

Preparation and Assessment of Antimicrobial Property of Resin Based Composites Impregnated with Proanthocyanidin and Titanium Dioxide



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OBJECTIVE: To synthesize three experimental resin based composites (RBCs) out of a commercial preparation (Control) by impregnating into each a fixed weight (0.01%) of titanium dioxide (TiO₂) and three different weight % (0.01, 0.02 and 0.03) of proanthocyanidin (PA) and to compare antibacterial property, from the zones of inhibition of streptococcus mutans (*S. mutans*), of the specimen restorations made in the experimental and control RBCs.

METHODOLOGY: In the first phase, *Streptococcus mutans* were isolated and identified from the oral cavity of patients and identified on the basis of morphogenic appearance of colonies and confirmed through catalase test and microscopic examination. Isolates were then incubated and cultured for sensitivity. Specimens for control and experimental RBCs were made through a metallic mold having 2mm thickness and 5mm diameter for all the 4 groups. Specimen restorations made in the commercial as supplied RBC acted as the control (Group A). Specimen restorations in the Group B, C, and D were made in each of the experimental RBCs modified with 0.01% proanthocyanidin (PA) and 0.01% titanium dioxide (TiO₂), 0.02% PA and 0.01% TiO₂ and 0.03% PA and 0.01% TiO₂. The antimicrobial property of all the specimen restorations in all the groups was evaluated by Bauer-Kirby (Disc Diffusion) method through the formation of zones of inhibition of *S. mutans* colonies on blood agar plates. Data were computed using SPSS version 21 for descriptive statistics and analyzed using one-way ANOVA with p value 0.05 taken as significant.

RESULTS: The range of the zone of inhibition for specimen RBC restorations (N= 12) containing PA and TiO₂ was 0.00 for Group A, 10-12mm for Group B, 12-14mm for Group C and 9-10mm for Group D. The mean inhibition zone for the experimental restoration specimens was 11±2mm for Group B, 12.7±1mm for Group C and 9.7±1mm for Group D compared to no inhibition zones (0.00 mm) with the control RBC Group A. The order of the inhibition zones from maximum to minimum was Group C > Group B > Group D > Group A. The differences between the mean values for the control and the experimental RBC restoration specimens were significant (p < 0.000). The specimen restorations in the Group D appeared under cured and could impact on their mechanical properties.

CONCLUSION: Irrespective of the mentioned concentrations, impregnating PA and TiO₂ rendered the RBC antimicrobial. RBC restorations made with the experimental RBC with 0.02% PA and 0.01% TiO₂ proved relatively more effective in terms of antimicrobial effect.

KEY WORDS: dental materials, resin based dental restorations, antibacterial resin based composite, proanthocyanidin, titanium dioxide.

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INTRODUCTION

The involvement of microbes in the process of dental caries is well-established due to the virulence of streptococcus mutans.¹⁻³ Dental resin based

composites (RBCs) suffers polymerization shrinkage, have lower strength and lack antimicrobial property. These have been noted to cause failure of the RBC restoration because of fracture as well as recurrent carries at the tooth-restoration interface.⁴ To address these issues, there is an ongoing research focus to produce RBCs with antimicrobial property. Antibacterial agents of bioactive nature, caries preventive additives, polymer antibiotic conjugates and quaternary ammonium salts were added in resins to elicit antimicrobial activity.⁴⁻⁹

The antioxidant action and free-radical scavenging

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effect of Proanthocyanidin (PA) have been established when applied to a surfaces.¹⁰ Cariogenic environment causing degradation of dentine has been prevented with PA.¹¹ The antibacterial action of Titanium Dioxide (TiO₂) is through photo catalysis with water to release the hydroxyl radical with subsequent formation of superoxide as an antimicrobial agent.¹²

Knowing the fact that there are inconsistencies in the literature regarding the antimicrobial property of RBCs, the search for making them antimicrobial is continue.¹³⁻¹⁴ Both PA and TiO₂ are naturally occurring and easily available, hence their incorporation into RBC for possible antimicrobial property of the resulting restorations justifies an investigation. When proanthocyanidin was incorporated in adhesive agent in different concentrations ranging from 1% to 6% the antibacterial activity was same as of the control which is devoid of Proanthocyanidin.¹⁵ Antibacterial activity of TiO₂ in concentrations from 0-10% was also evaluated in orthodontic composites which provided better antibacterial activity with concentration of 10%.¹⁶ Therefore, we have designed a comprehensive study that is looking at the effect of adding PA and TiO₂ to RBC in terms of enhancement of antimicrobial and mechanical properties. This report presents the results on the antimicrobial effect of incorporating different concentrations of PA and a fixed quantity of TiO₂ in RBC and assessing their antibacterial property of specimen restorations against *S. mutans* isolated from the oral cavity of subjects.

METHODOLOGY

Preparation of specimen restorations:

Group A was the control group in which no additives were added. In each group 4 specimens were prepared from a commercially available RBC, Nexcomp (Meta Biomed Co. Ltd) with 1% TiO₂ which was fixed for all the experimental groups. Group B, C, & D all were test groups which have 0.01, 0.02 and 0.03% PA. Sample size was calculated according to the following values the confidence level for this study was considered as 95% while power of the study kept 80 with a response distribution 60% and margin of error kept 5% the calculated sample size was 3 used in this pilot study.

The research protocol for the study was approved by the Board of Advanced Studies and Research (BASR) of the university (Riphah 25th meeting, dated, October, 18th, 2018) as well it was approved for publication of the research findings by the institutional review board (PRIME/IRB/2019-166.) The experimental work was conducted during the period of (01 May to 30 June, 2018) at the Department of Dental Materials and Microbiology Lab, Department of

Pathology, Peshawar Medical and Dental College (Pakistan). The materials used in the study including the experimental RBC are detailed in (Table 1).

Proanthocyanidin (PA) was characterized for purity using Fourier Transform Infrared Spectroscopy (FTIR) in Material Research Laboratory, Physics Department Peshawar University, Pakistan (Figure 1).

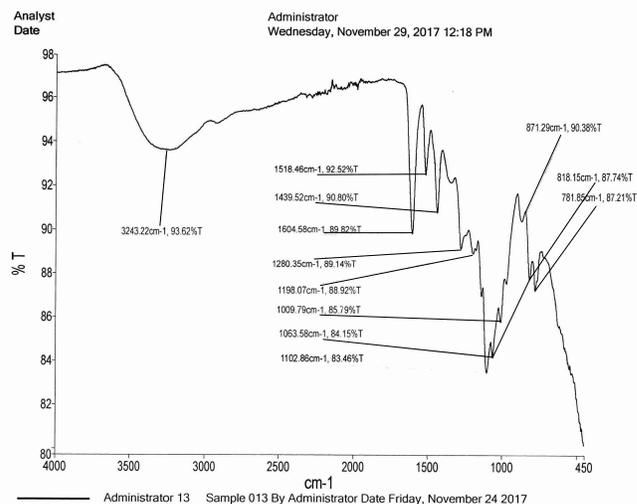


Figure 1

Each of the relevant weight % of PA & TiO₂ were first assimilated in the resin bonding agent to facilitate easy and thorough mixing and then impregnated into a commercially available light-cure nanohybrid RBC Nexcomp Nanohybrid Resin Composite Nexcomp contains Bis-GMA, Bis-EMA, UDMA, TEGDMA while Meta P&Bond Adhesive contains Bisphenol A Glycerolate dimethacrylate, Urethane Dimethacrylate, Pyromellitic glyceryl dimethacrylate, 2-Hydroxy ethyl methacrylate, Ethyl alcohol (Table 1). The

Table 1: Details of the materials used

Name of Material	Composition	Lot No	Manufacturer Name
Nexcomp Nanohybrid Resin Composite	Bis-GMA, Bis-EMA, UDMA, TEGDMA	Lot.No:NXC1706202	Meta Biomed Co. Ltd. Korea
Meta P&Bond Adhesive	Bisphenol A Glycerolate dimethacrylate, Urethane Dimethacrylate, Pyromellitic glyceryl dimethacrylate, 2-Hydroxy ethyl methacrylate, Ethyl alcohol	Lot.No:PNB1707182	Meta Biomed Co. Ltd
Proanthocyanidin Powder Capsules		17205	Olympian Labs., Inc. Phoenix., USA
Titanium dioxide Powder		Lot No: T1264PI1	Daejung Chemicals & Metals Co., Ltd. Korea

three experimental RBCs were prepared by adding in each a fixed weight % of TiO₂ (0.01%) and the various weight % of the PA (0.01%, 0.02% and 0.03%) to the commercially supplied RBC. The as supplied commercial RBC acted as the control RBCs.

The specimen restorations were prepared in each of the three experimental and the control RBC using a stainless steel mold according to ISO specification No. 4049. The mold facilitated the preparation of specimens having 2 mm thickness with 5 mm diameter. Specimen restorations were made by pouring each of the RBC into the mold and cured with a light curing unit (O-Light by DTE, 5 watts, Light Intensity from 1000 to 2300 mw/cm²) for 20 seconds from one side. The poured resin was kept pressed under a 1mm thick glass slab from both sides (Figure 2). After light-curing, the specimen restorations were recovered from the mold and examined and finished (Figure 2) and when considered satisfactory as no specimen was under



Figure 2

cured, no voids or cracks were present, they were then stored in dry sealed containers according to ISO 4049 specifications.

Assessment of antibacterial property of the specimen restorations

This was determined by Bauer-Kurby disc diffusion test using *S. mutans* from the oral cavity of patients who were negative for HbV and HcV infection. Swabs were taken from carious teeth of patients to collect *S. mutans*. Written consents of patients were obtained and duly signed by witnesses. The swabs were transferred to Microbiology Department Peshawar Medical & Dental College within

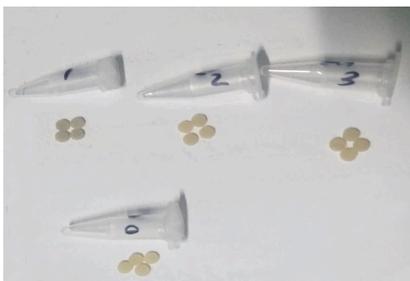


Figure 3

30 minutes in a sealed sterile tube. Strains of *S. mutans* were proliferated on sterile blood agar plates under optimum conditions and at 37°C for 24 hours and evaluated morphogenically-

confirmed through catalase negative test and finally confirmed through microscopic evaluation (Figure 3).

Colonies of *S. mutans* were tested for sensitivity by

placing the control and test specimen RBCs at different locations on blood agar plates having *S. mutans* colonies (Figure 4). They were incubated at 37°C for 24 hours. After incubation period circular inhibition zones if any appeared around the placed specimens were documented. Inhibition zone sizes were recorded through taking photographs by using a scale marked with millimeters and placing the scale over the blood agar plates (Figure 5) and the data were recorded in a pre-structured data collection sheet.

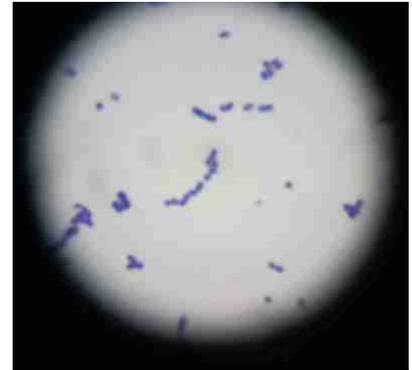


Figure 4



Figure 5

DATA ANALYSIS

Using SPSS Version 21, mean, mode and percentages for the data were computed. Data were also analyzed using one-way ANOVA to see the statistical significance of the within and between the specimen groups differences from the mean values with p value set as 0.05 considered significant. Post hoc test is not presented due to self-explanatory results of four groups of this pilot study.

RESULTS

The specimen restorations in the control group did not exhibit any antimicrobial property in-comparison to those in the experimental groups. Similarly, the antimicrobial property was maximum for the restoration specimens in group C. The order of antibacterial effect among the specimens in the various groups was Group C > Group B > Group D > Group A. (Table 2).

The mean size of the inhibition zone was 8.42mm for the specimens in groups B to D out of 13 mm. Maximum sizes of inhibition zones were found in two specimen restorations belonging to group C. None of the control group

Table 2: Inhibition zones for the various RBC specimen restorations.

RBC Specimen restoration group	Inhibition zone range (mm)	Mean \pm SD	mode
A	0.00-0.00	0.0 \pm 0.0	0.00
B	10.0-12.0	11.0 \pm 2.0	12
C	12.0-14.0	12.7 \pm 1.0	13
D	9.0-10.0	9.7 \pm 1.0	9

**Figure 6**

specimen restorations exhibited any antibacterial property (Table 2). Both the within group and between groups variations from the mean values were statistically significant ($p=0.000$). Different sized zone of inhibition were appeared which were then calculated (Figure 6).

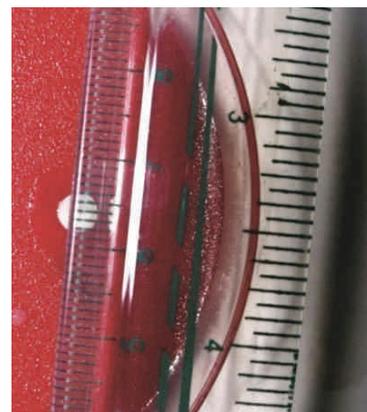
DISCUSSION

For assessing the antimicrobial property of RBC restorative materials, the method of disc diffusion has also been previously used.¹⁷ Studies on anti-microbial property of experimental RBCs have used standardized microbial strains species like ATCC35668¹⁸, ATCC 25175¹⁹ and a different strain of *S. mutans* (10449) has been used for assessing the antimicrobial effect of benzalkonium incorporated RBC.²⁰ A concern about these studies is that these have not mimicked the actual mode of action of the antimicrobial agent added to the resin. In this study strains of *S. mutans* collected from patient's mouth, making the testing method relatively more relevant because virulence/activity of standard cultures is questioned and reported as of low or no bacterial activity.²¹

At present, a definite methodology for testing antimicrobial properties of a material is lacking. Therefore, authors preferred the easy and commonly used method for this study.²² The disc diffusion method for establishing the antimicrobial efficacy of PA to *S. mutans* has also been used in another study.¹⁵ Quantitative methods like plate count method, flow cytometry and quantitative polymerase chain reaction for antimicrobial assessment are available but were not employed in the present study due to their technique sensitivity.²³ Results obtained through disc diffusion method are better than other protocols.²⁴ This was employed to verify the results obtained through direct contact test between microbial strains and the restorations and to validate the antibacterial action. Discs

prepared for experimental and control RBC specimens had the same dimensions as used by other investigator.²⁵ Furthermore, following the preparation of standardized specimen restorations and the selection of defect free specimens for testing obviated the need for using many specimens.

The incubation procedures employed for *S. mutans* strains were according to the Bergey's manual of determinative microbiology.²⁶ Due to vast discrepancy in literature for the growth medium and its yield, blood agar was used as nutrient medium for proliferation of the *S. mutans* colonies and their killing by the RBC specimen restorations. There is a positive correlation between genotype and phenotype evaluation of bacteria which confirms and validate the phenotypic evaluation of the bacterium.¹⁶ The colonies obtained after incubation were identified in the present study for morphology of the colony followed by confirmation through catalase test and finally through microscopic examination (Figure 2).

**Figure 7**

The findings of the present study are in accordance with those of a previous study, in which the range of size of zones of inhibition was 9.33mm to 12.67mm for antibacterial activity of resin containing 0.25% concentration of benzalkonium chloride.²⁰ Size of zones of inhibition always will be more than the size of discs if smaller than discs then it will be of no use and considered as no zone of inhibition. When a drug is placed in a medium where a bacterial culture is present if that drug is of antibacterial potential it produces areas of no growth around that drug specimen these areas are zones of inhibition. The findings of the present study are more encouraging as a comparable yet better antimicrobial effect has been achieved with a small concentration of PA (0.02%). Our finding of the lack of antibacterial property in the specimen restoration made in the control RBC are supported by the finding of another study where no zones of inhibition were also observed in case of specimen restorations in the control RBC.²⁷

In the present study, the RBC specimens in the control group exhibited no antibacterial effect as observed from no zones of inhibitions. In the present study a much lower concentration of TiO₂ and PA showed comparable antibacterial activity. In the present study lower concentration of PA (0.01, 0.02 and 0.03%) were used and found effective against *S. mutans*.

As titanium dioxide is highly opaque material therefore its concentration in the RBC was kept 0.01% so as to avoid the problem of shade change which was confirmed through another pilot study.

LIMITATIONS OF THE STUDY

An obvious limitation of this study may be the use of few samples and using a method for antimicrobial properties of the test RBCs restorations that does not mimic the real clinical situation in which the restorations are functioning. However, in case of in-vitro studies, using standardized dimension specimens that are defect-free, the use of few specimen restorations is justifiable. On the other side, in the present study, the specimen RBC restorations have been subjected to relatively harsher bacterial insult than that could be expected in the oral cavity. Despite this, it was encouraging to have seen enhanced antibacterial property in case of experimental RBC restorations containing PA and TiO₂.

Incorporation of additives in RBC may produce any effect on the mechanical properties which was not evaluated in current study. This aspect is being currently under investigation for the experimental RBCs used in this study. Another limitation, of this study is the addition of PA and TiO₂ to the commercial RBC, which have altered the actual formulation of the commercial RBC. Therefore, it is recommended that this aspect should be kept in mind in future studies by synthesizing the experimental RBC with the mentioned additives.

Another limitation of this study is that the antimicrobial effect has not been followed up over a larger duration of time and hence the sustained long term antibacterial effect of the RBC restorations cannot be predicted from the present study making observation at one time-point. It is recommended that the specimen restorations be tested repeatedly after simulated usage scenarios to give a clear picture of how much stable and long-lasting would be the observed antibacterial property as documented in this study.

CONCLUSION

Within limitations of this study, PA and TiO₂ imparted antimicrobial properties to the RBC especially PA 0.02% along with TiO₂ 0.01% which produced larger zones of inhibitions.

CONFLICT OF INTEREST

None declared.

AUTHORSHIP CONTRIBUTIONS

All authors (MUI, FG, MS) contributed equally to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; All authors have given final approval and have agreed to be accountable for all the aspects of the research work and its publication. FG had accepted the responsibility as the corresponding author but after his unfortunate death MUI is now corresponding author.

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