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Preparation and Evaluation of antimicrobial activity of novel calcium based dental cement

Assist. Prof. Neam N. Al-Yousifany[1], Assist. Prof. Dr. Makdad Chakmakchi[2], Prof. Dr. Amer A. Taqa[3]
[1,2]Dept. of conservative dentistry, College of dentistry, University of Mosul, Iraq
[3]Dept. of DBS, College of dentistry, University of Mosul, Iraq

ABSTRACT

The Aims: of the current study are to prepare new calcium based cement from seashell and assess the antibacterial viability of the new material and compare the result with biodentine cement.

Materials & Methods: calcium phosphate based cement have been synthesized from single species of seashell in a form of tricalcium phosphate and hydroxyl apatite , a mixture of mineral oxides extracted from the same shell was used to improve the properties of the prepared formula . The newly prepared cement was characterized by Fourier transform infrared (FT-IR) techniques. Antimicrobial activity was evaluated by agar diffusion test using the following strains: oral Streptococcus mutans, oral lactobacilli & Enterococcus faecalis, after 24 hours of incubation at 37◦C, zone of bacterial growth was observed and measured.

Results: The results showed that the prepared cement has antibacterial effect, these results were statistically analyzed using Oneway, ANOVA and T- test at p=0.01 levels.

Conclusions: Previous research has shown that the sea shells contain many types of metal oxides in different concentration , so in the current search the shell used for the preparation of a new type of dental cement with the same basic components of teeth structure, and within the limitations of the present study, the result revealed that the new cement has acceptable antibacterial activity.

Keywords: seashell, tricalcium phosphate, dental cement, antibacterial assay.

INTRODUCTION

The scientific investigations have been carried out to find products that have chemo-physical and biological properties that can induce tissue healing or replace dental structures that have been injured. For this reason, various calcium phosphate derivatives have been studied for more than 30 years to be used as a source of calcium and phosphate in orthopedics and dentistry (1).

Calcium phosphate are similar to bone and dentine in composition and in having bioactive and osteoconductive properties. In the biomaterial field, nowadays a great attention is driven onto calcium phosphates synthesis and obtaining of ceramics in different forms(such as cements, composites, and coatings) that mimics natural tissue properties, which can be used in many medical and dental applications (2).

Previous researches used different types of materials that are available naturally to prepare calcium phosphate powder, such as bovine bone(3,4,5) fish bone (6) and egg shell (7) . Problems do arise due to the variability of physical and chemical properties of the raw materials (8). Archaeological evidence has discovered ancient Egyptian used seashell to replace teeth (9).

Sea shell considered as waste material , in spite of their valuable economic minerals , analysis of different shell species revealed that they contain many types of , the most predominate one is calcium oxide followed by magnesium oxide , and trace amounts of iron oxide, MnO , Na_2O , K_2O , TiO_2 , CuO , ZnO, P_2O_2 , SrO and PbO(10).

Current trend in restorative dentistry has been greatly influenced by better understanding of advances dental materials science and increased demand for bioactive restorations and one of the bioactive functions proposed for dental materials is antibacterial activity. Research findings indicated that even the presence of any residual traces of infection in the site of restoration affects the success of restoration [11,12]. The bacterial contamination under filling proved to be the main reason for pulp inflammation thus emphasizing the importance of antibacterial properties of restorative materials. [13, 14]

The growth inhibitory effects of some types of dental cements are considered beneficial in preventing bacterial colonization. Thus, it is quite practical to evaluate the antibacterial activity of dental cements with different types of bacteria The study objectives were to prepare a new formula of calcium base dental cement from available natural material, and to assess its antimicrobial activities, then compare the results of estimations with similar available biomaterials.

MATERIALS AND METHODS

Single species of seashell were collected, cleaned, weighted, and stored in the clean dry container. Fifty grams of seashell were homogenized ground by mortar and pestle and electrical grinder, the powder was sieved using 25Mm pore size sieve, submitted to FTIR for analyzing. All FTIR were measured at room temperature cover the range from 400-4000 cm^{-1} using FTIR spectra (Using alpha Bruker spectrophotometer)

Tricalcium phosphate preparation

Seashells were heated at temperature range from 900 to 1200°C for 1 or 2 h with a rate of 5°C min-1 in the furnaces (Protherm, turkey) , for each temperature degree and time interval the product powder was identified by FTIR which revealed that 1000°C for 1h enough for complete removal of organic matter and transferred the mineral content into metallic oxides (mainly calcium oxide) .The resulted material was dissolved in nitric acid and precipitate with a dia-ammonium hydrogen phosphate solution , boil for 1/2 h and left overnight at room temperature , filtered , dried and calcinated in furnace at different temperature range from 1000 to1200°c for 2 or 3 h with a rate of 5°C min^{-1} whereas the cooling was performed as the furnace was left to cool down. The percentage of calcium and FTIR patterns for the calcinated powder were identified and compared with chemically prepared tricalcium phosphate (fig 1 and 2) , the powder heated at 1200 °C for 3h showed the higher level of calcium content reach to about 39 – 40 % while the level of "Ca" in the chemically prepared tricalcium phosphate not exceed 32%.

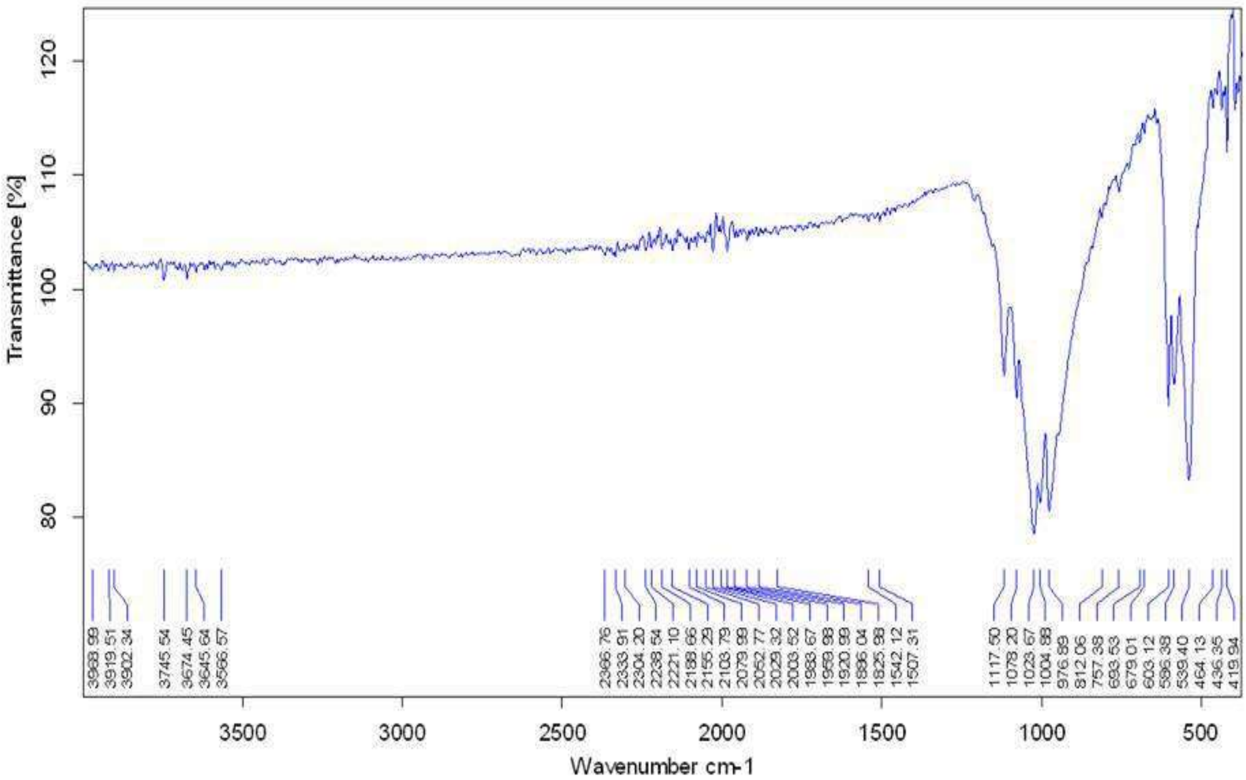

Fig (1): the FTIR of the chemically prepared tricalcium phosphate

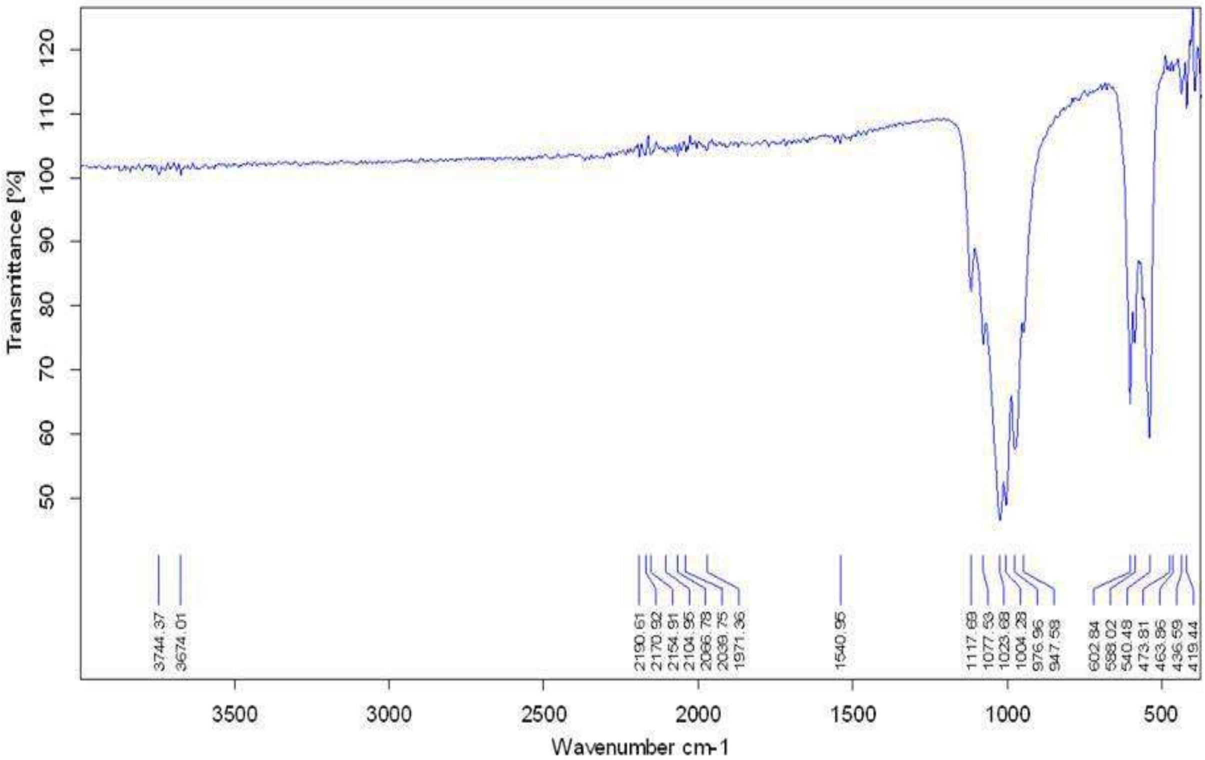

Fig (2): the FTIR for tricalcium phosphate prepared from seashell

Preparation of hydroxyapatite from seashell

Hydroxyapatite (HA) was synthesized from seashell according to (Taqa and AL Sandook 2002)[14] by slow addition of 30ml phosphoric acid to 50gm of heated shells powder with continuous stirring .then the powder was dried and sintered in air atmosphere at 1100°c for 2h.

Preparation of the cement powder

*Tricalcium phosphste 54 -55% (prepared from the seashell as previously mentioned), used as source of calcium and phosphate.
*mixture of mineral oxide 40-41% (mainly calcium oxide from heated seashell), to improve the mechanical properties.
*Seashells powder 3% (grinding without any chemical reaction).
*Hydroxyapatite HA 2% (prepared from cowry shell), to enhanced the biocompatibility of the cement.
*Bismuth subgallate 1% as radioopac material.

The prepared cement powder were sieved with mish size 25 Mm and mechanically mixed to produce homogenous nanoparticles.

Liquid used for the cement preparation was "Poly carboxylic acid"

Several pilot study were performed to select the best liquid that produced the most acceptable result during manipulation and setting , aqueous solutions of polyacrylic acid in a concentration of about 40 % show the most acceptable results.More than 3000 formula were performed to select the suitable one based on the behavior of material during manipulation andmicro hardness measure. The powder / liquid ratio were determined by trial 1/1 ratio by volume , 2P/ 1L ,1P/ 2L and evaluated for setting time and micro hardness , the ratio of 1\1 by volume was the perfect ratio give acceptable working time and harder than the other so this ratio will be used in this study. The preparation of all test specimens were done at 23+_ 1°Cand 50+_ 5% humidity.

Microbiological Part

The antimicrobial assay for novel dental cement was carried out in the Laboratory of Microbiology, Department of Dental Basic Sciences, college of Dentistry, University of Mosul. In the present study, we have two types of materials to be evaluated: 1st group – calcium based cement (the prepared material), 2nd group _ biodentine (Septodont, France)(control material). Three types of microbes were used for evaluation of antibacterialactivity of the studied cements (Streptococcus mutans ,oral Lacto bacilli andEnterococcus faecalis). Streptococcus mutans was isolated from the patient by tacking a swab from the carious teeth after a good isolation and then inoculated on Mitis-saliverious agar (HiMedia, Mumbai) and incubated for 48 h at 37^{o}C.

Oral lactobacilli were collected from carious teeth using a sterile swab and processed within an hour. Each swab was immersed in an aliquot (4.5 ml) of physiological saline and vortexes to disperse the adhering bacteria to the swab. A loopful of this suspension was streaked on the Rogossa agar (HiMedia, Mumbai) and incubated for 48 h at 37^{o}C. Oral Enterococcus faecalis was obtained from Department of Microbiology College of Sciences university of Mosul ,a single colony of bacteria was inoculated on selective media Enterococcus agar medium (HiMedia, Mumbai), and incubated aerobically at 37˚C for 24. All procedures were carried out under an aseptic laminar cabinet.

For all types of bacteria, single colony from the plates of each type of bacteria were transferred into brain heart infusion brothBHI broth (BHI,Lab M,UK) and incubated at 37°C for 24 hours and further isolation and identifications were done by: Gram's stain, Colony morphology. Catalase test

Agar diffusion methods

The antimicrobial effects of set specimens against Streptococcus mutans, Lactobacilli and enterococcus fecaliswere assessed with agar diffusion tests. McFarland 0.5 turbidity tubes were used to prepare suspensions of the strains in PBS at approximately 1.5×108 organisms/mL. Thirty discs shaped specimens (6mm in diameter x 1mm in thickness) were prepared in metalic mold, n=15 for each test group, a sterile cotton swab was used to evenly inoculate the entire surface of the agar plate, and then allowed to dry, discs were placed by a sterile forceps, then the plates incubated in anaerobic candle jar for 24-48 hours. After incubation, the diameter of the zone of inhibition were measured by digital vernia, the inhibition zone was measured at 3 different points and the mean value was used as the result of the specimens. The data were subjected to T test for evaluation of inhibition zone for each type of bacteria, Oneway ANOVA used to compare the results.

RESULT

Statistical analysis for antibacterial assay for each types of material separately were shown in table (1and2) The mean and standard deviation of the zones of microbial growth inhibition (mm) provided by the new material and the control one against three types of microorganism was shown in table(3). Agar Diffusion Assays demonstrated that all microbial species were inhibited by the two types of materials used in the currents study with different levels. Generally, the inhibition zones were lower against oral lactobacilli and it's slightly greater against oral streptococcus mutans and Enteroccocus fecalis.(Fig 3) Statistical difference was observed between the test material and the control one for all types of tested microorganisms.

Table (1): The results of antibacterial inhibition zone of the new calcium based cement

	Sum of Squares	df	Mean Square	F	p–value
Between Groups	424.319	2	212.160	685.335	0.000*
Within Groups	13.002	42	0.310		
Total	437.321	44			

* Significant differences at 0.01

Table (2): The results of antibacterial inhibition zone of the biodentine

	Sum of Squares	df	Mean Square	F	p–value
Between Groups	71.572	2	35.786	398.397	0.000*
Within Groups	3.773	42	0.090		
Total	75.345	44			

* significant differences at 0.01

Table (3): Mean of antibacterial inhibition zone of test materials

Microorganism	No	Ca. ph		Biodentine		t	df	p-value
		Mean	Std. Deviation	Mean	Std. Deviation			
Mutans S.	15	15.5580	0.44509	9.3480	0.22486	48.232	28	0.000*
Lactobacilli	15	11.9053	0.72456	6.6260	0.38495	24.921	28	0.000*
E.Faecalis	15	19.4260	0.45346	9.2520	0.26595	74.955	28	0.000*

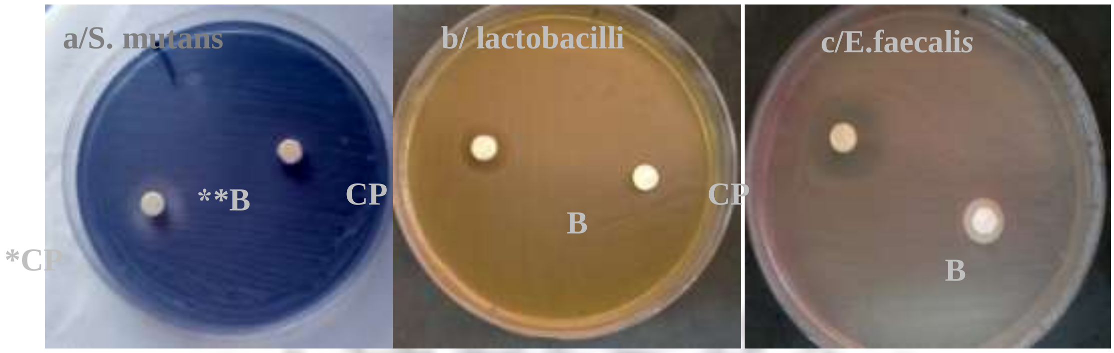

Fig. 3. Agar diffusion test of the two tested materials with different types of bacteria : a/ Streptococcus mutans , b/oral Lacto bacilliand c/ Enterococcus faecalis.

*** CP=new calcium based cement**
**** B= biodentine**

DISCUSSION

In the present study we prepared a recent dental material from seashell, its composed mainly from calcium and phosphate which are the most important inorganic constituents of biological hard tissues. They are present in bone and teeth to give these organs stability, hardness, and function. (2) Fourier-transform infrared spectroscopy (FT-IR, Scimitar Series model Varian 800) was used to determine the various functional groups in the synthesized and sintered calcium phosphate materials in the 4000–400 cm-1 range, IR is employed as a routine technique for determining the presence of functional groups and have been widely used in investigating biological synthetic apatites and related calcium phosphates [(15)].

The new material was evaluated for its antimicrobial activities because one of the requirements of an ideal dental material is that it should include good antimicrobial activity against oral cariogenic bacteria especially in case of those materials which placed in a direct contact with the pulp, periodontium or alveolar bone. [(16)]

Different types of microorganism were used for evaluations, we selected S. mutans and Oral Lactobacilli because they are considered to be the primary etiological agents for the onset of dental caries. Both of them can metabolize a wide range of carbohydrates to adhere to the tooth surface, forming biofilms. A direct relationship between S. mutans and increased caries rates has been reported. [(17)(18)]

We also choose the Enterococcus faecalis because of its inhibitory effect upon dentincollagen (type 1) producing by the odontoblast cell, previous study suggested that many types of the bacterial species among them the E. faecalis may have a direct influence upon the ability of the odontoblast to produce tertiary dentine. [(19)]

In this study, the size of samples were approximately 6 mm in diameter, whereas in most other studies, the diameter of the sample was 4 mm, we believed that 6 mm wells incorporate more amounts of cement and provide a much larger surface area for the diffusion of the soluble antibacterial agents and would ease in the determination of the antibacterial effect of the materials under study. Agar diffusion test (ADT) is commonly used to assess the antimicrobial action of dental materials .This method allows evaluation of the antimicrobial properties of different substances (cements, intracanal medications, and irrigating solutions) against a large number of microbial strains, at various concentration. [(20)]

This assay has ability to measure released water soluble components from the test materials, so the results mainly depend on the solubility and diffusion properties of both dental cements and media. For dental cements, ideal ones are expected to have low solubility and less diffusion properties.[(21)]

In the present study the two dental cements "the test material & biodentine" revealed antibacterial activities against different types of bacterial strain, analysis the result statistically show a significant differences between them. This differences in the results could be attributed to the molecular size, solubility, and diffusion of the materials through the aqueous agar medium. Although there was only few studies that evaluated the antibacterial activity of biodentine, researches believed that Biodentine forms calcium hydroxide, which later on dissociate into Ca^{++} and OH^{-}, this calcium is responsible for antibacterial activity of Biodentine.[22,23]

The antimicrobial activities of the test material against different types of microorganism may be related to mixture of mineral oxide (mainly calcium oxide , magnesium oxide and trace amounts of iron oxide , MnO , NaO , K2O , TiO2 , CuO , ZnO, P2O2 , SrO and PbO from heated seashell) [10] which added to the novel cement , previous study on the antibacterial activities of metallic oxide powders revealed that the antibacterial activity of CaO was the most one , followed by MgO and ZnO, [24] so the inhibition capacity of the new materials may be due to the high percentage of calcium available into the cement powder ,also present of other metallic oxide mainly magnisum , It was suggested that the mechanism of the antibacterial activity of the MgO relied on the presence of defects or oxygen vacancy at the surface of the nanoparticle which led to the lipid peroxidation and reactive oxygen species generation.[25].

CONCLUSIONS

Within the limitations of the current study, the results indicated that calcium phosphate cement can prepare from cheap available materials with acceptable antibacterial activity against different cariogenic bacteria this result is important especially in case of those materials which placed in a direct contact with the pulp, This activity appears to be variable and dependent on factors such as the chemical composition of the prepared materials which is extracted from seashell . Further, research regarding these bioactive materials is still required before used clinically.

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