ORIGINAL ARTICLE

Effect of Copper Dope Calcium Phosphate on Mechanical Properties of Modified Dental Composites

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ABSTRACT

Objective: To evaluate the mechanical, biological, and antibacterial properties of copper doped calcium phosphate (Cu-CaP) modified dental composites.

Study Design: Cross-sectional study.

Place and Duration of Study: The study was carried out at the Department of Dental Materials, Khyber Medical University Peshawar, Pakistan from February 2023 to January 2024.

Methods: The process involved adding ethyl alcohol, distilled water, silane, and octyltriethoxysilane to powdered silica, refluxing, centrifuging, washing, and preparing copper oxide nanoparticles and copper doped calcium phosphate. Composite was prepared using TEGDMA i.e. Triethylene glycol dimethacrylate, camphor quinone, and dimethyl aminoethyl methacrylate.

Results: The microscopic image of Copper oxide nanoparticles were gathered together with agglomerated structure, irregular shapes having voids, and size range of 80 nm to 5 µm. The shape was irregular with void spaces and composition was confirmed with Edx analysis. Scanning electron microscope image of copper doped calcium phosphate, shows a build-up of nanometric needles over entire base, with asymmetrical form and translucent appearance, ranging from 10 nm to 50 nm. The Energy dispersive X-ray (EDX) analysis demonstrated the presence of materials. The Fourier transmitted infrared microscopy of silica and salinized silica showed characteristics peaks of salinized silica at 1016 cm^{-1} and 1439 cm^{-1} with a main peak at 950 $cm^{-1,}$ while the Fourier Transform Infrared Spectroscopy of calcium phosphate, 2%, 4%, and 6% copper doped calcium phosphate in dental composite showed charcateristics peak at 1020 cm^{-1} which was due to increase of phosphates upon increasing copper doped calcium phosphate concentration. The compressive strength results of native dental composite and 2%, 4%, and 6% copper doped calcium phosphate in dental composite have values of 15.6 MPa, 18.72 MPa, 20.2 MPa, 49.15MPa while the flexural strength were of value 19.32MPa (dental composite) and that of 2%,4% and 6% have a value of 22.2MPa, 26.1MPa and 30.3MPa. The control group i.e. the dental composite had Vickers hardness of 14.55 MPa, while the experimental groups with 2% , 4%, and 6% copper doped calcium phosphate had values of 16.97 MPa, 21.49 MPa and 26.5 MPa respectively.

Conclusion: The Vickers's hardness, flexural strength, and compressive strength of experimental dental composite increased with an increasing percentage of copper doped calcium phosphate added as a filler in a proportion of 2%, 4% and 6% with salinized silica in the dental composite.

Keywords: *Control Groups, Copper, Flexural Strength, Phosphates*, *Silicon Dioxide.*

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Introduction

Dental composites offer a promising, aesthetic, tooth colored material in restorative dentistry that closely resembles natural teeth (also known as "dental composite resin"). Comprising of an organic resin matrix and an inorganic/organic filler phase, they have numerous applications as a restorative material, endodontic sealers, orthodontic devices.[1]

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Failures reported in dental composites were bulk fill fractures, secondary caries (due to formation of staphylococcus mutants like colonies on restoration surface) chipping, low flexural strength, increased surface roughness, high adhesion ability, caries susceptibility of restorative material and increased fracture toughness affecting the performance of both anterior and posterior composites.[2] The fundamental reason established was internal flaws; micro-cracks already present in a material that propagated preceding to cracks or fractures of the restoration.[3] Breakdown of the filler particles, external stresses, and interfacial de-bonding also contributed to their failure.

Several modifications have been done to improve the antibacterial properties (by addition of copper Nanoparticles and silver nanoparticles) as well as mechanical properties (through surface polishing to decrease surface adherence of biofilm thus preventing dental caries) of composites.[4] Graphene oxide was added with PVA (polyvinyl alcohol) and by reduction, graphene nanosheets were formed that when mixed with composite improved their mechanical properties.[5] In another study, silane modified glass fibers were added to PLA composite (i.e. poly lactic acid), and mechanical properties were drastically improved such as flexural modulus and flexural strength.[6] On addition of silicon nitride to calcium phosphate Nanoparticles, a composite with strong mechanical properties and better ability to prevent cavities was introduced.[7]

Copper is more frequently used in medicine and dentistry due to its low cost, antibacterial qualities, mechanical properties, low toxicity, accessibility, and efficacy as compared to silver nanoparticles.[8] Copper doped calcium polyphosphate promotes cytocompatibility, and mechanical strength of bone while enhancing angiogenesis and osteogenesis effects.[9] Hydroxyapatite (Ca10(PO4)6(OH)2) due to its nature of resembling bone minerals; physical and chemical properties are most commonly employed for prosthesis covering and/or replacing bone.[10,11] Calcium phosphate has gained more attention due to; its ability to repair and regenerate both hard and soft tissues, bioresorbable to produce a desired biological response (i.e as a temporary implant to eliminate another surgical procedure), slow delivery of growth factors as a coating in causing bone formation, and antimicrobial ions addition to avoiding biomaterial related infections.[12] It comprises calcium cations and phosphate anions that were first identified in bones in 1769.[13] Doped with K+, Mg2+, and Zn2+ nanocrystalline Calcium carbonate phosphate greatly improves performance for drug administration, hastens bone regeneration and bone tissue strengthening processes to about 5 times.[14] Metal ions of copper or oxidized cupric ions formed from copper nanoparticles possess antibacterial properties, hence, now being used as antimicrobial agents and to prevent MDR.[15] Copper nanoparticles have been prepared using methods like thermal reduction vacuum vapor deposition, microwave irradiation, chemical reduction, laser ablation and is being used as a useful strategy to encourage healing of damaged blood flow, secretion of various factors like basic fibroblast growth factor, vascular endothelial growth factor etc.[16,17] Its addition to dental amalgam showed increased compressive strength, corrosion resistance and decrease in gamma 2 phase.[18] A study was also performed to increase corrosion resistance, improve mechanical properties and other properties of titanium containing implants by coating it with diamond carbon film.[19]

In this study, copper doped calcium phosphate will be incorporated to modified dental composite to evaluate its influence on mechanical properties.

Methods

The study was carried out at the Department of Dental Materials Khyber Medical University Peshawar, Pakistan from February 2023 to January 2024 after taking approval from thr Ethical Review Committee of the University held on 12th January 2023 vide letter no: DIR/KMU-AS&RB/EC/IBMS/002135.

Silanization of silica was done by using 400 ml of ethyl alcohol along with 375 ml of distilled water (H2O) being added to 375 g of powdered silica, drop by

drop by stirring for 30 minutes. Following that, 200 ml of ethanol was mixed with a particular quantity of silane. 164 mmol of octyltriethoxysilane was mixed with 200 grams of ethanol and the solution was then poured to a nanoparticle mixture with a dropping funnel continuously stirring it for 45 minutes in order to fully coat each particle with silane. The reaction flask was refluxed for three hours at 78 °C and stirred which results in silane gets covalently bonded to the silica surface through condensation and hydrolysis, which were catalyzed by alkali subsequently. The solution was then centrifuged i.e. 4301 grams for 1 hour, with nanoparticles washed thrice in Ethanol followed by centrifugation.

Copper oxide nanoparticles were prepared by mixing 50 mmol solution of copper chloride dihydrate with 1% solution of amoxicillin (commercial). The resultant mixture was stirred and its pH was adjusted to 10 by adding 0.1% NaOH. The obtained precipitate was separated from the supernatant by centrifugation at 12000 rpm. The precipitate was dried and referred to as copper oxide particles, Copper doped calcium phosphate was prepared by mixing 5g of copper oxide nanoparticles with 95g of Calcium phosphate.

This mixture was then added to salinized silica to make up the respective filler component of 2%, 4% and 6% of copper doped calcium phosphate with salinized silica respectively.

The prepared respective copper doped calcium phosphate salinized silica (2, 4, and 6%) was added in 70% with the gold standard composition of dental composite (30%) containing organic matrix (60% Bis-GMA, 40% diluent co-monomer).TEGDMA (0.54 mole fraction) as a co-monomer was used. Camphor quinone as an initiator and dimethyl aminoethyl methacrylate as a co-initiator were used. Groups were made with respect to ratios of copper doped CaPO4 with salinized silica fillers as discussed in table-1.

To prepare the composite, the above sample was loaded in the respective mould and the mould was placed on a glass sheet and covered with Mylar strips to prevent the potential of oxygen inhibition layer formation. Using light curing equipment, the specimen was then polymerized for 40 seconds on each side making sure the compiste are properly cured.

Table-1: Division of dental composites into groups

Abbreviation	Groups
Control	S-Silica +composite
C2	2%Cu-CaP+ S-Silica +composite
C4	4%Cu-CaP + Silica +composite
C6	6%Cu-CaP + Silica +composite

FTIR-ATR spectrophotometer was used to analyse the Cu-CaP. Thin pastilles transparent in the concentration of 1mg of each tested substance were vacuum pressed under a pressure of 200 MPa and FTIR spectra of tested samples was obtained, analysed using a spectrophotometer. Analysis using FTIR was performed at concentrations of 2%, 4%, 6%. Their composite in wave-number range of 4000-450 cm^{-1} and 32 scans with resolution of 2 cm^{-1} was obtained.

Scanning electron microscopy SEM is a method of investigating a sample's surface morphology, its structure, composition, and electrical conductivity.

This beam of energy varies from 500eV to 50keV but the most commonly used beam are between 20 to 30keV. Energy dispersive X-ray (EDX) analysis was performed to analyse the particle size, shape, and diameter of Cu-CaP.

This non-invasive technique, X-ray diffraction (XRD first introduced and discovered by Max von Laue in 1912, gives information about the crystallographic feature, and physical and chemical properties of the sample. Powder XRD patterns were acquired using the x-ray diffractometer and monochromatic Cu-K radiation (wavelength equals 1.54) using a scale factor 0.02° every 1s, scans were collected in the 2θ range, between 20 and 80 degrees.

To determine their mechanical parameters, research samples were placed on VHN tests, CS tests, and FS tests.

The criteria used to assess compressive strength is ISO 6874 standard. Mould was washed with ethanol

to get rid of possible impurities and Dental composite was inserted in Teflon moulds with dimensions 4 mm in diameter and 6 mm in height. Samples were placed in UTM and set to 1 mm every minute and a weight of 5 to 50 KN.

Formula: Compressive stress = F/ A where F is the applied force and A is the area of the sample.

The flexural feature of restorative materials indicates longevity of restoration as it fractures under compressive as well as tensile force. The 3-point bending test was used with samples (2 mm x 22 mm x 25 mm) fabricated in mold and kept in filtered water. The universal testing device used a three-point bending test with a pace 0.5 mm every minute that determined flexural strength measured through MPa.

A diamond shaped indenter was utilized to conduct tests by ISO/CD6507-1. Three indentations of weight 0.49 N and duration 15 s were created on the specimen. The resulting indentations were given a Vickers Hardness Number (VHN) after being microscopically calibrated.

Results

Fourier transmission infrared (FTIR) spectroscopy, The peaks corresponding to the material's various functional groups are shown in FTIR. Figure.1 shows the Ftir of silica and salinized silica. Graph A is the graph of silica while Graph B is the graph of salinized silica. There is a peak shoulder of salinized silica at 1016 cm^{-1} and 1439 cm^{-1}. The main peak of both silica and salinized silica is recorded at 950 cm^{-1}. The peak at 2347 cm^{-1} is related to adsorbed CO_2 that is present in both silica and salinized silica.

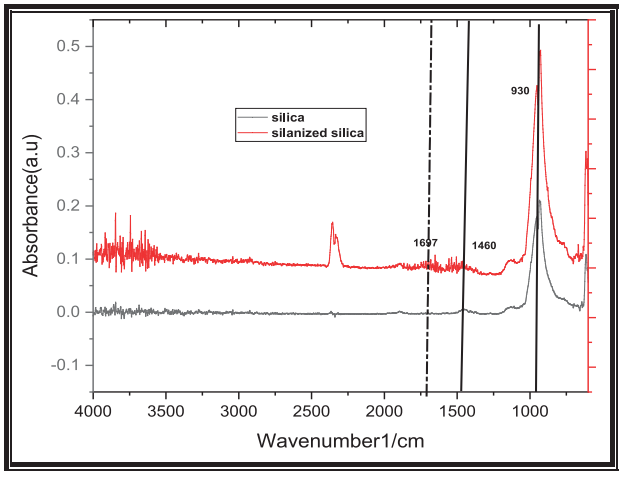

Fig.1: Ftir Analysis of Silica and Salinized Silica

Figure.1 and Figure.2 indicates FTIR of salinized silica, calcium phosphate, 2% copper-doped calcium phosphate in silica on dental composite, 4% copper-doped calcium phosphate in silica on dental composite, and 6% copper doped calcium phosphate in silica on dental composite. The peak at 1020 cm^{-1} corresponds to the functional group of phosphate in calcium phosphate. The peak at 922 cm^{-1} represents the characteristic peak of silica. The SiO peak is present in all four samples; as the level of copper doped calcium phosphate increases, the phosphate peak becomes more visible.

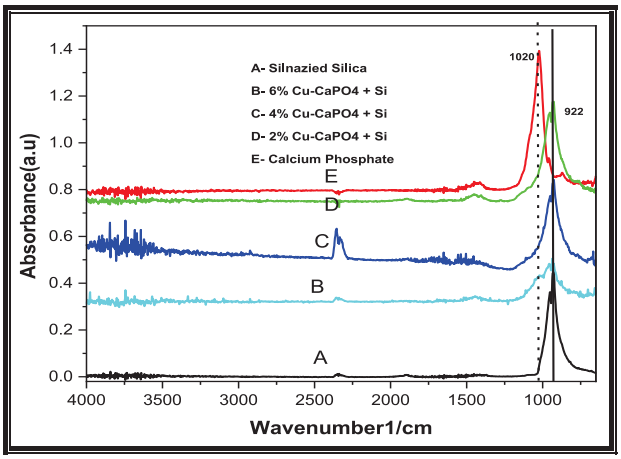

Fig.2: Ftir Analysis of Salinized Silica, Dental Composite.2,4,6% Copper Doped Calcium Phosphate in Dental Composite

The SEM image of copper doped calcium phosphate shows a build-up of nanometric needles over the entire base, with an asymmetrical form and translucent appearance as seen in figure.3. The particles were agglomerated, hence small particles were measured in image J software that ranged from 10 nm to 50 nm. The EDX analysis demonstrated the presence of Ca, P, and Cu in the copper doped calcium phosphate materials.

XRD gives information about the crystallinity of a material. The XRD showed patterns of crystallographic structure of the material as given below in figure.4.

Through XRD diffraction, data was collected at 10-20 degrees. Tetragonal crystalline structure was determined with a characteristic peak at 211 of calcium phosphate present in copper doped calcium phosphate.

The experimental groups of dental composites with

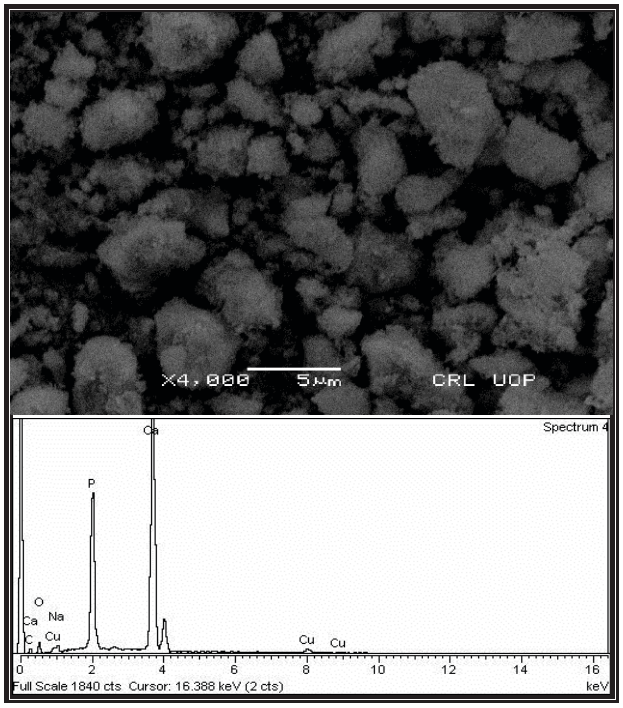

Fig.3: Showing SEM Image and EDX analysis of Copper Doped Calcium Phosphate

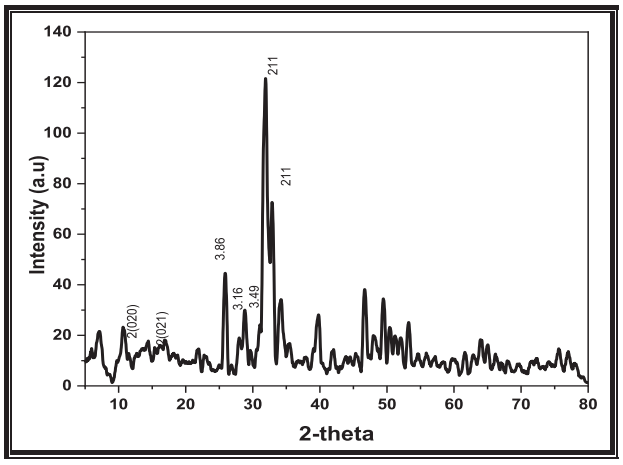

Fig.4: Shows XRD pattern of Copper doped calcium phosphate

copper doped calcium phosphate of the proportion of 6%, have the highest compressive strength value of 49.15 MPa followed by dental composite with copper doped calcium phosphate added along filler in a proportion of 4%, have a value of 20.2 MPa, that of 2%, compressive strength has a value of 18.72 MPa. The lowest value was achieved by the control group i.e. the dental composite having the compressive strength of 15.6 MPa.

The dental composite as the control group had a flexural strength of 19.32±2.236, whereas the experimental groups' flexural strengths were 22.2± 2.121 for dental composite with copper doped calcium phosphate added as a filler in a proportion of 2% and 26.1±4.301 for dental composite with copper doped calcium phosphate added along filler in a proportion of 4%. On the other hand, dental composite containing 6% copper doped calcium phosphate has a flexural strength value of 30.3±0.707.

Discussion

The degree of polymerization constitutes one of the main factors that affects the clinical performance of the dental composite. Fourier transformation infrared spectroscopy serves as the most accurate and reliable approach for calculating the degree of conversion (DC) of dental composite as well as its process. Ismail Ab Rahman et al. stated that a 30-degree deviation in bond angles of Si–O–Si between tetrahedral silica and non-crystalline silica.[20] When the degree of conversion of experimental bis-GMA/TEGDMA composites with copper doped calcium phosphate in SiO2 filler was determined, FTIR results were similar, with the values rising as the concentration of copper increased. These results were in agreement to our previous study and as the copper doped calcium phosphate concentrations were increased in dental composite samples, the peak became more visible.

SEM images possess a significant depth of field and a unique 3D image that helps comprehend the surface structure of a sample because of an extremely focused electron beam. Abbas Eslami et al. published in their research paper that SEM can be used to access the surface morphology or topography of copper oxide Nanoparticles with an average copper oxide particle size of 50 to 70 nm, with particle segregation and having a high homogeneity which correlates with our study.[21] SEM images of copper doped calcium phosphate were in accordance with Camille Pierre et al. research paper which showed presence and form of copper oxide nanoparticles adsorbed on calcium phosphate.[22]

Mr. S. Suresh et al. determined the particle size of copper oxide nanoparticles and their uniformity. The polycrystalline nature of copper oxide nanoparticles was determined.[23] Transmission Electron Microscopy (TEM) analysis were also performed to see the

morphology of copper oxide Nanoparticles. The presence of copper nanoparticles doped on calcium phosphate had been confirmed by EDX and correlated with Camille Pierre et al. CuO was identified and confirmed by the EDS findings which were similar to EDS findings by Mr. S. Suresh et al. XRD determines the diffraction peaks of solids that may be either crystalline of different polymorphic forms or non-crystalline (i.e. amorphous).[24] Prasanta Kumar Raul et al. demonstrated the presence of distinctive peaks of copper(II) oxide by X-ray diffraction (XRD) pattern in 2 theta range of 20° to 70°; which were similar to our results.[25]

Nenad L. Ignjatovic et al. in their research paper analyzed the diffraction peaks with the strongest peak at (211) and (300), which were attributed to that of calcium phosphate found at 2θ of 31.70 along with 32.96o respectively. The crystalline materials showed strong diffraction peaks whereas amorphous substances exhibit diffuse and halo diffraction patterns.[26]

Chewing forces emphasize the importance of compressive strength. One of several indicators used to evaluate a material's strength under various force conditions, a greater value indicates a stronger material. The forces during masticatory processes correspond to the compression strength of composite materials. The dental adhesive composite group containing calcium phosphate doped with 2% copper has the lowest compressive strength, whereas the group containing calcium phosphate doped with 6% copper has the highest compressive strength.

Aysu Karakaş Aydınoğlu et al. worked on dental composites to improve its mechanical properties i.e. the compressive strength of dental composite and found out that its degree of polymerization was significantly affected by the salinization process that affects the organic phase and inorganic interface.[27] Ana Josefina Mojarras-Ávila et al. also worked on improving the compressive strength of dental composite with the result that the concentration of monomer and, consequently the type of the copolymer used that constituted the matrix, has an impact on the modulus of elasticity and flexural strength.[28] By improving the quantity, size, and form of filler, the mechanical properties of the dental adhesive composite were improved.

The mechanical characteristics of the dental composite are greatly influenced by the quantity and dimensions of filler particles. Berthelot JM et al. in their research paper worked on mechanical durability i.e vicker hardness by adding fillers such as zirconia or silica to the polymer matrix of dental composite and found out that their properties were greatly improved as compared to un-reinforced polymers.[29] SH Park et al. worked on surface polishing of dental composites and concluded that the celluloid striped finishing of dental composites had less Vickers hardness value relative to dental composite with polished surfaces.[30]

Chinelatti MA et al. also worked on improving the hardness of dental composite and concluded that the hardness of micro, Mini fill composites were increased than flowable composites.[30]

In this study, Vickers hardness increases with increased concentration of copper doped calcium phosphate. The controlled group has the lowest hardness value and the group with 6% copper doped calcium phosphate has the highest value. Overall, as the amount of copper doped calcium phosphate increases, it increases the hardness of the dental composite.

Conclusion

Based on results obtained from modified dental composite with copper doped calcium phosphate, it was concluded that Mechanical properties i.e. compressive strength, Vickers hardness, and flexural strength improved on increasing the concentration of copper doped calcium phosphate.

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Conflict of Interest: The authors declare no conflict of interest.

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Authors Contribution

WA: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading

FA: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading

BZB: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading

AK: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading

SK: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading

SAA: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading