

Assessment of Anti-Bacterial Effect of Faujasite from Patients with Periimplantitis

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ABSTRACT

Objective: To assess the concentration of Faujasite that inhibit growth of the anaerobic bacteria that growth around implants with signs and symptoms of implant failure.

Design: Experimental in vitro study, antimicrobial effect of faujasite nanoparticles on the anaerobic bacteria isolated from patients with peri-implantitis.

Material and methods: Ten partially edentulous subjects (six women, four men), aged 32– 90 years, who had one or more implants with peri-implantitis, a total of 15 implants were diagnosed with peri-implantitis. Samples of Subgingival bacterial were obtained with sterile paper points from infected implants of everyone.

Results: Revealed that Minimum Inhibition Concentration of Faujasite nanoparticles for Total anaerobic bacteria was (3%), this concentration showed growth after re-culturing on plain BHI-A media.

Discussion: Antibacterial property at 3% concentration of Faujasite nanoparticles, because of metallic contents such as aluminium ions were found in faujasite framework, which induced for better minimum inhibition concentration quantity, in addition the state of oxidation for the Si has an important property in the antimicrobial activity.

Conclusion: The antibacterial activity increased with the increasing in the Faujasite concentration from 3% and above, and there was no effect of Faujasite nanoparticles at concentration 1%, 2%, and 2.5%.

Key words: Gaspack, Sabouraud dextrose agar, Oxiod

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INTRODUCTION

Faujasite is one type of zeolite, so it is a porous aluminosilicate agent with pore size between 3 to 10 Å in diameter. These pores can trap cations (Ag, Zn), water, and organic molecules and exchange them by other cations. By this way zeolite can display appealing features such as antimicrobial effect. Its main constituents are phosphorus, aluminium, oxygen, silicon, etc., which they are repeated in the structure. Zeolites are divided into synthetic and natural groups. Zeolites are widely used in various sciences. Faujasite has been used in industry, agriculture, Animal feed and poultry, Water pollution treatment, biochemistry and so on. Recently it was taken into consideration in medicine. They are widely used in medicine such as wound dressing, drug delivery, as an antibacterial agent, anticancer agent, for osteoporosis treatment, treatment of various diseases, tissue engineering and so on. Zeolite has been also used in dentistry especially for antibacterial properties [1]. Microbial colonization and antibacterial movement on

metallic and ceramic implanted materials have been accounted for under in vitro and in vivo tests. Titanium itself has no antibacterial movement, however there is a plausible danger of plaque development on titanium inserts. In any case, hardly any investigations have been led on a superficial level alteration of titanium inserts to repress the colonization of oral microbes [2].

MATERIAL AND METHOD

Preparation of culture media

Brain heart infusion agar (BHIA)

The readiness of this media was by the guidance of Oxoid Laboratories Company by suspending 47 gm of powder in 1000 ml refined water at that point blended well utilizing attractive stirrer to guarantee disintegration of the entire amount of the powder.

The media was sanitized by the maker's headings. To sanitized media can be used autoclave at 121°C for fifteen minutes after it was poured in sanitized petri dishes, then put aside to cool until use [3].

Brain heart infusion–blood agar (BHIB.A)

Arrangement of this media was by the guidance of Oxoid Company. After cleansing, this media allowed to cool at 45°C and afterward add 5-7% ml of blood and the media was poured in sterilized petri dishes to cool at room temperature, set and were then put away in the fridge until use [4].

Prepared of brain heart infusion broth BHI.B

According to manufacture, the media was prepared by using 34.5 gm of it, dispersed in one thousand ml of distilled water, and locked with capped bottles. Cleansing was finished via autoclave at 15 lbs pressure, 121°C for 15 minutes [5].

Prepare of muller Hinton agar

By Follow the instruction of producer, it was used 35 of agar powder in 1 liter of water refining until total powder of agar was disintegration to sanitize it by autoclave [6].

Method of sterilization

Sterilization was done via autoclave at 121 °C and pressing factor of 15 pounds/inch² for 15 minutes included media, water, and Phosphate buffer solution (PBS) but mouth reflect, kidney dishes, and all spotless glasses were managed by dry air broiler at 180 C° for 60 minutes. Chairs and floor of the lab were sanitized by Dettol clean arrangement [7].

Sample and culture

Anaerobic microbes were secluded from the mouth of 10 patients in the school of dentistry with indications of embed disappointment and bone loss. Anaerobic microorganisms were obtained from the mouth by a swab [8]. This procedure incorporates a delicate scouring of pockets around 11 mm by a sterile q-tip, and afterward along these lines immunizing an essential separation medium, for example, Sabouraud dextrose agar (SDA) [9]. At 37°C for 24 - 48 hrs., swabs were purified on Sabouraud dextrose agar and brooded forcefully, then at 4°C was stored for extra investigation [10].

Culture and analysis of microbes

In glass universal tubes containing 5 ml of Phosphate buffer solution, the sample of anaerobic bacteria mixed in vortex for 2-3 minutes, bacteria were taken by sterile loop and trace it, then incubated for 96 hrs. in blood agar. At 37°C, also plan tubes containing 10 millilitres of BHI broth were inoculated and incubated for 48 hrs. At 37°C All plats and tubes were used gas pack to incubate it in anaerobic condition.

Maintenance of bacterial isolate

States from bacterial confines were gotten from the bacterial agar media and moved to 10 ml of sterile BHI stock and hatched in anaerobic for 24 hrs. at 37°C. These supplies were put away in cooler until utilized and this methodology was repeated twice a month to month [11].

Activation of isolated anaerobic microorganisms

By the expansion of clear, disconnects to ten millilitres of sterile BHI stock which were brooded vigorously for 24 hours, at 37°C and anaerobically for anaerobic microscopic organisms, Inoculums of bacterial were done [12].

Determination of viable count

Absolute reasonable check CFU/ml were finished utilizing sequential weakening with PBS and 0,1 ml from 10⁻³ - 10⁻⁵ were vaccinated on BHI.A brooded anaerobically for 24 hrs. at 37°C [13].

Gram's stain

In the sterilization condition, Gram's stain was put to the distraction parts of blood agar. A little inoculum from a disconnected province was taken, emulsified in a drop of typical saline on a glass slide to frame a suspension, spread, dried and heat fixed. Gram's staining performed beginning with violet for 1 moment and afterward washed with water, stained with Iodine then, cleaned, removed color with ethanol liquid for half an hour. Counter stained with safranin for an extra moment, washed and dried. Under a light magnifying lens with 100X amplifier, the slides were analysed for staining properties, cells' morphology, and course of action as shown in Figure 1 [14].

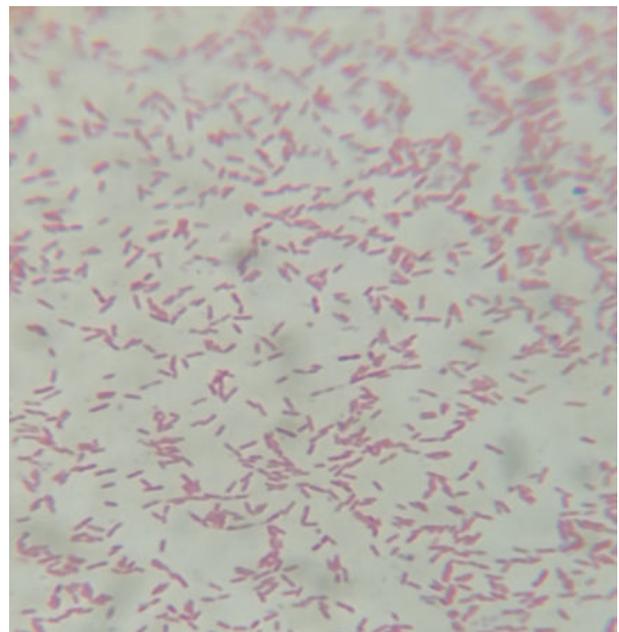


Figure 1: Colonies of anaerobic bacteria under light microscope.

Calculate the activity of nanoparticles Faujasite against anaerobic bacteria

In this study, the antimicrobial effect of the Faujasite NPs on total anaerobic bacteria was defined by the agar diffusion method using ten numbers of isolated anerobic bacteria.

Procedure

Muller Hinton agar media was put about twenty-five millilitres in separated partition of the sterile Petri dishes and waited until set. Brooded at 37 °C for 24hrs. to ensure the media was sterilized. There was activated about 0.1 ml of all-out anaerobic (dilution 10-1) cell forming unit/ml confine was spread on MH agar plates and left for twenty minutes at room temperature, at that point wells of equivalent size and profundity made with clean hardened steel Cork drill in the MH agar 6mm in breadth were set up in the agar. Three holes in each plate, each one was loaded very well up with Faujasite Nanoparticles in various fixations concentration of 3,7,10%. Plates left in the room temperature for 10 minutes, and subsequently brooded in an in an anaerobic container with a gas pack for 24hrs at 37°C. Restraint zones were across the breadth of each hole. The breadth of restraint around the wells containing the test materials was estimated and recorded after the hatching under aseptic conditions. By the Vernia caliper can be measured the area of inhibition zone.

Determination of minimum inhibition concentration (MIC) for anaerobic bacteria

All the selected concentrations dispersed separately with BHI-A to obtained 25 millilitres of agar after pouring it in suitable Petri dishes to become hard, then brooded with 0.1 millilitre of anaerobic bacteria which was activated separately. At 37°C including the control plates (negative control which contained BHI-A with microbial inoculums without the addition of the Faujasite nanoparticles and the plates which contained BHI-A and different concentrations of the Faujasite Nanoparticles without microbial inoculums) which were incubated for one day. Examination all the prepared Petri dish to notice

Table 1: Total viable of anaerobic bacteria.

Concentration of faujasite	Total viable of bacteria
1%	growth
2%	growth
3%	No growth
4%	No growth
5%	No growth
6%	No growth
7%	No growth
8%	No growth
9%	No growth
10%	No growth

Mean of MIC for 10 samples of each 3, 7, and 10 % of Faujasite was 15.9,21, and 25 in sequential as shown in Table 2.

According to manufactured information Faujasite has antimicrobial properties. FAU zeolite has classified into two types- Y and X - depending on the Si/Al percentage: Y

growth of bacteria. The lowest concentration killed the microorganisms could be consider minimum inhibition concentration as seen in Figure 2.

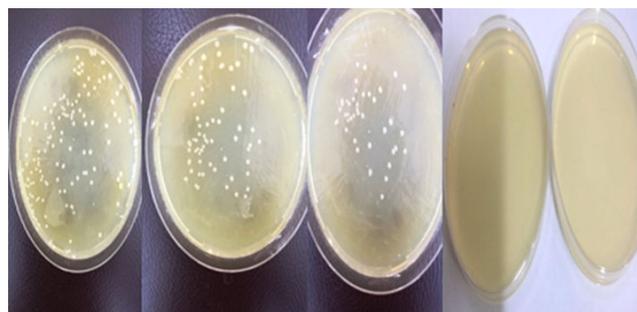


Figure 2: Bacterial growth before the lowest concentration of faujasite that killed microorganism.

RESULTS AND DISCUSSIONS

Antibacterial activity of Faujasite nanoparticles on microorganisms (agar well diffusion method). Results revealed that Minimum Inhibition Concentration of Faujasite nanoparticles for Total anaerobic bacteria was (3%), this concentration showed growth after re-culturing on plain BHI-A media (there was no effect of Faujasite nanoparticles at concentration 2%, 2.5%) number of isolates within the MIC and the concentrations of the Faujasite nanoparticles as shown in Table 1.

The suitable tally of anaerobic bacteria (CFU/ml) an aggregate of 10 segregates were analysed all examples were weakened 1:10 in ordinary saline (0.9% NaCl). Sequential weakening (10-1 to 10-5) were made in ordinary saline and 100µl were plated on blood agar. The plates were hatched at 37°C for 72 hrs., utilizing gas pack. Various delusion were considered and detailed settlement framing units/ml (CFU/ml).

(Si/Al 2.5) and X (Si/Al 1.5) were suitable for multiple usage to build the proficiency of ionic trade [15]. The morphology and molecule size of the examples were explored utilizing SEM portrayal, as demonstrated in Figure 3. From the picture, the morphology of the FAU powder doesn't show a reasonable molecule shape which demonstrates the indistinct idea of the example [15].

Table 2: The minimum inhibition zone.

Inhibition zone	3 % of faujasite	7% of faujasite	10% of faujasite
Sample 1	15 mm	21 mm	25 mm
Sample 2	17 mm	23 mm	23 mm
Sample 3	16 mm	23 mm	22 mm
Sample 4	14 mm	20 mm	27 mm
Sample 5	14 mm	21 mm	26 mm
Sample 6	16 mm	21 mm	27 mm
Sample 7	18 mm	18 mm	25 mm
Sample 8	18 mm	19 mm	25 mm
Sample 9	15 mm	22 mm	25 mm
Sample 10	16 mm	22 mm	25 mm
Mean	15.9	21	25
S.D.	1.4	1.58	1.56

A particle shape was noticed in the specimen with 432.8-615.9 nm., the average particle size was noticed in 243.9 nm that explained in many old surveys.

Average particle size was showed about 432.1 nm in this survey. All specimens were noticed broad T-O (T=Si or Al) in the wavenumber’s area of 900–1100 cm⁻¹ which was interpreted an amorphous crystal faujasite characteristic.

Examines was acted in faujasite focuses indicated great antimicrobial action, especially against anaerobic microscopic organisms [16]. In the concentration of 1, 1.5, 2 and 2.5 % of faujasite, there were not detected any antibacterial property to these concentrations but at 3% concentration, The MIC became clearly, so these results were occurred because of metallic contents, in addition aluminium ions were found in faujasite framework [17] which induced for better MIC quantity.

The state of oxidation for the Si has an important property in the antimicrobial activity as shown in previous studies, but this study was used ranging from 3% to 10% of faujasite, so the antimicrobial capability of zeolite as well as its MIC quantity were evaluated in tested microbial strain. Agar dilution method was utilized to evaluate the antibacterial property of faujasite by using indicator strains [18].

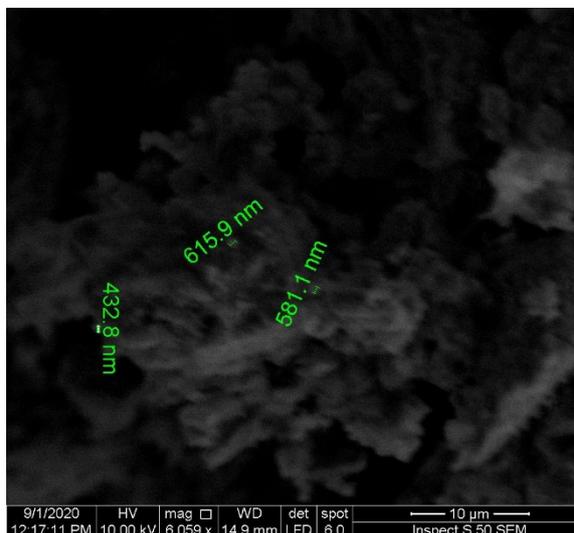


Figure 3: SEM of faujasite (particle size in nanometre).

Faujasite is aluminosilicates with a uniform microporous structure, researchers have demonstrated zeolites to be non-poisonous, feasible transporters, controlled delivery specialists, and adjuvants for drugs. High-silica Faujasite might be saved on microbes, accordingly, killing the arrival of hurtful particles along these lines, it is the impact on the movement of bacterial by the damage of the living framework or the impact on the external layer of the cell by particle trade with the faujasite through the pores that present in the system of the design [19]. The property of Faujasite basicity was occurred due to found of the aluminate anion (AlO₄) in their framework. Fundamental locales are related with the oxygen ions near the cations that make for the negative charge [20].

CONCLUSION

The lower MIC value was recorded at 3% of Faujasite. The potential of activity antimicrobial was associated to its contents include ions of aluminium, Na, Mg, and ions exchange in the framework of faujasite. The ion exchanged in synthetic faujasite structure has a significant role for the anaerobic bacteria inhibition. Antibacterial activity increased with the increasing in the Faujasite concentration from 3% and above, and there was no effect of Faujasite nanoparticles at concentration 1%, 2%, and 2.5%, so MIC of Faujasite nanoparticles that obtained antibacterial properties was 3% of faujasite in concentration.

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