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Comparative Assessment of Conversion Degree, Resin Tag Depth, and Mineral Deposition in Adhesive Resin Enhanced with Inorganic Nanofillers such as Cerium Dioxide and Tantalum Oxide Nanoparticles

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Abstract

The World Health Organization (WHO) characterizes dental caries as the deterioration of tooth enamel resulting from acids generated by microbial activity on carbohydrates. Untreated dental caries can lead to pain, eating and sleeping difficulties, and systemic health issues, ultimately diminishing the quality-of-life Composite restorative materials moderately became one of the most used restorative materials for anterior restorations and also is used more extensively for restoring posterior teeth which are having largest stress-bearing areas. The annual failure rate is 2.2-3.6% which is seen in posterior restorations, in the past, the predominant cause of failure in bonded restorations was attributed to material wear. The data was obtained and tabulated in MS-Excel sheets, where the normality was evaluated using the Shapiro-Wilk test and the initial data analysis involved Using descriptive statistics, which encompass central tendency measures like the mean and standard deviation, to provide insights into the dataset's characteristics. In this study, the experimental adhesives underwent evaluation for mineral deposition through Raman spectroscopy. The evaluation will rely on variations in the absorbance of the phosphate Raman peak. Our hypothesis suggests that the adhesive resins created have the capability to promote the deposition of calcium phosphate, potentially assisting in maintaining the hybrid layer's integrity.

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INTRODUCTION

The WHO defines dental caries as the erosion of the tooth enamel caused by acids produced by microbial action on carbohydrates. Untreated dental caries can lead to pain, eating and sleeping difficulties, and systemic health issues, ultimately diminishing the quality of life.[1] Composite restorative materials moderately became one of the most used restorative materials for anterior restorations and also is used more extensively for restoring posterior teeth which are having largest stress-bearing areas. The annual failure rate is 2.2-3.6% which is seen in posterior restorations, in the past, the predominant cause of failure in bonded restorations was attributed to material wear. However, contemporary research suggests that the primary reason for restoration failure is secondary

caries. Hydrolytic degradation and enzymatic activity within dentin are recognized as the primary factors contributing to the failure of adhesive resin.

Restorative materials have been evolved to improve their mechanical, physical, and bonding properties, but incorporation of different nano fillers gives a therapeutic effect and that would be a true revolution. Other provocations for the restorations are oral micro-organisms, which adhere on restorative materials or amass in bonded interfaces [2]. This poses a risk to the restoration's viability, as microleakage of fluids, microorganisms, and bacterial toxins pave the path to secondary caries. Bonded restorations accumulate more biofilm and are susceptible to accelerated bacterial degradation, a process contingent on their chemical composition.[3] The objective of this paper is to assess and contrast various physical and mechanical properties of adhesive resin formulations augmented with different types of fillers.[4]

LITERATURE REVIEW

Development of dental caries adjacent to restorations are known as secondary caries. It is defined as a carious lesion occurring at the margins of cavity after dental restorations and it is considered the most frequent reason for failure of the composite restoration in primary as well as permanent teeth.[5] The primary reason for this failure is the structure of the dentin, containing four major component that is dentin matrix; dentinal tubules; mineral (i.e. hydroxyapatite) and dentinal fluid which makes heterogenic composition and bonding to the resin relies on organic component according to Loguercio AD et.[6]

As Breschi L et al [7] stated resin-dentin bonds exhibit lower durability compared to resin-enamel bonds. Moisture plays a crucial role in achieving successful bonding, yet it detrimentally impacts long-term bonding effectiveness. The immediate bond strength can be high but after aging gradual decrease is seen in resin-dentin bond according to van Dijken JW et al [8], especially adhesive system with etch-and-rinse showed continuous loss of bonded restorations with time. This degradation can weaken bond destroying the hybrid layer and leading to cause space between dentin and direct restorations according to J. Sharan et al. [9]

The marginal integrity that is the hybrid layer can be maintained by strengthening the bond between tooth and the dentinal surface and bonding agent. The integration of inorganic fillers into the composition may augment the adhesive's mechanical characteristics and enhance its clinical effectiveness. Adhesives with incorporation of inorganic fillers showed improved chemical and mechanical properties. It reduces the organic content of the resin. This may result in adhesives being more resistant to hydrolysis, as water sorption primarily affects the organic matrix according to Garcia IM et al. [5]

Nanotechnology stands as a burgeoning field with the potential to diagnose and treat diseases. The application of nanotechnology in dentistry is known as nanodentistry or nanodontics. In the endeavor to thwart dental diseases, dentistry employs a variety of materials, and nanotechnology holds considerable promise for enhancing their properties. Dentistry encompasses a range of nanostructures, such as nanoparticles, dental nanorobots, nanorobotic dentifrice, and nanoneedles. Among these, nanoparticles emerge as particularly promising materials for nanodentistry as stated by J. Sharan et al. [9]

In this study the incorporation of nano inorganic fillers was done to improve the strength of the experimental adhesive resin. The fillers used in this study are Cerium dioxide and Tantalum pentoxide. In previous studies Cerium dioxide in different volume percentages was evaluated for radiopacity and extent of conversion and Tantalum pentoxide were evaluated for Extent of conversion and other mechanical properties by Garcia IM et al [3] and Garcia Isadora et al. [5] which showed decreased in extent of conversion for cerium dioxide category above 1.44vol% and tantalum pentoxide category above 5wt%. The particle size used in these studies were macro sized particles.

Within the realm of metal oxides, specifically metal oxide nanoparticles like cerium dioxide (CeO2), a rare earth metal located in the lanthanide series of the periodic table, exhibit notable efficacy in pathogen elimination owing to their substantial antibacterial properties. CeO2 nanoparticles demonstrate remarkable durability and controlled release of metal ions, distinguishing them from other metal oxide nanoparticles. Furthermore, they exhibit antioxidant characteristics attributed to their reversible transfer from a reduced to an oxidized state, a process that can be reinitiated according to Garcia IM et al [3]

CeO2 nanoparticles in literature show an encouraging impact against the oral microflora. Previous studies have shown that the incorporation of CeO2 nanoparticles into acrylic resins enhanced mechanical properties. [3,5] There is no literature showing addition of cerium dioxide nanoparticles in adhesive resin hence selected as one of the materials in our study.

Tantalum is a transition metal which has high atomic numbers. Tantalum has properties like radiopacity and biocompatibility. In literature by Garcia IM et al [5] tantalum has shown bioactivity and affinity to phosphate categories and has shown deposition of appetite on its surface. It is also considered as bioactive which shows hydroxyapatite growth. Tantalum oxide has been used in medicine and in dentistry because of its high fracture toughness and high workability. However, as far as we are aware, there are no documented instances of utilizing nanoscale tantalum oxide (Ta2O5) in adhesive resins.

The concentration of nanoparticles used in this experiment is 2wt% as per the literature by Esteban Florez FL et al [4] which shown notable results and also illustrates if we incorporate more than 2wt %[1.92gms/100gms] there will be deterioration of physical properties of adhesive resin. The particles have been added to 5^{th} generation adhesive resin which is commercially available by *ivoclar* which comes in 5gms, hence 0.115gm/ 5gms of adhesive were formulated.

Formulated experimental adhesives were evaluated for the Extent of conversion. After photoactivation the aliphatic carbon double bonds are changed into carbon single bonds which indicates the polymerization of the resin or conversion of monomer into polymers. If the percentage of monomer is more, it reduces the mechanical properties of the adhesive. The addition of 2wt% of cerium dioxide and tantalum pentoxide showed notable extent of conversion as compared to control category, all categories exhibited values exceeding 90%, consistent with the extent of conversion observed in commercial adhesives in the literature by Gaglianone LA et al [10] There are various factors which influence extent of conversion. The addition of fillers in an adhesive resin decreases the light transmition in filled resin that may change the extent of conversion as stated by Emami N et al. [11] Absorption of light before photoactivation was less in Category B which is increased after photo activation. In category C absorbance of light before photoactivation is more and decrease in absorbance was seen after photoactivation.

The variances observed may be attributed to variations in resin composition. Discrepancies in the findings of this study could stem from multiple factors, including the type of photo initiator/light source employed, the presence of monomer molecules, and the proportion of inorganic particles integrated into the adhesive resin as per de Souza MO et al [12] Consequently, the presence of higher refractive index inorganic fillers compared to the co-monomer blend diminishes light penetration into the matrix, resulting in reduced extent of conversion of the adhesive resin. Moreover, the addition of fillers may alter the material's viscosity as said by Porto IC et al [13] Probably it explains the no notable difference observed in category B and control category and category C.

The experimental adhesives then evaluated for Depth of resin tags. Following cavity preparation using a bur or similar instrument, residual organic and inorganic components create a smear layer of debris on the surface. This smear layer forms a dentinal plug, obstructing the dentinal tubules and

reducing dentin permeability by 86% by blocking their orifices as per Pashley et al [14] The smear layer consists of hydroxyapatite and denatured collagen. To eliminate this layer, the dentinal surface underwent etching with 37% phosphoric acid. This process revealed a collagen fibril network with interfibrillar micro-porosities, providing space for adhesive resin penetration and interdiffusion into the dentinal tubules. This resultant layer, where the adhesive resin establishes micromechanical interlocking with dentinal collagen, is referred to as the hybrid layer. This layer consists of a combination of resin, collagen fibers, dentin surface structure, and intertubular structures. Within this layer, resin tags form and infiltrate the dentinal tubules, enhancing bond strength. The adaptation of resin tags within the dentinal tubules is influenced by their thickness and length, with closer adaptation resulting in stronger bonds. The formation of the hybrid layer is crucial for dentin bonding. The strength of the resin-dentin interface relies on the quality and depth of the hybrid layer, with longer and more uniform hybrid layers correlating with improved bond strength as stated by Collares, F.M et al [15]

In this study Category B and category C showed larger depth of resin tags as compared to control category in scanning electron microscopy, which shows that after incorporation of nanosized cerium dioxide and tantalum pentoxide the quality of hybrid layer was improved hence better bonding was shown in experimental categories.

The experimental adhesives were then evaluated for Mineral deposition. Acid-etching induces dentin demineralization, activating matrix metalloproteinases (MMPs), which, coupled with the vulnerability of polymers to hydrolytic degradation, particularly in non-infiltrated collagen regions at the hybrid layer's base, could contribute to degradation of the bonding interface as said by Hashimoto M et al. [16]

To overcome this situation, one solution would be to formulate an adhesive system that will induce the mineral deposition which will fill this exposed collagen fiber region with minerals. The antibacterial mechanism of CeO2 nanoparticles has been thoroughly investigated, particularly concerning their antibacterial efficacy in dental composite resin has been studied by Varghese et al [17], but literature on its application in adhesive resin is nil. Zamparini F et al [18] conducted research aimed to assess the chemical-physical properties and apatite-forming capability of three premixed calcium silicate materials containing monobasic calcium phosphate (CaH4P2O8) bioceramic, tantalum pentoxide, and zirconium oxide. These materials exhibited notable ion release and demonstrated significant abilities to nucleate a layer of B-type carbonated apatite. The integration of bioactive materials has been suggested as a strategy to replenish denuded collagen, aiming to mitigate hybrid layer deterioration. Profeta AC et al [19] and Melo MA et al [20] has evaluated Calcium silicates and calcium orthophosphates on adhesives aiming at their remineralization effect. Collares et al [15] Previous studies have investigated the induction of mineralization through niobium pentoxide and demonstrated its promising potential for remineralization. Bioactive materials are expected to interact with tissues, prompting the formation of hard tissue.

MATERIALS AND METHODS

The current experimental investigation was conducted at the Department of Pediatric and Preventive Dentistry, School of Dental Sciences, KIMSDU, Karad, following the formal approval of the Institutional Ethics Committee (Protocol Number).

- **Duration of study-** 21 months

Accordingly, the total sample size was 108.

The teeth samples selected for the study included 36 human premolars and 36 molars that were extracted for therapeutic purposes.

- **Inclusion criteria:** sound teeth extracted for orthodontic purposes.
- **Exclusion criteria:** decayed teeth removed for reasons other than treatment.

Materials Used In this Study are:
- Adhesive Resin
- Cerium Dioxide nanoparticles
- Tantalum Oxide nanoparticles

Materials required for preparation of sample teeth:
- Rubber cups for polishing Thymol crystals
- Diamond Discs for sectioning

Materials for assessment of extent of conversion
- Curing gun

Materials for assessment of depth of resin tags:
- HCL
- Sodium Hypo chloride.

Materials for assessment of mineral deposition
- Simulated body fluid

Sampling technique
- Randomized sampling technique.

OBSERVATIONS AND RESULTS

The data was obtained and tabulated in MS-Excel sheets, where the normality was evaluated using the Shapiro-Wilk test and the initial data analysis involved Using descriptive statistics, which encompass central tendency measures like mean and standard deviation, to provide insights into the dataset's characteristics. Paired student t- tests/Wilcoxon Matched Pair test was performed between Extent of conversion before photoactivation and after photoactivation that is KNH1 and KNH2. The data of extent of conversion and depth of resin tags, Raman spectroscopy underwent analysis using ANOVA (Analysis of Variance) or the Kruskal-Wallis test across different study categories, followed by Tukey's post hoc test.

Extent of conversion

Table 1. Intra Category Comparison of FTIR Absorbance Spectrum**.**

Degree of Conversion	**Absorbance (a.u.) before photoactivation**		**Absorbance (a.u.) after photoactivation**		**Mean difference**	**Wilcoxon Matched Paired Test Value**	**p- value**
	Mean	*SD*	*Mean*	*SD*			
Category A	3.1	0.1	2.88	0.12	0.22	53	0.0039*
Category B	2.78	0.08	3.26	0.11	-0.47	-78	0.0005*
Category C	4.47	0.12	3.72	0.13	0.22	78	0.0005*

**Notable when p<0.05, Category A-Control, Category B-Cerium Dioxide, Category C-Tantalum pentoxide*

FTIR- Fourier Transform Infrared Spectroscopy, a.u.-Arbitary unit.

The FTIR absorbance spectrum of adhesive resins among study categories is shown in Table. 1, where intra category analysis between absorbance before photoactivation and absorbance after photoactivation was done and it was found that all the study categories showed statistical significance difference with ($p<0.0001$, S) and the mean difference of absorbance spectrum before photoactivation and after photoactivation was 0.22 arbitrary unit in category A and Category C with value $p<0.05$,that shows the absorbance of light was more before photoactivation indicating more monomer content and it reduced after photoactivation indicating conversion of monomer in polymer. The mean difference of

absorbance spectrum before photoactivation and after photo activation in Category B showed an increase in absorbance after photoactivation. So, we could state that Category A and Category C showed less absorbance after photactivation indicating reduced number of monomers with mean difference 0.22 and p=0.0039, p=0.0005 respectively as compared to category B.

Table 2. Inter Category Comparison of Extent of Conversion of Adhesive Resin incorporated with Nanofillers.

FTIR	**Absorbance before<Photoactivation**		**Absorbance After Photoactivation**	
	Mean	***SD***	***Mean***	***SD***
Category A	3.1	0.1	2.88	0.12
Category B	2.78	0.08	3.26	0.11
Category C	4.47	0.12	3.72	0.13
Kruskal Wallis Test- value	31.19		30.951	
p-value	<0.0001*		<0.0001*	

**Notable when p<0.05, Category A-Control, Category B-Cerium Dioxide, Category C-Tantalum pentoxide*

FTIR- Fourier Transform Infrared Spectroscopy

The inter category analysis between absorbance before photoactivation and absorbance after photoactivation was done and it was observed from table 2 that absorbance spectrum before photoactivation was 3.1±0.1 in Category A, 2.78±0.08 in Category B, and 4.47±0.12 in Category C respectively. Also, the absorbance spectrum after photoactivation was 2.88±0.12 in Category A, 3.26±0.11 in Category B, and 3.72±0.13 in Category C respectively. The inter category absorbance spectrum was shown statistically significance with (p<0.05) both before and after.

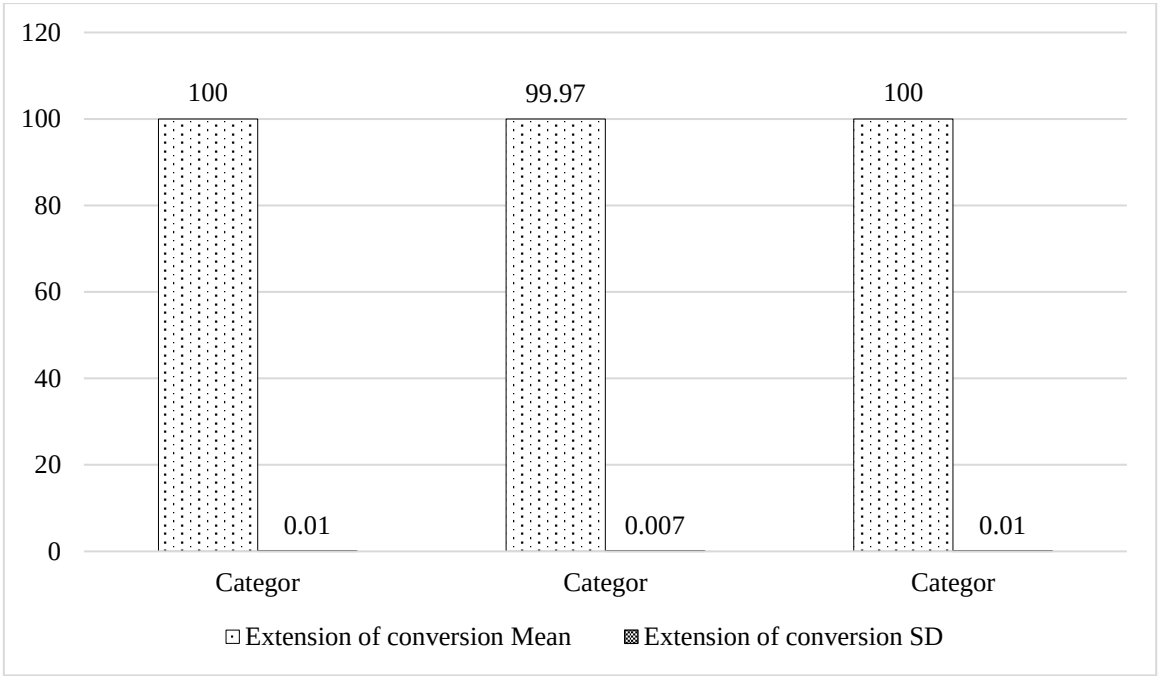

Figure 1. Column Chart Showing Mean, SD of Extent of Conversion.

The inter category analysis between extent of conversion is done after calculation of extent of conversion using the below formula and found that there was statistical significance with p<0.05. Category A, Category B and Category C showed promising extent of conversion compared to

commercially available adhesive resin which is shown in Figure 1.

Equation to measure Extent of conversion,

$$DC\% = 1 - \left[\frac{C_{aliphatic} / C_{aromatic}}{U_{aliphatic} / U_{aromatic}} \right] . 100$$

Depth of resin tag

The inter category analysis of depth of resin tags was done and it was observed in Fig.2 that mean depth of resin tags was 7.79±2.71 mm in Category A, 15.035±3.16 mm in Category B, and 10.15±3.03 mm in Category C. The inter category depth of resin tags was showed that statistically significance with ($p<0.05$). Showing more depth of resin tags in Category B and Category C.

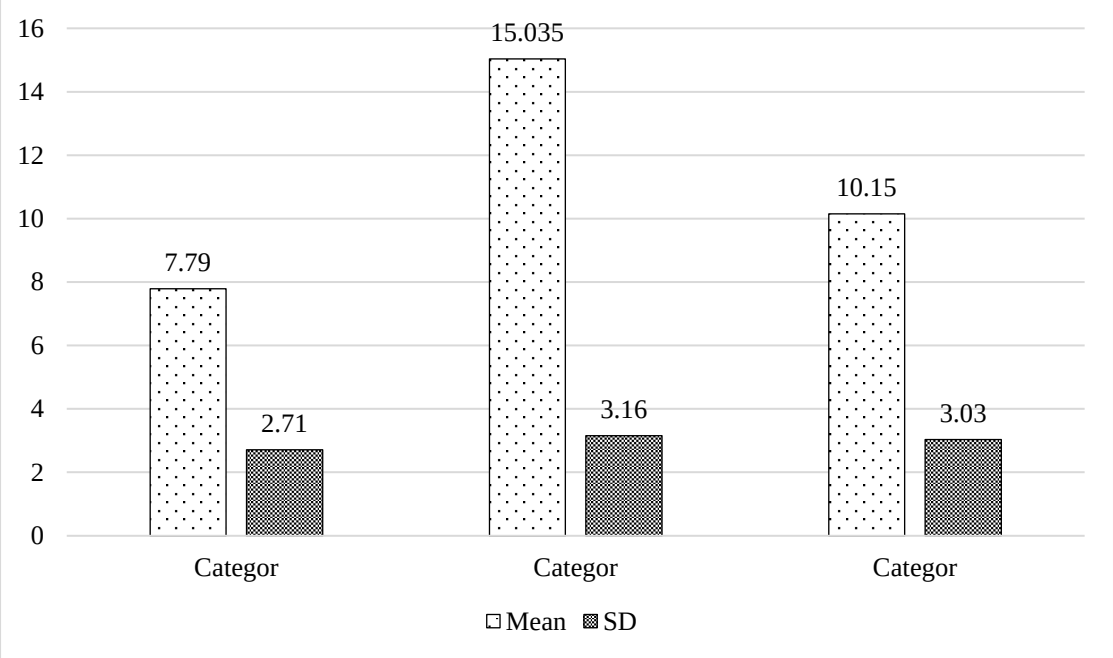

Figure 2. Column Chart Showing Mean, SD of Depth of Resin Tags

Table 3. Pairwise Comparison and Evaluation of Depth of Resin Tags.

Depth of Resin Tags	Mean Difference	Unpaired T- value	P -value
Category A Vs. Category B	7.23	6.027	<0.0001
Category A Vs. Category C	2.35	2.004	0.0575
Category B Vs. Category C	-4.88	3.86	0.0008*

The pairwise comparison of depth of resin tags was done by using unpaired t- test and stored in Table 3. Category A was compared to Category B and mean difference was 7.23 mm with $p<0.05$ but there was no notable difference observed between Category A and Category B with $p>0.05$ and mean difference was 2.35 mm. When Category B compared to Category C statistical significance was observed with mean difference -4.88 mm.

Evaluation of Mineral Deposition

The inter category analysis of mineral profile using Raman spectroscopy was done and it was observed from Fig.3 that mean mineral deposition was 9834.29±4.76 in Category A, 13235.5±2.77 in

Category B, and 13264.69±2.75 in Category C.

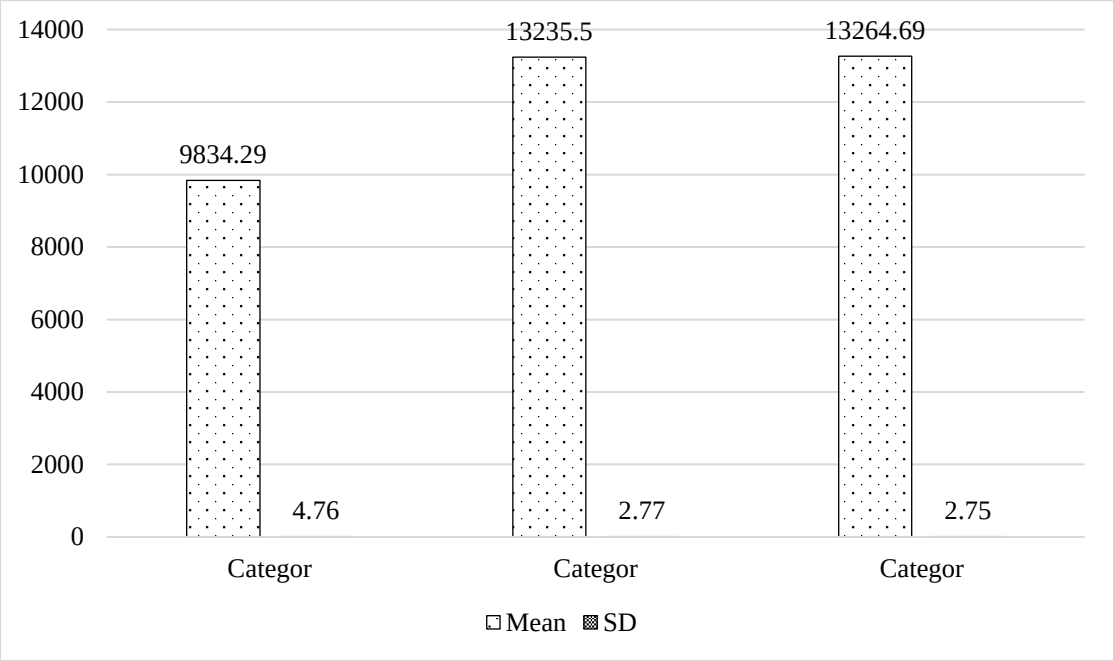

Figure 3. Column Chart Showing Mean, SD of Mineral Profile Using Raman Spectroscopy of Adhesive Resin Incorporated with Filters.

Table 4. Pairwise Comparison and Evaluation of Mineral Profile Using Raman Spectroscopy

Mineral profile using Raman spectroscopy	Mean Difference (a.u.)	Unpaired T -value	P -value
Category A Vs. Category B	3401	2636.7	<0.0001*
Category A Vs. Category C	3430.4	26661	<0.0001*
Category B Vs. Category C	29.183	25.86	<0.0001*

The comparative analysis of mineral profiles utilizing Raman spectroscopy was done by using an unpaired t-test and data were stored in Table 4. When Category A compared to Category B the mean difference found was 3401 with $p<0.05$. When category A compared to Category C the mean difference was 3430.4 with p value $p<0.05$. Also, it was observed that statistical significance with mean difference 29.183 when Category B compared to Category C with $p<0.05$.

CONCLUSION

In this study, the experimental adhesives underwent evaluation for mineral deposition through Raman spectroscopy, with assessment based on changes in the absorbance of the phosphate Raman peak. The Raman spectra obtained for Phosphate ion was at 960cm^{-1}. There is no literature showing the spectra for calcium ions hence spectra for phosphate ions were evaluated, which showed increase in mineral deposition after incorporation of nanosized cerium dioxide and tantalum pentoxide. Tantalum pentoxide showed more phosphate deposition as compared to cerium dioxide category. Therefore, we hypothesize that the developed adhesive resins possessed the ability to facilitate calcium phosphate deposition on their surfaces and potentially aid in sustaining the efficacy of the hybrid layer.

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