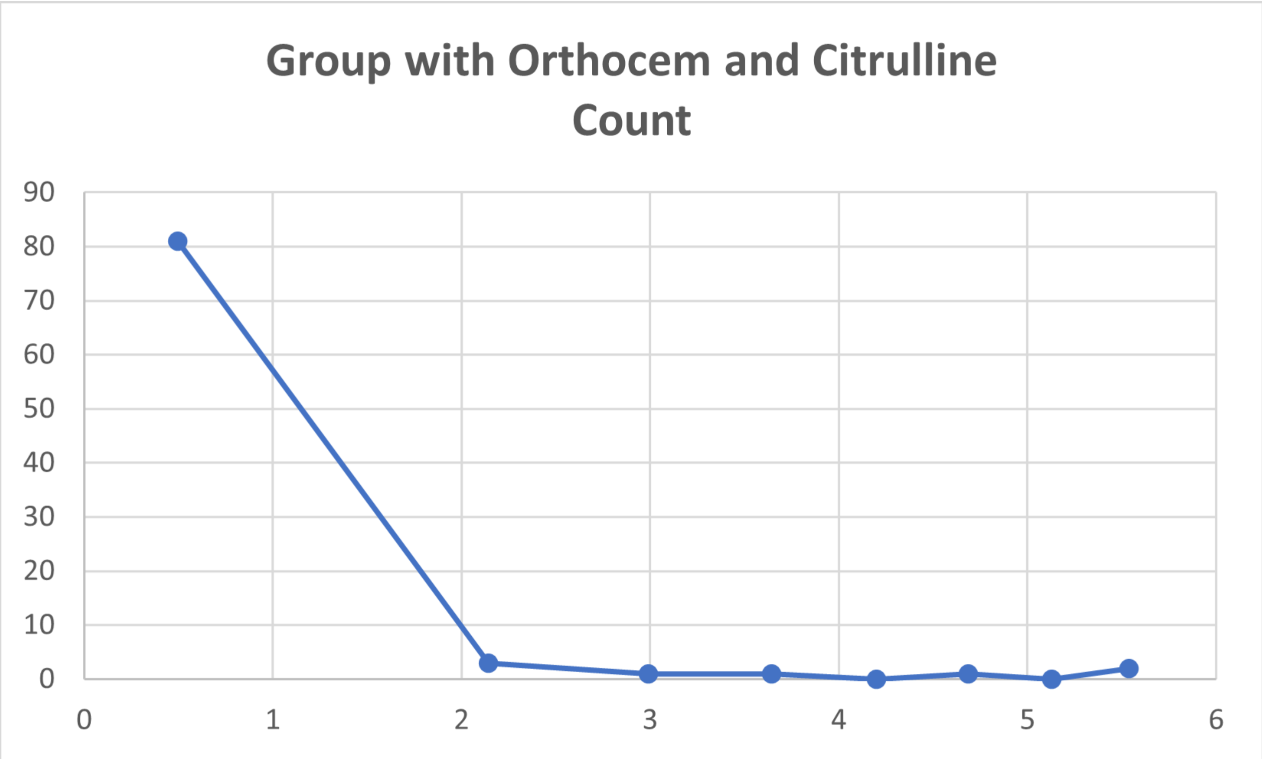
Graph 5.2: Distribution curve of particles obtained from the Scanning Electron Microscope of Group with Orthocem and Arginine (Mean particle size: 5.424 ± 12.148 µm)



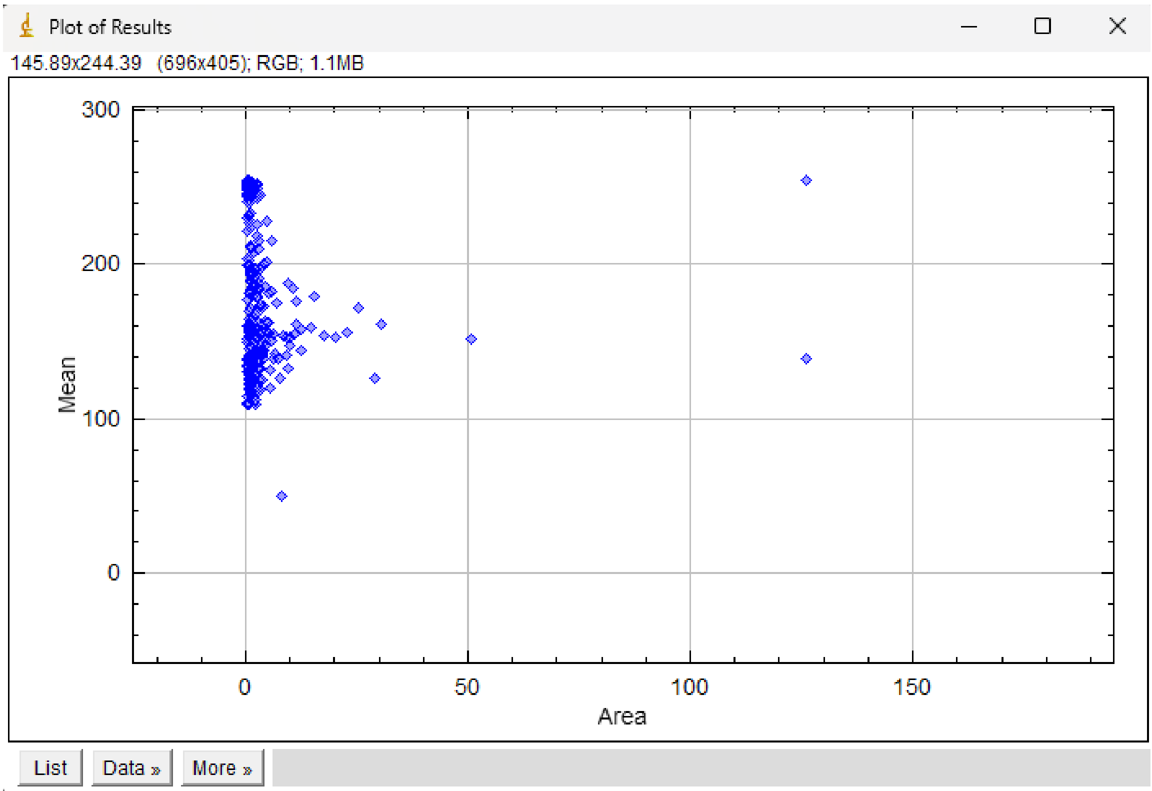
Graph 5.3: Plot of Area of particles obtained from the Scanning Electron Microscope of Group with Orthocem and Citrulline (Mean particle size: 1.699 ± 3.81 µm).



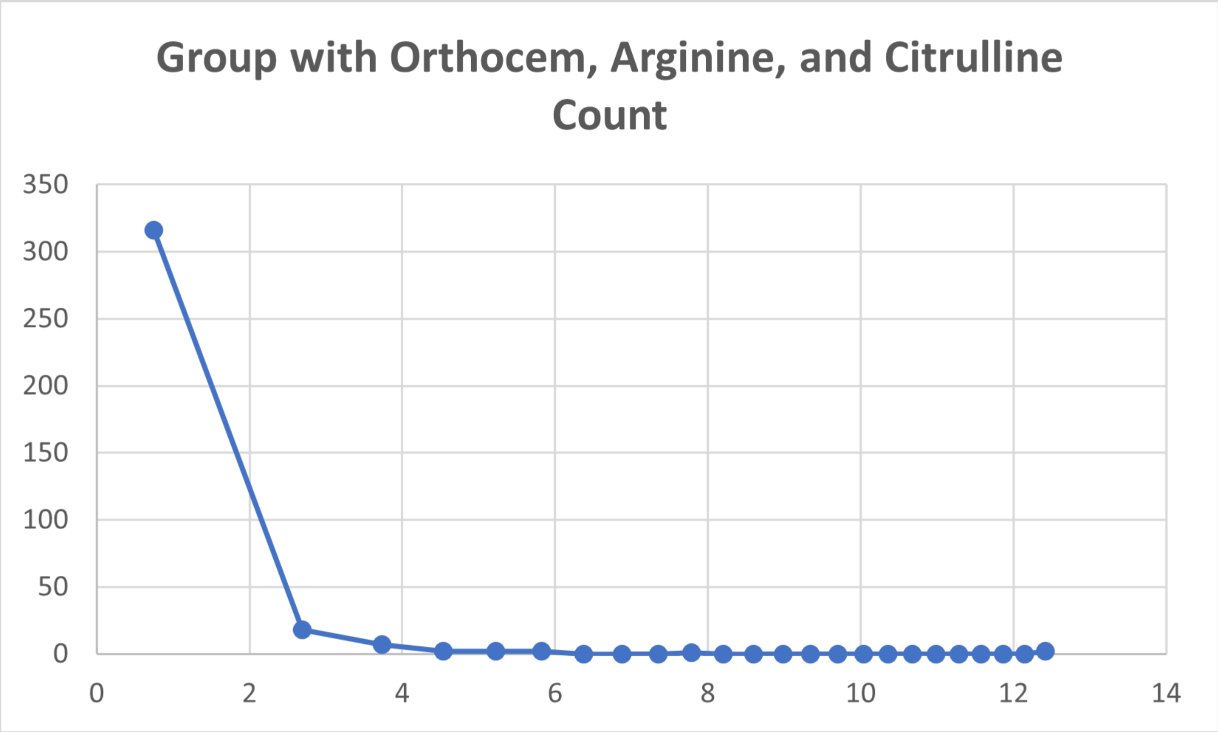
Graph 5.4: Distribution curve of particles obtained from the Scanning Electron Microscope of Group with Orthocem and Citrulline (Mean particle size: 1.699 ± 3.81 µm)



Graph 5.5: Plot of Area of particles obtained from the Scanning Electron Microscope of Group with Orthocem, Arginine, and Citrulline (Mean particle size: 2.979 ± 8.089 µm)



Graph 5.6: Distribution curve of particles obtained from the Scanning Electron Microscope of Group with Orthocem, Arginine, and Citrulline (Mean particle size: 2.979 ± 8.089 µm)



- The mean particle size for the Adhesive with only Orthocem is 0.0229 ± 11.211 μm.
- The mean particle size for the Adhesive with Orthocem and Arginine is 5.424 ± 12.148 μm.
- The mean particle size for the Adhesive with Orthocem and Citrulline is 1.699 ± 3.81 μm.
- The mean particle size for the Adhesive with Orthocem and the combination of Arginine and Citrulline is 2.979 ± 8.089 μm.
- The Graphs of the plot of area and particle distribution curves represent the above findings.

It can therefore be concluded that

- The maximum mean area was seen with Group with Adhesive and Arginine.
- The minimum mean area was seen with Group with Adhesive and Citrulline followed by Group with Adhesive and the combination of Arginine and Citrulline

.

Table 6- Intergroup comparison of the Shear Bond Strength (MPa) between different group

Comparison groups	Sum of Squares	df	Mean Square	F	p-value
Group O vs Group A vs Group C vs Group A+C	332.962	3	110.987	356.759	.000*

*p value <0.05 statistically significant, <0.01 highly significant

Interpretation – In our study, Intergroup comparison of the Shear Bond Strength (MPa) between different groups was performed using One-way Analysis of Variance (ANOVA). This comparison showed statistically significant differences (p-value <0.05) between the shear bond strength of all the groups

Table 7- Pairwise multiple post hoc Intergroup comparison of the Shear Bond Strength (MPa) between different groups

(I) Groups	(J) Comparison Groups	Mean Difference (I-J)	p-value
Group O	**Group A**	2.04760*	.001*
	Group C	3.42160*	.000*
	Group A+C	2.80960*	.000*
Group A	**Group O**	-2.04760*	.001*
	Group C	1.37400*	.000*
	Group A+C	.76200*	.000*
Group C	**Group O**	-3.42160*	.000*
	Group A	-1.37400*	.000*
	Group A+C	-.61200*	.000*
Group A+C	**Group O**	-2.80960*	.000*
	Group A	-.76200*	.000*
	Group C	.61200*	.000*

*p value <0.05 statistically significant, <0.01 highly significant

Interpretation – In our study, Pairwise multiple post hoc Intergroup comparison of the Shear Bond Strength (MPa) between different groups was performed using Tukey's post hoc test. This comparison showed statistically significant differences (p-value <0.05) for shear bond strength between all the groups.

Table 8- Intergroup comparison of the Adhesive remnant index between different groups

Group	Comparison groups	Chi-square test p-value
Group O	**Group A**	.001*
	Group C	.021*
	Group A+C	.000*
Group A	**Group O**	.001*
	Group C	.001*
	Group A+C	.000*
Group C	**Group O**	.0021*
	Group A	.001*
	Group A+C	.341
Group A+C	**Group O**	.000*
	Group A	.000*
	Group C	.341

*p value <0.05 statistically significant, <0.01 highly significant

Interpretation – In our study, Intergroup comparison of the Adhesive remnant index between different groups was performed using Chi-square test. This comparison showed statistically significant differences (p-value <0.05) for index scores between all the groups; except for group A+C and group C.

Table 9- Intergroup comparison of the Tensile Strength (MPa) between different groups

Comparison groups	Sum of Squares	df	Mean Square	F	p-value
Group O vs Group A vs Group C vs Group A+C	172.116	3	57.372	38.638	.023*

*p value <0.05 statistically significant, <0.01 highly significant

Interpretation – In our study, Intergroup comparison of the Tensile Strength (MPa) between different groups was performed using One-way Analysis of Variance (ANOVA). This comparison showed statistically significant differences (p-value <0.05) between the Tensile strength of all the groups

Table 10- Pairwise multiple post hoc Intergroup comparison of the Tensile Strength (MPa) between different groups

(I) Groups	(J) Comparison Groups	Mean Difference (I-J)	p-value
Group O	**Group A**	3.18000*	.000*
	Group C	5.64700*	.000*
	Group A+C	4.19800*	.000*
Group A	**Group O**	-3.18000*	.000*
	Group C	2.46700*	.000*
	Group A+C	1.01800	.260
Group C	**Group O**	-5.64700*	.000*
	Group A	-2.46700*	.000*
	Group A+C	-1.44900	.054
Group A+C	**Group O**	-4.19800*	.000*
	Group A	-1.01800	.260
	Group C	1.44900	.054

*p value <0.05 statistically significant, <0.01 highly significant

Interpretation – In our study, Pairwise multiple post hoc Intergroup comparison of the Tensile Strength (MPa) between different groups was performed using Tukey's post hoc test. This comparison showed statistically significant differences (p-value <0.05) for Tensile strength between all the groups.

Table 11- Intergroup comparison of antimicrobial test (*10^5 CFU/ml) between different groups

Comparison groups	Sum of Squares	df	Mean Square	F	p-value
Group O vs Group A vs Group C vs Group A+C	2.421	3	.807	115.115	.001*

*p value <0.05 statistically significant, <0.01 highly significant

Interpretation – In our study, an Intergroup comparison of the antimicrobial test (*10^5 CFU/ml) between different groups was performed using One-way Analysis of Variance (ANOVA). This comparison showed statistically significant differences (p-value <0.05) between the Antimicrobial properties of all the groups

Table 12- Pairwise multiple post hoc Intergroup comparison of the antimicrobial test (*10^5 CFU/ml) between different groups

(I) Groups	(J) Comparison Groups	Mean Difference (I-J)	p-value
Group O	**Group A**	.50167*	.000*
	Group C	.71500*	.000*
	Group A+C	.82833*	.000*
Group A	**Group O**	-.50167*	.000*
	Group C	.21333*	.001*
	Group A+C	.32667*	.000*
Group C	**Group O**	-.71500*	.000*
	Group A	-.21333*	.001*
	Group A+C	.11333	.121
Group A+C	**Group O**	-.82833*	.000*
	Group A	-.32667*	.000*
	Group C	-.11333	.121

*p value <0.05 statistically significant, <0.01 highly significant

Interpretation – In our study, Pairwise multiple post hoc Intergroup comparison of the Antimicrobial Test (*10^5 CFU/ml) between different groups was performed using Tukey's post hoc test. This comparison showed statistically significant differences (p-value <0.05) for Antimicrobial properties between all the groups.

DISCUSSION

[6.1] Rational Behind the Study

This in-vitro study was conducted to incorporate the antimicrobial properties by adding the amino acids, arginine and citrulline, to the orthodontic resin adhesive. The added orthodontic resin was then compared to the control orthodontic adhesive without any additions.

Since the study involved the addition of new components to the orthodontic adhesive, changes in its physical properties were to be assessed, with the understanding that there should not be any significant changes to the already established physical properties of the commercially available adhesive.

Although the use of arginine has been debated since the early 1980s, its actual application in dentistry has yet to be established. There have been several researchers from various fields who have studied the effects of arginine. This study was one such attempt to understand the effects of arginine, citrulline and the combination of arginine and citrulline and incorporate the amino acid into the adhesives

[6.2] Incorporation of Amino acids into the adhesive system

The per cent weight of amino acid to be mixed with adhesive was calculated. The amino acids and adhesives were carefully weighed using an analytical weighing scale. The two components were of different states of matter, a Semi-solid/quasi-solid orthodontic adhesive and Solid amino acids. To incorporate the amino acids into the orthodontic adhesive, there was a need to achieve a homogenous mixture. To obtain a homogenous mix, a dual-axis centrifugal mixing device had to be used. this device was obtained through MSN Enterprises (Thane), which handles the distribution of the Hauschild Speed Dual Axis Centrifugal mixer (150.1 FVZ). This centrifuge uses the concept of planetary motion to rotate its container in its axis and in the opposing centrifugal axis [Fig 6]. A unique holder was made specifically for this study as the materials to be mixed were of smaller quantities. The materials were placed in the mixing container and the weight was rechecked. The centrifugal mixing device was run at 3000 rpm for 1 minute at a temperature below 37℃. The mixed material was then stored in a light-opaque container to prevent light contamination, which could have caused undesirable polymerization. This process resulted in a homogenized mixture of amino acids and the adhesive on which further tests were done.

[6.3] Interpretation of Physical Properties

[6.3.1] Interpretation of Shear bond strength

Jain M et al reported that the values of shear bond strength of adhesives non-specifically were in the range of 3.2 MPa to 41.3 MPa. In 1975, Reynolds pointed out that in the in-vitro setting a 6 MPa to 8 MPa is clinically acceptable, therefore, this range was considered standard. However, this range has never been tested clinically to ascertain this range as the Gold standard. Thus, the authors considered that using such standard values is to be taken cautiously. [23]

Silva et al conducted a study where the investigation was done on the shear bond strength of metal and ceramic orthodontics brackets bonded with the different orthodontic adhesives. They reported that the shear bond strength values for Orthocem on the metal bracket revealed mean values of 7.8 MPa with a standard deviation of 3.6. [24]

In the current study, we compared and evaluated the shear bond strength for the four groups. We discovered that the group with Orthocem and Arginine [3.44 ± 0.393 MPa] had the greater shear bond strength among all the experimental groups, but lesser than the Control group [5.49 ± 0.771 MPa]. The least SBS was shown in the group with Orthocem and Citrulline [2.07 ± 0.273 MPa] followed by the group with Orthocem and the combination of Arginine, and Citrulline [2.68 ± 0.648 MPa] [Table 1]. This variation in the SBS test with the addition of arginine was relatively acceptable. The addition of Citrulline to the adhesive showed a significant 40% reduction in SBS when compared to the control group. [Graph 1]

[6.3.2] Interpretation of Tensile strength

Reynolds IR reviewed direct orthodontic bonding and reported that the recommended range of tensile strength was 5.9-7.8 MPa. The range given by Reynolds is widely accepted and several studies have followed the same range to evaluate tensile strength.[25]

In the current study, we compared and evaluated the tensile strength for four groups. We discovered similar to shear bond strength the group with Orthocem and Arginine [5.9930 ± 0.8102 MPa] had the greatest tensile strength among all the experimental groups, but lesser than the control group [9.1730 ± 1.5794 MPa]. The least tensile strength was shown in the group with Orthocem and Citrulline [3.5260 ± 0.6405 MPa] followed by the group with Orthocem and the combination of Arginine and Citrulline [4.9750 ± 1.5420 MPa] [Table 3]. This variation in the tensile strength in the Tensile strength test with the addition of arginine was relatively acceptable. The addition of citrulline to the adhesives showed a significant 40% reduction in tensile strength test when compared to the control group [Graph 3]. The intergroup comparison of the groups of Adhesive and the combination of Arginine and Citrulline with the Group with adhesive and Arginine and the group with Adhesive and Citrulline was not significant [Table 10].

The tests to evaluate the Physical properties of experimental adhesives in the current study show that the first Null hypothesis is rejected due to the alteration of the physical properties from the control group due to the addition of the amino acids. However, the addition of arginine compound to the adhesive shows promising results and is closer to the accepted values. The SEM explains the significant reduction in the physical properties due to the addition of the citrulline. This could be attributed to the inability to form a homogenous mixture with Orthocem because of citrulline's crystalline nature. Further research in this aspect should be carried out to study the unfavourable physical properties after the addition of the amino acids.

[6.4] Interpretation of Adhesive Properties using Adhesive Remnant Index

In the current study, the adhesive property of the adhesive groups was studied using the Modified Adhesive Remnant Index. The Modified Adhesive Remnant Index was developed by Bishara and Trulove in the year 1990 [26]. Similar to the original ARI index, this index measures the amount of adhesive material which is still bound to the enamel surface. After the Shear bond strength test, the debonded teeth were then checked for the presence of adhesive. The scores used to test are as follows:

Score 0 = There is no adhesive retained on the buccal surface of the tooth

Score 1 = 1% to 25% of adhesive retained on the buccal surface of the tooth

Score 2 = 26% to 50% of adhesive retained on the buccal surface of the tooth

Score 3 = 51% to 75% of adhesive retained on the buccal surface of the tooth

Score 4 = 76% to 99% of adhesive retained on the buccal surface of the tooth

Score 5 = Complete composite retained on the surface with the impression of the bracket base

Lon et al conducted a study aimed at understanding the shear bond strength of three different adhesives. They reported that for the Orthocem group (n=15) the ARI scores were, 86.7% in Score 3 (n=13), and 13.3% in Score 2 (n=2). Score 2 is 25% of adhesive retained on the tooth surface and Score 3 is No adhesive retained on the tooth surface.[27]

Santos L K et al studied the comparative analysis of ARI of Orthodontic adhesives. They reported that for the Orthocem group (n=10) the ARI scores were, 20.0% (n=2) in Scores 3, 2, and 1. 40% in Score 0 (n=4). Score 0 is no adhesive retained on the buccal surface of the tooth, Score 1 is less than half retained on the buccal surface of the tooth, Score 2 is more than half retained on the buccal surface of the tooth, and Score 3 is when all the adhesive is retained on the buccal surface of the tooth.[22]

In this study, the ARI scores contradict some studies done prior with Orthocem We compared and evaluated the Modified Adhesive Remnant Index for groups. The group with Orthocem showed maximum samples showing Score 5. The group with Orthocem and Arginine and the Group with Orthocem and Citrulline showed maximum samples showing Score 2. For the Group with Orthocem and the combination of arginine and citrulline, maximum samples showed a Score of 4. The group with Adhesive and Arginine and the group with Adhesive and Citrulline showed that most of the samples displayed 25% - 50% Adhesive material on the tooth's surface [Table 2]. The intergroup comparison of the Group with adhesive and Citrulline and the Group with Adhesive and the combination of Arginine and Citrulline was not significant [Table 8].

[6.5] Interpretation of Antimicrobial Test

- Antimicrobial property was tested by counting and calculating the colony forming units per ml. The lowest mean CFU/ml was shown in the group with adhesive and the combination of Arginine and Citrulline ($0.2967 \pm 0.049*10^5$ CFU/ml) followed by the group with Adhesive and Citrulline ($0.410 \pm 0.095*10^5$ CFU/ml) and then Group with Adhesive and Arginine ($0.623 \pm 0.109*10^5$ CFU/ml), when compared to the control group ($1.125 \pm 0.066*10^5$ CFU/ml) [Table 4]. The group with only citrulline showed the most promising result compared to other experimental groups. Intergroup comparison of the Group with Adhesive and the combination of Arginine and Citrulline with the Group with Adhesive and Citrulline was not significant [Table 12].
- The tests to evaluate the Antimicrobial properties of experimental adhesives in the current study show that the Second Null hypothesis is rejected due to the alteration of the Antimicrobial properties of the experimental adhesives due to the addition of the amino acids. However, the addition of citrulline compound to the adhesive shows promising results.

[6.6] Interpretation of test conducted using Scanning Electron Microscope (SEM)

- The samples which were tested were not polished to simulate a clinical situation where the composite is not polished after bonding the bracket. The unpolished samples had porosities which reasonably did not have much effect on the combination of Orthocem and Arginine due to the resin matrix covered with arginine particles within these porosities. Citrulline however had a more crystalline structure and bound the arginine particles to the citrulline structures. This could show that the physical property of the adhesive with citrulline component was significantly compromised by 40%.
- To evaluate and compare the surface morphology and particle size of the four groups, a Scanning Electron Microscope was employed. The mean particle size of all the groups was obtained. The group with Orthocem and Arginine gave the mean area of 5.42 ± 12.14. The group with Orthocem and Citrulline gave a mean area of 1.69 ± 3.81. The group with Orthocem and the combination of Arginine and Citrulline gave a mean area of 2.97 ± 8.08 [Graph 5]. The maximum mean area was seen with Group with Orthocem and arginine followed by the combination of Arginine and Citrulline. The least mean area of the particles was seen for adhesive with citrulline among the experimental groups. [Graph 5.1-5.6]

The results obtained in this study contradict the study done by Geraldeli et al. The study by Geraldeli et al showed no changes in the physical properties of the adhesives due to the addition of the amino acid, arginine.[13], the reason could be attributed to the sample size variation.

LIMITATIONS OF THE STUDY

- Considering that this is an in vitro study, it is not possible to reproduce every clinical scenario.
- The oral cavity of a patient consists of saliva, the effect of which on the adhesive could not be considered in this study.
- The release of Amino acids from the experimental adhesive could not be measured.
- This study was unable to precisely determine the force necessary for the adhesive-to-enamel interface to fail in a clinical setting.

SCOPE OF THE STUDY

The combination of arginine and citrulline in the adhesive showed a significant increase in the antimicrobial properties. However, the addition of these amino acids altered the physical properties of the adhesives. So, the further scope of the study is as:

- To research a methodology to efficiently incorporate the amino acids into the adhesives with a null effect on physical properties.
- Another scope is to conduct studies on the release of the amino acids from the cured adhesives.

CONCLUSION

The following conclusions can be drawn from the current study

1. Physical Properties
    a. Shear bond strength and tensile strength were altered due to the addition of the amino acids.
    b. The addition of Arginine to adhesive had their physical properties near to acceptable standards.
    c. Addition of Citrulline to the adhesive had a 40% decrease in the physical properties compared to the Control groups.

    Hence, it can be concluded that the addition of Arginine, Citrulline and a combination of Arginine and Citrulline to the adhesives alters the physical properties of the adhesive.

2. Adhesive Properties
    a. The group with Orthocem showed maximum samples showing complete composite retained on the surface with the impression of the bracket base.
    b. The group with Orthocem and Arginine and the Group with Orthocem and Citrulline showed maximum samples showing 26% to 50% of adhesives retained on the surface of the tooth
    c. The group with Orthocem and the combination of arginine and citrulline, maximum samples showing 76% to 99% of adhesives retained on the surface of the tooth.

Hence, it can be concluded that the addition of Arginine and Citrulline individually to the adhesives had the debonding occur at the bracket-to-enamel interface better than Orthocem adhesive.

3. Antimicrobial Properties
    a. There was an increase in the antimicrobial properties of the Experimental group.
    b. The most promising result was shown in the group with Orthocem and Citrulline.

Hence, It can be concluded that the addition of Arginine, Citrulline and a combination of Arginine and Citrulline to the adhesives increased the antimicrobial properties of the adhesive.

4. Surface morphology and Particle size
    a. The maximum mean area of the particles was seen in the group with Orthocem and Arginine.
    b. The minimum mean area of the particles was seen in the group with Orthocem and Citrulline followed by the group with adhesive and the combination of Arginine and Citrulline.

Hence, It can be concluded that the addition of Arginine, Citrulline and a combination of Arginine and Citrulline to the adhesives increased the number of particles and particle size of the adhesive.

SUMMARY

This invitro study was aimed at evaluating and comparing the effects of the addition of arginine, citrulline and a combination of arginine and citrulline to commercial orthodontic resin adhesive on the tensile, shear bond strength and antimicrobial properties.

The two amino acids, Arginine and Citrulline were incorporated with Orthocem adhesive using a Dual Axis Centrifuge. A total of three experimental groups were made; 1, Adhesive and Arginine; 2, Adhesive and Citrulline; 3, Adhesive and a combination of Arginine and Citrulline. The experimental groups were compared with the control group; Orthocem.

Brackets were bonded to the extracted premolars. They were subjected to a shear bond strength test using a Universal testing machine. The teeth specimens were then scored using the Modified Adhesive remnant index. Hour-glass specimens were prepared for each group and subjected to a Tensile strength test using a Universal testing machine. Discs were fabricated for each group and subjected to antimicrobial sensitivity tests by counting the colony-forming units. Similar Discs of each group were imaged using a Scanning electron microscope at 500x and 5000x to study the particle size and surface morphology.

The results of the above tests showed that

- The group with Orthocem and citrulline showed the least bond strength compared to the control group. The group with Orthocem and Arginine showed having the most shear bond strength among the experimental groups.
- The group with Orthocem showed maximum samples with complete composite retained on the surface with the impression of the bracket base. The group with Orthocem and Arginine and the Group with Orthocem and

Citrulline showed maximum samples showing 26% to 50% of adhesives retained on the surface of the tooth. In the group with Orthocem and the combination of arginine and citrulline, maximum samples showed 76% to 99% of adhesives retained on the surface of the tooth.

- The Group with Orthocem and Citrulline showed the least Tensile strength compared to the Control group with Orthocem. The Group with Orthocem and Arginine showed having the most Tensile strength compared to other experimental groups.
- The group with only citrulline showed the most promising result compared to other experimental groups for antimicrobial properties.
- The maximum mean area was seen with Group with Adhesive and Arginine. The minimum mean area was seen with the Group with Adhesive and Citrulline followed by the Group with Adhesive and the combination of Arginine and Citrulline. The Surface morphology was seen to be rough in the groups with Citrulline.

Adding arginine and citrulline to the Orthocem resin adhesive influenced the physical properties and decreased the growth of Streptococcus mutans for all the experimental groups. The addition of citrulline has shown promising results with increasing the anti-microbial properties of the adhesives. Further research on incorporating citrulline without affecting the physical properties can provide an alternate method for controlling the occurrence of White spot lesions in orthodontic treatment.

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ANNEXURE – I

ETHICAL CLEARANCE

Research and Ethics Committee
KLE VK INSTITUTE OF DENTAL SCIENCES
A Constituent Unit of KLE Academy of Higher Education & Research
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
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CERTIFICATE

EC/NEW/INST/2021/2435
Research & Ethics Committee

This is to Certify that the synopsis titled Comparison of Orthodontic Resin adhesives incorporated with Arginine and citrulline- An Invitro study of Physical adhesive and antimicrobial properties *Submitted by Dr.* [redacted] *P. G. Student / Staff, Guided by* [redacted] *from Department of* Orthodontics and Dentofacial Orthopaedics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 3/4/24

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

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Research & Ethical Committee
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BELAGAVI.

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Research and Ethical Committee
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