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Biophysical Characterization of MTA Plus and Chitosan Conjugate for Biomedical Applications

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ABSTRACT:- Objective: The aim of the study was to evaluate the conjugation of MTA plus (Mineral Trioxide Aggregate Plus) and chitosan using FTIR, XRD and study the surface morphology of the conjugate with AFM.
Material and Methods: MTA Plus was mixed with acetylated chitosan and a conjugate was formed. This conjugate was analyzed by FTIR, XRD, and AFM to study its biophysical properties and surface structure.
Results: The conjugate formed was very much similar in composition and chemical changes or dergradation were not significant.
Conclusion: The conjugate formed can be alternate material in non-surgical and surgical appliactions with better properties.
Clinical Significance: The Conjugate formed using Chitosan has shown promising results. Hence, this conjugate will be the next material of choice as root end filling material and for pulp capping. The properties of chitosan will be synergistic with the root end materials and helps in faster and better healing of lesions.

Key words: Chitosan, Microscopy atomic force MTA Plus, Spectroscopy Fourier Transform Infrared, X-Ray Diffraction Spectroscopy

I. INTRODUCTION:

Protection of pulp, keeping the tooth alive, pulp-dentin and bone regeneration, healing of the periapical tissues, repair of perforations/defects using alternative materials are the targets in dentistry. These will prolong the life of the tooth. For this reason it is essential to use a material which can closely relate with bone/pulp and help in regeneration. Mineral trioxide aggregate is one such material. Mineral trioxide aggregate (MTA) was introduced in the 1990s for use as a root end filling material in surgical endodontics. Its application has broadened rapidly in areas such as vital pulp therapy, perforation repair, retrograde filling, and apexification. Several studies have reported the superior effects of MTA regarding sealability, biocompatibility, and hard tissue–forming capacity [1-3]. Although popularly used material it has certain disadvantages like longer setting time and poor handling properties [4,5].

Newer MTA like materials are introduced in the market to overcome the disadvantages of MTA. Recently, one such material was introduced by Prevest Denpro namely MTA Plus. Manufacturers claim it is similar to MTA but with finer particle size. A study has shown that MTA Plus had good antifungal but poor antibacterial against *Enterococcus Faecalis* [6]. Hence a novel approach of mixing MTA Plus with another bioactive material was thought.

Materials those interact with the biologic tissues are called as Biomaterials [7]. Such materials are widely used in the medical, biomedical and other fields. One such material is chitosan. Chitosan is a straight chain cationic polysaccharide obtained through partial deacetylation of chitin during which a high molecular weight chitin is converted to low molecular weight chitosan [8]. It has its applications in medical, biomedical, dental and other fields. Chitosan is biodegradable with hemostatic, bacteriostatic and wound healing properties [9]. Bioactivity is one of the most important properties of chitosan. It associates with calcium phosphate mineral and maintains high bioactivity because of its functional and structural versatility [10]. It is used as alternative for

the replacement of tissues, including bone tissue, as they have compatibility with the biologic tissue and lesser chances of immune rejection [11].
Studies on chitosan has been performed to evaluate the flow characteristics of sealers, antibacterial property, smear layer removal and root canal disinfection [12-14].
Hence the aim of this study was to evaluate the conjugation of MTA plus and Chitosan using various biophysical techniques. This study was performed to use Chitosan as a biomaterial along with MTA plus and to ensure this novel conjugate meets the needs of dentists for various applications.

II. MATERIAL AND METHODS:

I. Materials:

1. Chitosan of Mol.wt 350 Kda, Deactylation >75% was obtained from Sigma Aldrich (Cat No. 101700976).
2. MTA Plus was supplied by Prevest DenPro, Jammu, India.
3. Glacial acetic acid was purchased from Sisco research laboratories, Mumbai, India

III. METHODOLOGY

1. Preparation of Chitosan gel: Due to high acetylation rate Chitosan was dissolved in various percentage of acetic acid. 2% acetic acid in water was used to dissolve Chitosan.
2. Preparation of MTA plus and Chitosan conjugate: The 2% Acetylated Chitosan gel was used to mix with MTA plus powder, until desired consistency was obtained.
3. Biophysical Analysis: The Chitosan and MTA plus conjugate was subjected to FTIR, XRD, AFM analysis.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR):
FTIR spectra of the MTA, Chitosan and MTA-acetylated Chitosan mixture was obtained using KBr pellets (NICOLET 6700, USA FTIR spectrometer) with spectral resolution from 4000 to 400cm−1.

X- RAY DIFFRACTION (XRD):
To investigate the crystallinity of MTA plus and Chitosan conjugate by XRD analysis using (Philips XPERT-PRO) with Cu-Ka radiation (λ1- 5405980 nm) and 45 kV and 40 mA voltage and current respectively was used in continuous mode. The scan range was 5-70° with a scan speed of 2°2 per minute and the step size 2θ 0.001.

ATOMIC FORCE MICROSCOPY (AFM)
The disc surfaces were analysed with a dynamic force mode atomic force microscope (Nanosurf, Flex AFM). The spring cantilever's length of 125 μm, width of 40 μm and thickness of 2 μm were set. The surfaces were analysed at room temperature, and 256 × 256pixel resolution images were obtained. These images were analysed with image processing and analysis software to measure surface roughness.

IV. RESULTS:

FTIR: The characteristic absorption peaks of various groups in chitosan and acetylated chitosan is shown in figure 1 a and b respectively. There materials showed –OH groups, amine groups and C-H groups at different wavelength.
The characteristic absorption peaks of MTA Plus and MTA Plus chitosan conjugate are shown in figure 1 c and d respectively. The Chitosan/MTA plus complex, almost corresponds to MTA plus pellet and hence conjugation was confirmed.
XRD: MTA Plus showed large peaks representing bismuth oxide, calcium silicate oxide, calcite was observed at 27.39, 33.22 and 29.40 respectively. Peaks at 46.58 and 52.14º represents tricalcium aluminate. There are no noticeable differences in the composition and crystalline structure between the MTA plus and MTA plus/Chitosan complex.
AFM: MTA plus appeared to have flat and uniform base layers that were filled with submicron-sized pits and nano-scale projections, evenly distributed across their surfaces (figure 3 A). MTA Plus chitosan conjugate showed shallow depressions across their surface with bilbous structure and irregular surface with surface roughness measurements that were in the μm scale (figure 3 B).

V. DISCUSSION:

MTA Plus was introduced to overcome the disadvantages of Mineral Trioxide Aggregate. It is claimed to have a finer particle size than other commercially available versions. MTA Plus is available with water as

dispensing liquid or salt-free polymer gel in place of water as the mixing vehicle to improve its washout resistance.

Chitosan is a polymer with hydroxide and amine groups with pKa near to 7 and generally doesn't dissolve in water. Chitosan is soluble in dilute acids, such as acetic acid, formic acid, lactic acid, as well as inorganic acids, after prolonged agitation. In the present study acetic acid of various percentages was used to dissolve chitosan through acetylation.

The biophysical characterization of MTA plus and Chitosan conjugate was confirmed and evident through amine group shifts to 1583 cm^{-1} from 1650 cm^{-1} and disappearance of tertiary amine groups. The C-H vibration shifts from 2934 cm^{-1} to 2922 cm^{-1} and characteristic CH deformation of β- glycosidic bond was recorded. Aliphatic amine shifts from 1054 cm^{-1} to 1545 cm^{-1} with N-O asymmetric stretch was not found in Standard Chitosan spectra. This free electron pair of nitrogen in the amino groups is responsible for the adsorption of metallic cations. The peaks obtained in this study are similar to previous studies [15].
Similar spectra of Chitosan were reported in use of chitosan- chrome derivative for biomedical applications. The results of the FT-IR spectra confirm the presence of MTA plus and acetic acid to form a conjugate [15].

The FTIR spectra of MTA plus and its conjugate with Chitosan is as follows. The peaks of Calcium carbonate shifted from1459 cm^{-1} to 1450 cm^{-1} for conjugate. C-N Aliphatic amines at 1249 cm^{-1} for MTA Plus shifted to 1054 cm^{-1} for MTA Plus chitosan conjugate and at 875 cm^{-1} in both the samples representing calcium silicate. The peak of 1021 cm^{-1} is typical phosphate symmetric stretching. The decrease of NH2 peak intensity, increase in phosphate group peak intensity increased and the carbonate group peak became more distinctive at 1450 cm^{-1}, indicating that the cement surface can promote differentiation process of stem cells [16]. Previous studies on the FTIR spectra of MTA and chitosan has shown similar results [16]. There is no doubt about the identity of MTA plus peaks whose diffraction pattern is super imposable with the IR pattern of Chitosan/MTA plus complex. X-ray diffraction analysis of MTA plus revealed intense peaks of bismuth oxide (27.3) calcium silicate oxide (33.22) and calcite (29.40). MTA Plus showed peaks at 46.58 and 52.14 which represented tricalcium aluminate considering the production of ettringite in the samples [17]. The findings of our study are in confirmation with findings where the setting characters of MTA plus was characterized using XRD [18].

The XRD patterns of MTA plus/Chitosan conjugate showed similar peaks although with same intensity and positions. Thus, there was no noticeable differences in the composition and crystalline structure between the MTA plus and MTA plus/Chitosan conjugate.

AFM examination at the submicron scale showed that the MTA plus appeared to have flat and uniform base layers that were filled with submicron-sized pits and nano-scale projections, evenly distributed across their surfaces. In contrast, the MTA plus/ chitosan conjugate had much larger μm-sized ridges and shallow depressions across their surface with bilbous structure and irregular surface with surface roughness measurements that were in the μm scale. This porous sturcuture of the conjugate formed is helpful in cell transplantation and tissue regeneration [19]. The surface structure evaluated for chitosan in previous studies has shown similar pattern [19]. Thus, the surface of MTA plus/ chitosan conjugate is better developed than the surface of MTA plus.

VI. CONCLUSION:

Chitosan may serve as an alternative chelating agent for use with various root canal sealers. It has both chelating effects and positive effects on the bonding of root canal sealers. Within the limitations of the study the acetylated chitosan and MTA Plus conjugate almost corresponds to MTA Plus when studied under FTIR and XRD. There were no structural changes with the conjugate formed. AFM results indicate that this conjugate formed can be an excellent material in tissue engineering. Hence a modified or newly developed material inspired from the existing one can overcome a weakness of its predecessor. Chitosan offers great opportunities for a bright future. Therefore, intensive studies may be essential in future to bring new possibilities.

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Figures:

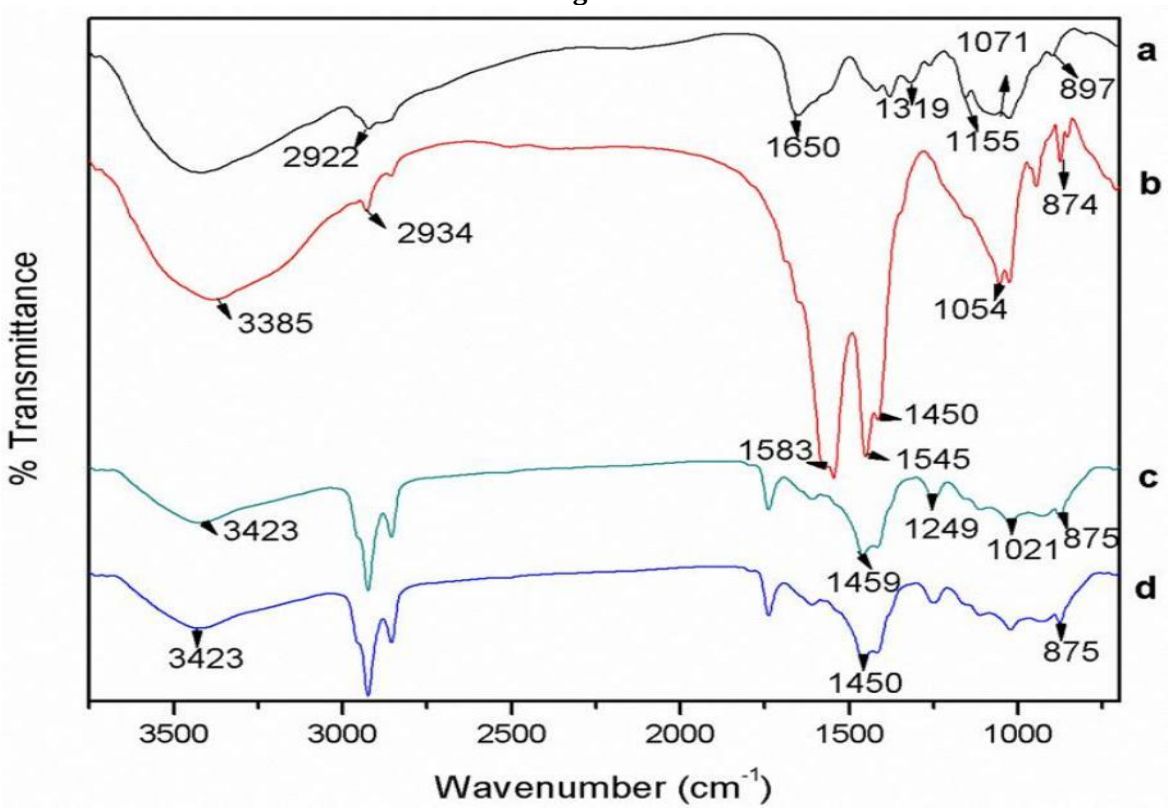

Fig 1: FTIR spectra of Chitosan (a), Chitosan in 2%Acetic acid (b) MTA plus (c) and Chitosan/MTA plus complex (d).

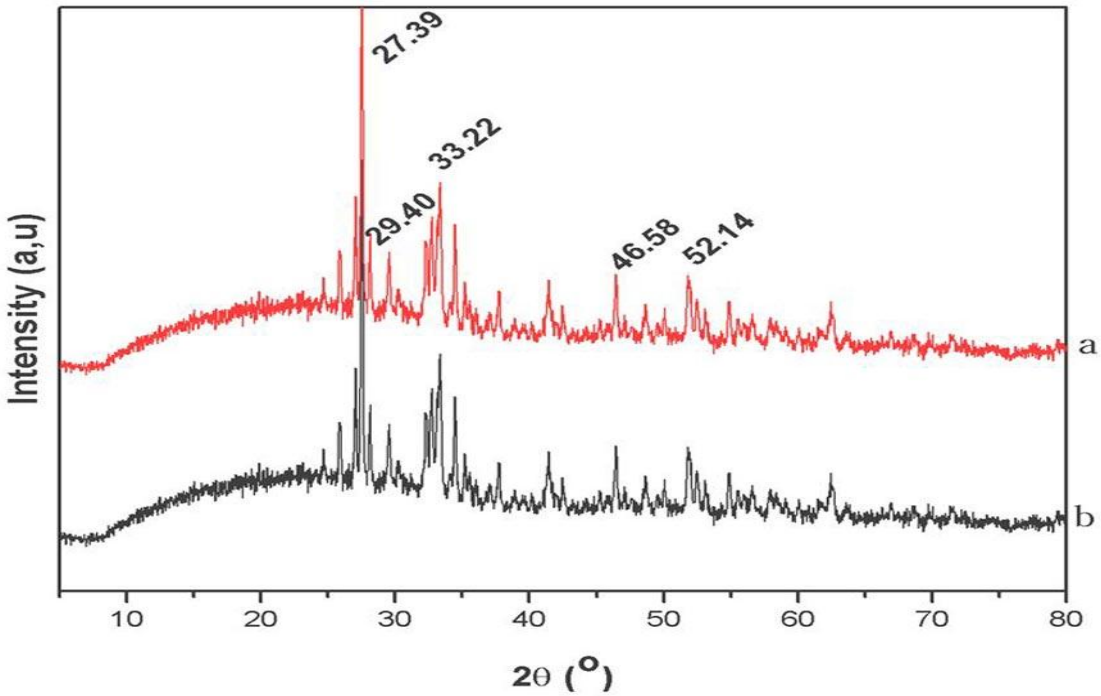

Fig 2: XRD pattern of MTA plus (a) and MTA plus/Chitosan complex (b)

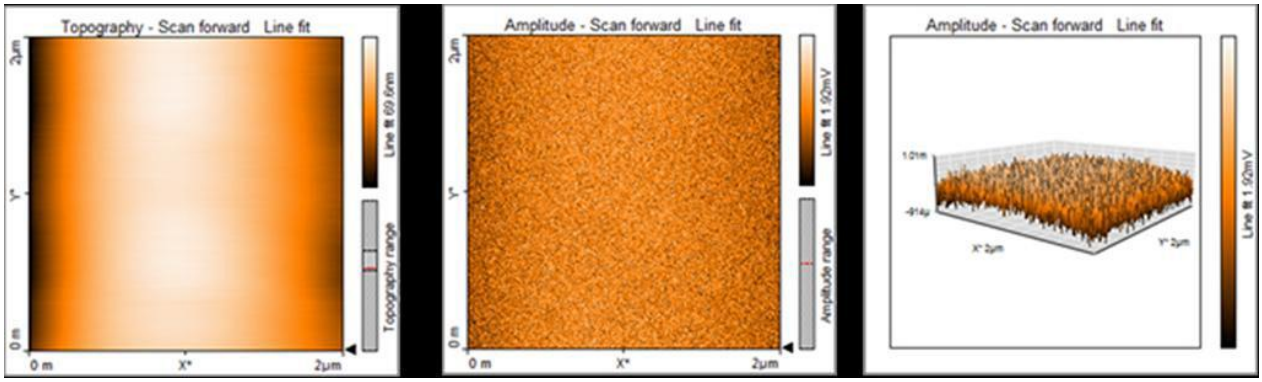

Figure 3A: Surface structure of MTA plus

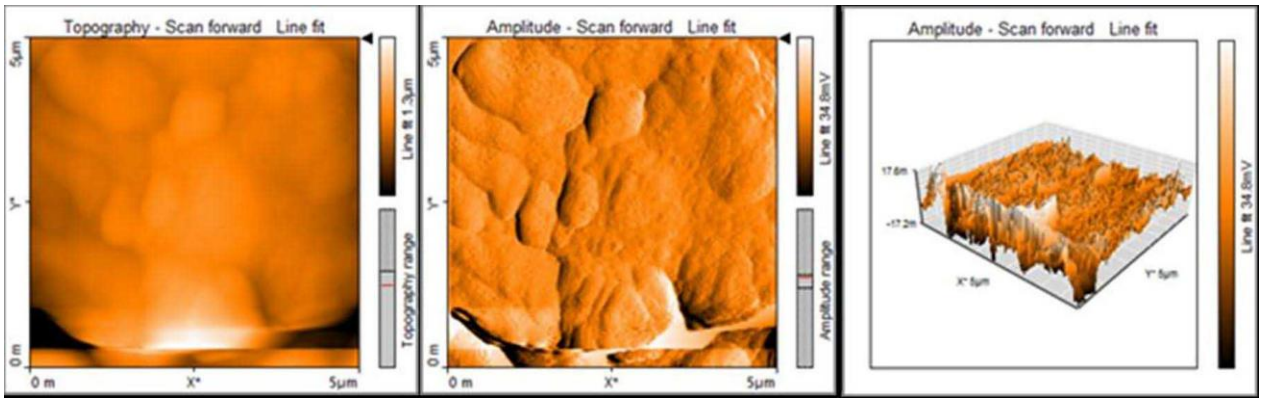

Fig 3B: Surface structure of MTA plus/chitosan complex