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Coating Orthodontic Miniscrew with Chlorhexidine Hexametaphosphate Nanoparticle (An in vitro-study)

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Abstract

Introduction: Inflammation associated with insertion of orthodontic miniscrews (OMS) may lead to their failure, therefore inhibition of microbial buildup is preferable. Chlorhexidine hexametaphosphate has offered persistent, slow release of active chlorhexidine over time, and hence a good anti-microbial agent.

Aims of study: To prepare and characterize CHX-HMP antimicrobial nanoparticle for coating and characterization of the stainless steel and titanium orthodontic miniscrews.

Materials and Methods: Suspension of 5 mM CHX-HMP nanoparticles were prepared. The formulated suspension was subjected to examination by AFM and FTIR. Sterilized miniscrews were immersed in the prepared suspension for half a minute, then inserted in deionized water for 10 seconds to eliminate any unbound material and then left to dry in air for at least 1 hour. The OMSs were examined by using SEM and FeSEM.

Results: The AFM micrographs displayed that the characterization of nanoparticles expressed a relative homogeneity in terms of particle size and in their topographical distribution with the regular shaped particles being well aligned vertically with mean particles size 45.2 nm. FTIR showed the presence of C=O bond in chlorhexidine digluconate and its absence in the mixed nanoparticles indicate the replacement of gluconate by hexametaphosphate. SEM and FeSEM showed that all coated OMS (stainless steel and titanium) revealed nanoparticles of spherical shape with perfect homogenous distribution with no agglomeration meaning that the coating of OMS with the prepared colloidal suspension have a good dispersion of nanoparticles.

Conclusion: OMS could be coated with antimicrobial CXH-HMP nanoparticles to assist reducing infection associated with the insertion of OMS.

Key words: orthodontic miniscrew, chlorhexidine, CHX-HMP nanoparticle.

Introduction

One of the most challenging phases during orthodontic treatment is controlling the loss of anchorage [1]. Orthodontic miniscrews (OMS) provide several advantages for both orthodontist and patient because of their simple insertion and removal, improved patient comfort, and advantageous cost-benefit ratio. Commercially offered OMSs

show a success rate of 60-75% [2,3]. Numerous things lead to the success percentage of OMS that may be associated with the shape, clinician factors and even patient. Another issue associated with the patient to reduce the miniscrews survival rate was poor dental hygiene [4,5,6].

Most available OMSs are titanium, but stainless steel OMSs are usually found. In spite of the distinct features of these two materials, they both justify the biomechanical requirements of devices used for orthodontic anchorage. Since it is difficult to maintain peri-implant hygiene, it is essential to screen the microbiological colonization in this area. Consequently, the authors directed to regulate the development and establishment of the microbial colonization activity in orthodontic miniscrews during their installation time. The understanding of the qualitative and quantitative features of these microorganisms must assist in reducing the inflammation, enhancing oral hygiene and subsequently rising the long-term success of orthodontic miniscrews [7,8,3].

Prevention of microbial accumulation is emphasized over treatment. The antibacterial activity of chlorhexidine (CHX) belongs to the biguanide pharmaceutical class, which is effective against gram-negative, gram-positive bacteria, and yeast. Numerous investigations were undertaken to create hexametaphosphate (HMP) nanoparticles as CHX-releasing materials [9-14]. CHX hexametaphosphate (CHX-HMP) is a soluble CHX salt that, upon contact with aqueous media, produces a slow release of soluble active CHX over an extended period; the duration of the release and the subsequent concentration of CHX in the surrounding environment are dependent on factors such as fluid flow [15].

At low doses, CHX exerts its effect by damaging membranes, especially phospholipid bilayers, while it causes cytoplasm to congeal at high doses. CHX does not enhance the development of bacterial resistance; therefore, CHX digluconate is frequently used in medicine in general and dentistry in particular [16].

CHX has a broad-spectrum antimicrobial activity as well as plaque removal by its cationic action. It has positive charges that react with the negative charge of microbial cell surface. This activity can increase the permeability of cell membrane by disrupting the osmotic barrier and impeding the membrane transport. It also has the capability to bind to the outer membrane of bacterial plaque and inhibit their attachment to epithelial cells. Due to its actions, it can prevent formation of new plaque rather than decreasing the deposits in pre-existing plaque [10,16].

The magnitude and rate of releasing CHX from CHX-HMP functionalized materials depend on several factors like the local conditions, the doping of CHX-HMP and the matrix where the CHX-HMP is embedded; the duration of release may be ranged from days to months or even years [10,17,18,14]. Therefore, this study aimed to investigate the ability of coating and characterization of the orthodontic stainless steel and titanium miniscrews with CHX-HMP antimicrobial nanoparticle.

Materials and methods

1. Nanoparticle synthesis

The nanoparticles (NPs) were prepared from sodium hexametaphosphate (Sodium HMP) nanoparticle in a crystalline powder form and CHX (CHX) 20% aqueous solution. The molecular weight of HMP is 611.77 gm/mm; so, 100 mL of 10 mM Sodium HMP was prepared by dissolving 0.61177 gm from the powder in 100 ml of double ionized distilled water (DDW). The molecular weight of CHX is 897.8 gm/mm; the CHX digluconate was provided as aqueous solution, so 100 mL of 10 mM aqueous CHX digluconate was prepared by diluting the 20% solution according to the dilution equation:

$$M1 \times V1 = M2 \times V2$$

$20/100 \times V1 = 0.8978/100 \times 100$

$V1 = 4.49$ mL

So, 4.49 mL from the 20% solution supplied by the company (Sigma Aldrich, Germany) was put in a glass container (sterilized by ultrasonic cleaner to exclude any contamination) and filled to 100 ml with DDW. This procedure was done under constant stirring and under ambient conditions. Mixture of the two reagents with rapid stirring lead to the immediate creation of a colloidal suspension of CHX–HMP NPs by a magnetic stirrer using to get a homogeneous suspension with a total concentration of 5 mM of both CHX and HMP. According to previous studies, the minimum inhibitory concentration was dose-related where by the specimens of CHX-HMP-5 displayed a further release of nanoparticles [10,19].

2. Characterization of Nanoparticles

After preparing nanoparticles, the particle size, shape and structure in the colloidal suspensions was characterized by using atomic force microscope (AFM) and Fourier-transformation infrared spectroscopy (FTIR).

A. Atomic Force Microscopy (AFM)

In order to obtain accurate AFM images for the nanoparticles in the colloidal suspension, one drop of the colloidal suspension was first applied to a flat surface of a special glass slides (Mica glass), spread evenly, and then blown dry by using pure nitrogen. The glass slide was then mounted on the AFM imaging Tab., and images were taken [19].

B. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was utilized for the chemical characterization of the created colloidal suspension to confirm the chemical reaction between the components of nanoparticle. This was done by comparing the FTIR charts of Sodium hexametaphosphate powder and CHX digluconate liquid nanoparticles with FTIR chart of the created colloidal suspension after being dried. The samples were prepared by using the classical KBr (Potassium bromide) pellet method FTIR measurement (the scan range was 400-4000 cm^{-1}).

3. Miniscrews preparation and coating

The orthodontic miniscrews (OMS) were divided into 4 groups: 2 control groups (one of stainless steel and one titanium). The other two experimental (coated) groups were stainless steel and titanium OMS coated with the CHX-HMP NP.

Each miniscrews was supplied from the manufacturer (Dentose, Korea) in a small pouch. According to the manufacturer instructions, the miniscrews were sterilized by autoclave steam sterilization at 121ºC for 20 minutes. To coat the sample with nanoparticles, each sterilized miniscrew was carefully removed from the package to prevent any contamination and inserted directly in a test tube containing 10ml of the previously prepared nanoparticle colloidal suspension for 30 seconds being stirred on a rapid stirrer device. Then, it was picked up with a sterilized pair of tweezers and inserted in deionized water for 10 seconds to eliminate any unattached material then leave to dry in a biosafety cabinate for at least 1 hour before further use to remove excess water. Finally, it was inserted in a sealed tube with a clear label till the day of test [9,12].

A. Scanning Electron microscope (SEM)

SEM was applied to study the morphological and topographical surface characteristics and the size of nanoparticles on the surface of the coated miniscrews [19,20]. Image J software program was used to measure the size of nanoparticles on miniscrews surface.

B. Field emission scanning electron microscopy (FeSEM):

To get a clearer image and nanoparticle size measurement, FeSEM scan was used for the coated OMS. FeSEM is an advanced technology used to capture the microstructure image of the material.

Results

Characterization of the prepared colloidal suspension

A. AFM findings

The AFM micrographs show that the nanoparticles characterized by relative homogeneity in terms of particle size and in their topographical distribution. They also show that the particles have regular shapes and well aligned vertically as seen in the 3D and 2D AFM micrographs (Fig. 1. A). From the histogram, the particles size range between 10-58nm with an average of 45.2 nm (Table. 1, Fig. 1.B). In general, the material had a nanoscale size, so it falls within the range of mesoporous materials because it has an average size of less than 50 nanometers.

The distribution of nanoparticles ranged in size in relation to the particles surface area in nm^2 (Fig. 2). It exhibited that the particles with 49 nm had the higher surface area which was about 0.06 nm^2 and the surface area of nanoparticles ranged between 0.01-0.05 nm^2.

Table 1: AFM grain statistics for the prepared colloidal suspension obtained from the AFM micrographs.

Number of Particles	**353**
Coverage	54.25 %
Density	3.502 e^{+10} particles/mm^2
Mean size of particles	45.2 nm
Total projected area	14.49 nm^2
Root mean square height (Sq)	7.886 nm
Maximum height (Sz)	64.71 nm
Arithmetic mean height (Sa)	6.199 nm
Developed Interfacial area ratio (Sdr)	13.53 %

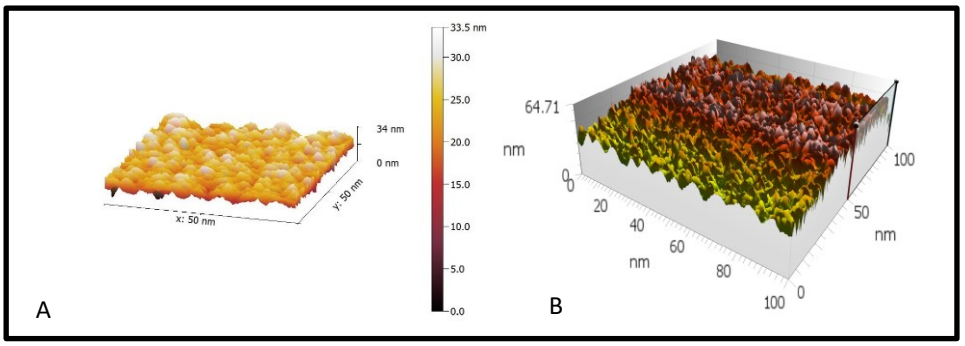

Figure 1: 3D AFM topographical micrograph for nanoparticles of prepared colloidal suspension at 100nm and 50nm respectively.

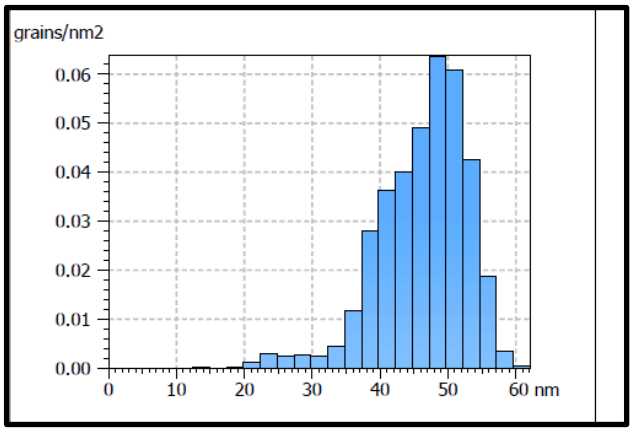

Figure 2: The distribution of nanoparticles sizes in relation to the particles surface area in nm^2 by AFM.

FTIR findings

The FTIR charts for the components of the prepared nanoparticles was studied to determine the chemical interaction between the CHX digluconate and sodium hexametaphosphate in the resultant colloidal suspension. Fig. (3.A) shows the FTIR spectra of CHX digluconate liquid of the nanoparticle before mixing, which shows the presence of characteristics transmittance bands at 3327 cm^{-1} for the O-H group, 2937 for CH_2 asymmetric group and 2860 cm^{-1} for C-H methanediyls group. More band were found at 1720 cm^{-1} for C=O, 1641cm^{-1} for C=O, 1604 for $(CH3)_2NC(=N–H)C(CH_3)_2$. For the aliphatic guanidine absorptions, 1533 (C=N), 1417 cm^{-1} (C=C aromatic), 1490 and 1373 cm^{-1} (C-H methanediyl), 823 cm^{-1} (C-H aromatic), and 717 cm^{-1} (C-Cl aromatic) were found. However, the FTIR spectra of sodium hexametaphosphate crystalline powder alone (Fig. 3.B) showed peaks at 1276 cm^{-1} for the P=O, 1095 cm^{-1} for the P-O, and 879 cm^{-1} for P-O-P.

Figure (4) shows the FTIR spectra of CHX hexametaphosphate colloidal suspension of the prepared nanoparticle in which the FTIR spectra were assessed at range between 400-4000 cm^{-1} in the transmittance mode. It shows broad peaks at 3392 and 3385 cm^{-1} mostly referring to OH bond and may cover the NH bond, 2933 cm^{-1} (CH_2 asymmetric bond) and 2860 cm^{-1} (C-H methanediyls). Yet, for the aromatic guanidine absorptions, peaks were found at 1614 cm^{-1} for the ArNHC(=NH)NHAr), 1531 cm^{-1} for C =N and 1417 cm^{-1} for the C=C aromatic, 1492 and 1375 cm^{-1} for the C-H methanediyl, 1257 cm^{-1} for the P=O, 1089 cm^{-1} for the P-O, 877 cm^{-1} for the P-O-P, and 721 cm^{-1} for the C-Cl aromatic groups.

In the FTIR charts, there are specific characteristic bands which indicate the chemical interaction between CHX digluconate and sodium hexametaphosphate. The presence of C=O in CHX digluconate and its absence in the mixed nanoparticles indicate the replacement of gluconate by hexametaphosphate. In addition, the presence of phosphate and Cl groups in the resultant colloidal suspension of the prepared nanoparticles may indicate the interaction between the sodium hexametaphosphate and CHX digluconate after mixing.

Characterization of OMS coated by nanoparticles

A. SEM findings

Scanning electron microscope images showed the surfaces of both Titanium and Stainless steel OMS before coating with CHX-HMP nanoparticle, and represented the original material of OMS without any interference to see the topography of the surface before coating with nanoparticles (Fig. 4 A-D). Figure (4 E-F) demonstrates SEM images for the stainless steel coated OMS revealing the nanoparticles with a

spherical shape and perfect homogenous distribution. The images showed no agglomeration and this means that the coating of OMS with the prepared colloidal suspension has a good dispersion of nanoparticles. Figure (5 A&B) showed that the particle size was around 32-65 nm and the average particle size was 49.77 nm.

SEM images for the titanium coated OMS revealed nanoparticles of spherical shape with perfect homogenous distribution. The images showed no agglomeration meaning that the coating of OMS with the prepared colloidal suspension have a good dispersion of nanoparticles (Fig. 4 G-H). The particle size was 30-65 nm and the average particles size was 49.08 nm according to Image J software (Fig. 5).

B. FeSEM findings

Figure 6 shows several FeSEM images at different magnification for the stainless steel and titanium OMS coated with the nanoparticle. It showed that the nanoparticle had spherical shape with normal and homogenous distribution of nanoparticles on the coated OMSs surfaces.

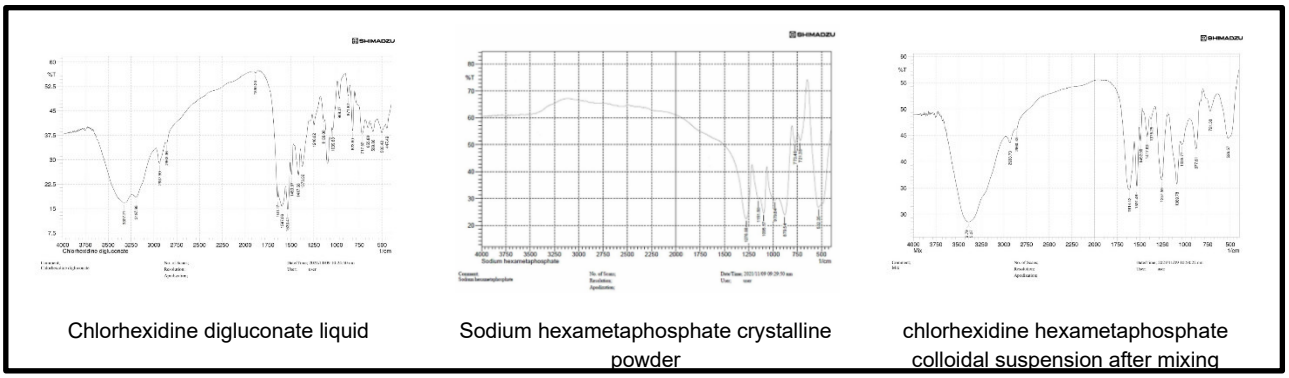

Figure 3: FTIR characterization chart for the nanoparticle with spectra measured at a range between 400-4000 cm^{-1}.

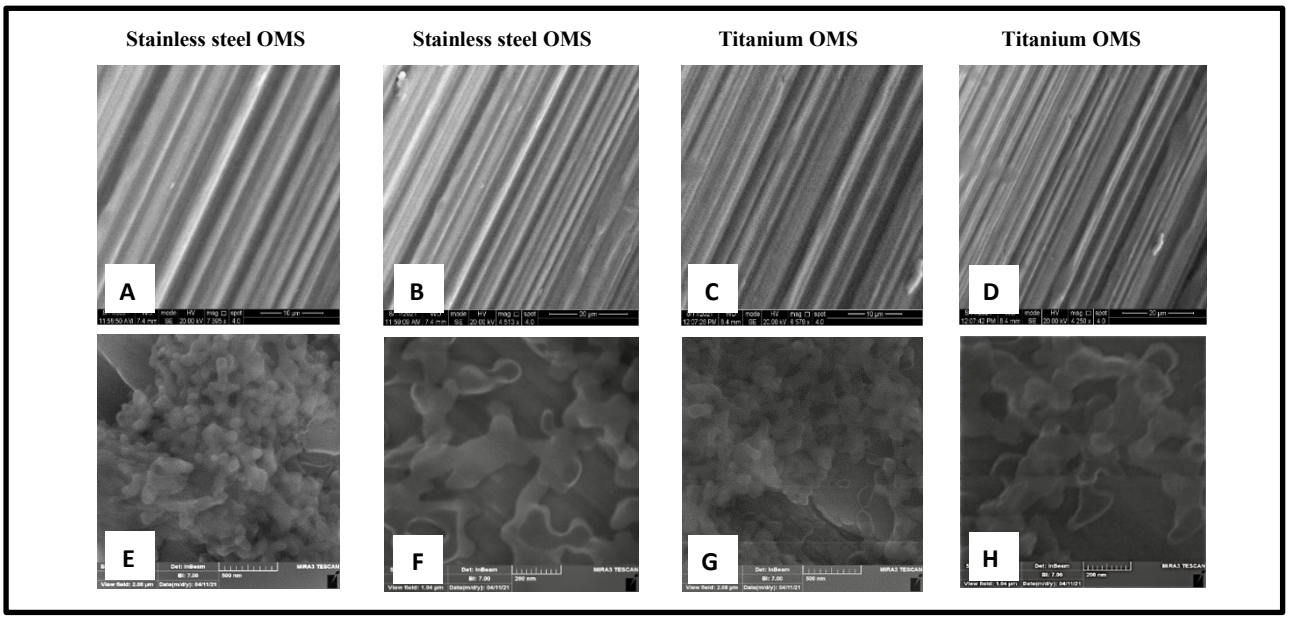

Figure 4: SEM graph for (A-B) uncoated stainless steel OMS, (C-D) uncoated titanium OMS; (E-F)coated stainless steel OMS, (G-H) coated titanium OMS.

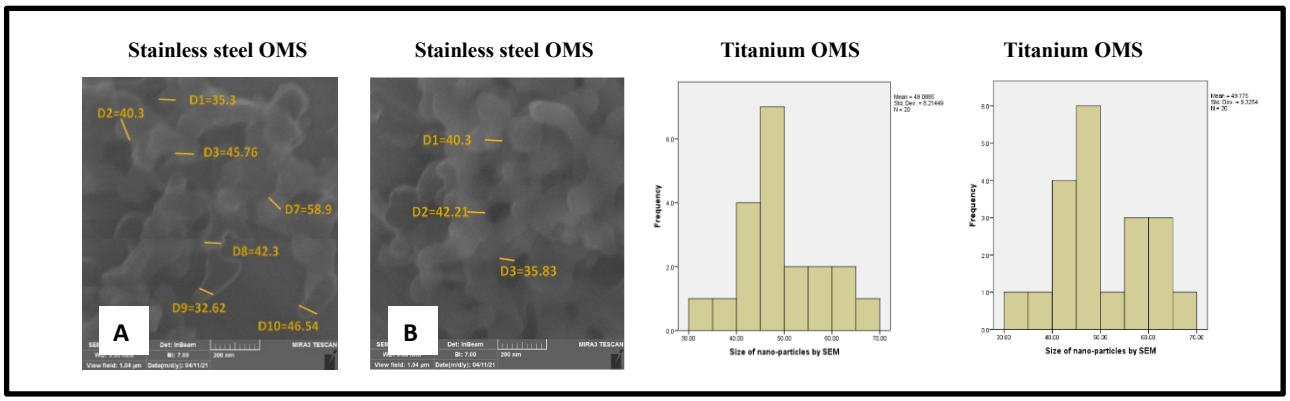

Figure 5: SEM measurement by Image J software to determine the size nanoparticles on the coated OMS for (A) stainless steel and (B) titanium OMS.

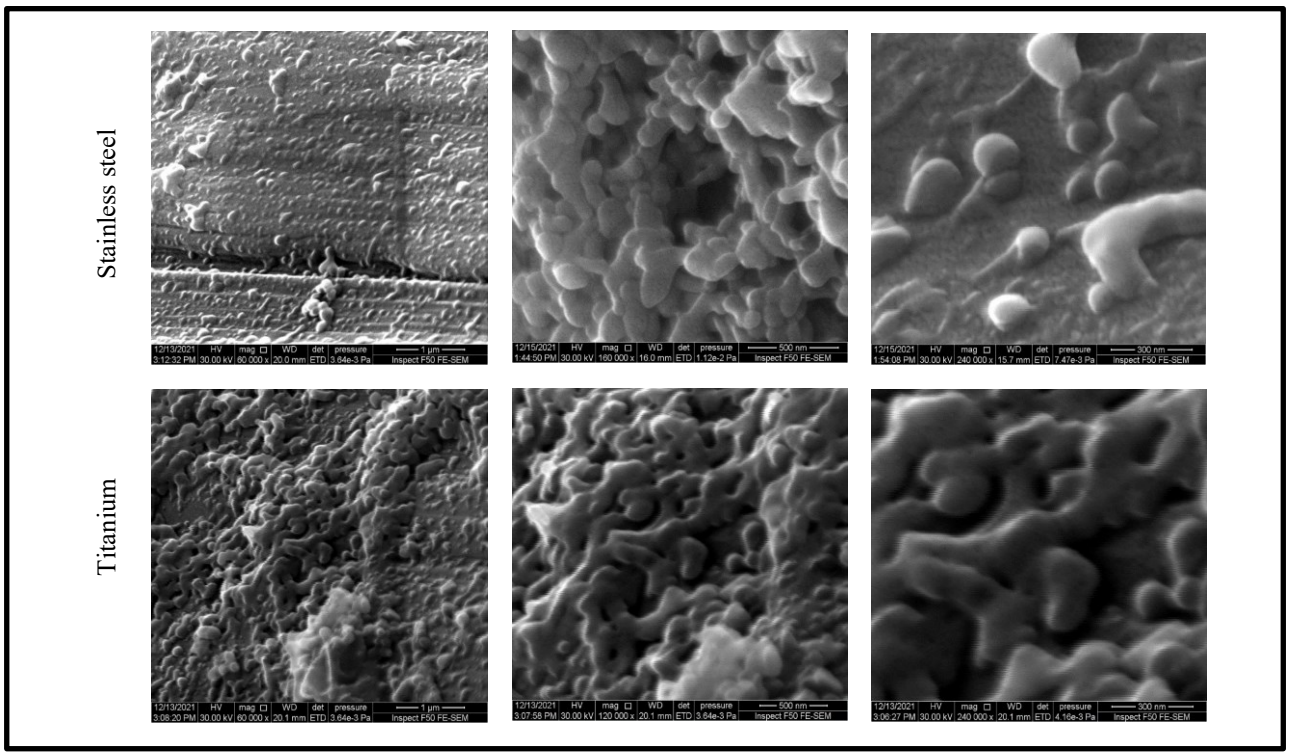

Figure 6: FeSEM images for coated OMS different magnification power.

Discussion

CHX is an effective antimicrobial means against gram-negative, gram-positive bacteria and yeast. The effectivity of CHX is due to damage to phospholipid bilayers in cell membrane at low concentration and at high doses it produces congelation of cytoplasm. As a broad-spectrum antimicrobial and antifungal agent, CHX is broadly used in medicine and dentistry in a CHX digluconate form, it does not stimulate the progress of bacterial resistance [13].

Barbour *et al.* and Wood *et al.* developed materials that release CHX by utilizing HMP nanoparticles in conducted studies [9,21]. Sodium HMP is a cyclic inorganic phosphate, because of its capability to prevent the establishment of extrinsic stains and inhibit the formation of dental calculus it is commonly used in the dental and food dentistry fields. According to a Wood *et al.* , CHX-HMP NP measuring ~49 nm in diameter were worked to coat dental implants [9].

In the present study, the colloidal suspension of nanoparticles was prepared according to the manufacturer's instructions that has also been used in previous studies [9,10,13,21]. The prepared colloidal suspension was characterized by using AFM to evaluate the particle morphology, distribution, and size because it is one of the most effective and proved methods to characterization especially for nanoparticles smaller than 50nm [22]. The mean size of CHX-HMP nanoparticles was found to be 45.2 nm; it was well distributed, homogenous, and had regular shapes. Unfortunately, no previous study used AFM to characterize these nanoparticles.

The prepared colloidal suspension was differentiated by FTIR spectroscopy to evaluate the chemical interaction between the CHX and hexametaphosphate nanoparticles, as one of the valuable method to identify such chemical reaction [23]. The presence of C=O in CHX digluconate and its absence in the mixed nanoparticles indicate the replacement of gluconate by hexametaphosphate. This was in agreement with Duckworth *et al.* whose FTIR results showed the lack of gluconate in the isolated CHX-HMP [11].

Scanning electron microscope (SEM) was used to test the nanoparticle coated OMSs because it is one of the best-approved tools for providing direct characteristic image of surface coated with nanoparticles. The size of nanoparticles ranged between 30-65 nm with an average of 49.08 nm for titanium coated OMSs and 49.7 nm for stainless steel coated OMS, which confirmed the FeSEM result. This was in agreement with previous studies which identified the size of prepared nanoparticles on the coated orthodontic power chain in which the size of nanoparticles ranged between 37-70 nm [13]. Another study by Wood *et al.* determined the size of nanoparticles on the CHX-HMP coated titanium dental implant with TEM which was about 49 nm [9].

In conclusion, stainless steel and titanium OMSs can be coated successfully by antimicrobial CXH-HMP nanoparticles that assist in reduction of the infection associated with the insertion of OMS.

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