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Evaluation of Cavity Adaptation of Silver Nanoparticle Modified Biomineralizing Material

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Abstract

Objectives: To investigate the internal cavity adaptation of various concentration of silver nanoparticle modified MTA and Biodentine material using SEM Analysis. **Methods:** Eighty human extracted maxillary premolars were collected for orthodontic reasons which are of free of dental caries, cracks and any defects. On the occlusal surface class I deep cavities were prepared using tungsten carbide burs and confirmed its depth using williams periodontal probe. Eighty cavities were prepared and randomly divided into 8 groups. Silver nanoparticles were synthesized by $NaBH_4$ reduction of $AgNO_3$ and PEG 2000 as a capping agent. The Ag Nanoparticles are divided into different concentrations (0,2,5,10 wt %) with the use of weighing machine. Different concentration silver nanoparticles were mixed and added with MTA and Biodentine and applied in the pulpal floor with a thickness of 1mm. Groups were divided into Biodentine with 0wt% added Ag NP (n=10), Biodentine with 2wt% added Ag Np (n=10) , Biodentine with 5wt% added Ag Np (n=10) , Biodentine with 10wt% added Ag Np (n=10) and MTA with 0wt% added Ag Np (n=10) , MTA with 2wt% added Ag Np (n=10) , MTA with 5wt% added Ag Np (n=10) , MTA with 10wt% added Ag Np (n=10). All the specimens were thermocycled for 24hrs. Teeth were sectioned through center of restoration in bucco-palatal direction with a diamond disc. The specimens which are obtained from each tooth were subjected to Scanning Electron Microscopy for evaluation of internal cavity adaptation of dentin with pulp protecting material. Results: SEM analysis report showed that, the Silver Nanoparticle additives to the bio-mineralizing pulp protecting material in comparison with conventional types, showed better cavity adaptation in 5 to 10wt % concentration. **Conclusion:** A minimum of 5 to 10 wt% of silver nanoparticle has a significant role to improve the physicochemical behavior of pulp protecting bio-mineralizing materials for the better cavity adaptation.

Keywords: biomineralizing material, internal cavity adaptation, silver nano particles.

Introduction

A variety of dental materials have been introduced to provide pulp protection from physical, mechanical, chemical and biologic irritants. Direct pulp capping refers to placing the pulp protecting material on exposed pulp where odontoblast layers are breached to enhance reparative dentin formation, which are mediated by odontoblast like cells differentiated from dental pulpal stem cells at the materio-pulpal complex. It is important to optimize direct pulp capping techniques improve biocompatibility of materials and enhance biological response of pulp tissues to maximize regeneration of reparative dentin. A long term hermetic seal of the restoration is necessary to provide protection of pulp-dentin complex.

It is important to optimize direct pulp capping techniques improve biocompatibility of materials and enhance biological response of pulp tissues to maximize regeneration of reparative dentin. A long term hermetic seal of the restoration is necessary to provide protection of pulp-dentin complex. The main reason for biologic failure of the restoration is poor ability of restoration to adapt with the inner cavity surface. In restorative dentistry, micro leakage is defined as the leakage of ions, fluids, bacteria and bacterial by-products through the tooth-restoration interface. It leads to failure of restoration through partial or total loss of restorative material by causing secondary caries and pathological changes in the pulp. The materials such as Dental Amalgam, Resin based composites, Ceramics and Metals are used to restore missing parts of teeth. Biologically, these materials are not expected to behave entirely like enamel and dentin. The use of bases and liners are necessary not only to protect the vital pulp and dentin from the potential toxic effect of restorations but also to provide good adaptation of tooth-restorative interface.[1]

The Bio ceramic materials in restorative dentistry can be considered as a magnanimous entity which has changed the prognosis of many cases which were once considered as next to impossible. A remarkable biocompatible material, MTA with improved clinical applications was pioneered by Dr. Mahmoud Torabinejad and the Bio dentine a new bioactive calcium silicate based cement launched as a dentin substitute. The introduction of MTA and Bio dentine was a breakthrough for replacement of Calcium hydroxide and became the material of choice as a pulp protecting agent due to its advantages of biocompatibility, less pulpal inflammation, predictable hard tissue barrier and radio opacity. [2]

The quality of dental biomaterials has been improved by the emergence of nanotechnology. The basic idea of in co-operation of nanotechnology is to employ individual atoms and molecules to construct functional structures. It can alter the molecular arrangements of macroscopic properties of a material. Nanoparticle affect the hydration process and fill empty spaces and is beneficial in producing and constructing advanced biomaterials with improved physicochemical, mechanical and anti-bacterial properties of dental materials. The adaptation of pulp protecting materials to the pulpal floor and the occurrence of gaps affect the longevity of the restoration. [3] Even though the bio-mineralizing pulp protecting material proved its clinical efficacy, the time period between the liner placement and bio-mineralization is crucial. Especially the situations like ultra conservative removal of carious tissue, often referred to as partial caries removal. In this procedure, practitioner removes most but not all of the infected dentin, seals the cavity and proceeds with the restoration. Cariogenic bacteria which are present under the restoration which can proliferate and leads to secondary caries. [4] The space which are present between the restoration and pulpal floor which serve the zone for the multiplication of cariogenic bacteria. In this study synthesized silver were nano particles, incooperated into the pulp protecting material to check its adaptability with the dentin.

AIM

To investigate the internal cavity adaptation of various concentration of silver nanoparticle modified MTA and Biodentine material using SEM Analysis.

Materials And Methods

Eighty human extracted maxillary premolars were collected for orthodontic reasons which are of free of dental caries, cracks and any defects. On the occlusal surface class I deep cavities were prepared using tungsten carbide burs and confirmed its depth using Williams periodontal probe. Eighty cavities were prepared and randomly divided into 8 groups. Synthesis of Silver nanoparticles.

Silver nitrite dissolved in pure water and kept in ice bath for 15 degree celcius. Poly Ethyl Glycol 2000 added as a capping agent and Sodium borohydrate was added drop by drop until a black precipitate is formed. Then the black precipitate was washed with deionized water and it was dried at 80 degree celsius vacuum for 2 hours. [10,11]

The silver nanoparticles were divided into different concentrations (0,2,5,10 wt%) with the use of weighing machine. MTA and Biodentine were mixed according to the manufacturer recommendation by incooperating different concentration of silver nanoparticles and applied into the pulpal floor with a thickness of 1mm. Thickness of 1mm of pulp protecting agents were standardized by measuring the depth of the cavity using williams periodontal probe followed by the remaining portion of cavity was restored with composite resin. Groups were divided into Biodentine with 0wt% added Ag NP (n=10), Biodentine with 2wt% added Ag Np (n=10) , Biodentine with 5wt% added Ag Np (n=10) , Biodentine with 10wt% added Ag Np (n=10) and MTA with 0wt% added Ag Np (n=10) , MTA with 2wt% added Ag Np (n=10) , MTA with 5wt% added Ag Np (n=10) , MTA with 10wt% added Ag Np (n=10). All the specimens were thermocycled for 24hrs.

Each cycle consist of 15s of immersion in 15 degree celsius water bath, 10s dwell time, 15s immersion in 55 degree celsius water bath. Teeth were sectioned through center of the restoration in bucco-palatal direction with a diamond disc. The specimens which are obtained from each tooth were subjected to Scanning Electron Microscopy for evaluation of internal cavity adaptation of dentin with pulp protecting material.

Results

Scanning Electron Microscopy has been done to evaluate the cavity adaptation with pulp protecting material. Statistical analysis done using ANOVA. All the groups showed statistically significant of $P<0.0005$ with the control group (0wt% AgNP).

Discussion

The restoration of compromised dental structure requires attention to function, esthetics and biology. This can be achieved with current restorative materials and techniques.[1,2,3] For many years it was believed that restorative materials were toxic to the pulp. Based on that assumption, the use of bases and liners covering the vital dentin for pulp protection was considered essential to the success of the restoration. This recommendation was based on studies that linked pulp reactions to the low PH of dental materials. However, it is now believed that the main reason for the biologic failure of restorations is not related to PH or other attributes of the restorative material, but rather to the poor ability of restorations to seal the tooth-restoration interface leading to marginal leakage of bacteria and toxins. In restorative dentistry, the most common causes of pulp injury, before, during and after a restoration is placed are presence of bacteria in the dentin-pulp complex, exposure of patent dentinal tubules, depth of tooth preparation, dentin dehydration and heat generation. Pulp protecting agents are applied on to the pulp when it is exposed by traumatic injuries, mechanical factors or dental caries in order to allow pulp healing and to maintain the vitality of pulp and its functions. An ideal pulp protective material should satisfy the following requirements such as, it should stimulate reparative dentin formation, maintain pulp vitality, it should not cause secondary caries, it should be bactericidal or bacteriostatic, it should resist masticatory forces during the life of restoration, it should be osteo-inductive, radio opaque and adhere to internal cavity surface. [5,6]

Bioceramics offer a new treatment options for improving prognosis in many operative procedures. It surpasses many traditionally used materials such as calcium hydroxide. Research is being done to improve the properties of bioceramics to provide quality treatment.

Mineral Trioxide Aggregate was first proposed for pulp capping in 1996. It primarily composed of calcium oxide in the form of tricalcium silicate, di calcium silicate and tricalcium aluminate. When MTA reacts with water it forms calcium hydroxide and so it is actually the formation of calcium hydroxide that provides MTAs biocompatibility. It helps in the formation of thicker dentin bridge, with low inflammatory response, hyperemia and pulp necrosis compared to calcium hydroxide cement. There are several disadvantages with MTA, as well. It has shown high solubility, demonstrating 24% loss after 78 days of storage in water. The presence of iron in the grey MTA formulations may darken the tooth. The prolonged setting time of MTA is approximately 2 hours and 45minutes. This requires the pulp capping with MTA either be done in a two-step procedure, placing a temporary restoration to allow the MTA to set before placing the permanent restoration, or using a quick setting liner to protect the MTA during permanent placement of restorations. [7,8]

Biodentine is also known as dentine in a capsule, biocompatible and bioactive dentin substitute. It was made commercially available in 2009. This also has biocompatibility, good antimicrobial activity, stimulates tertiary dentin, good handling property, less soluble and produce tighter seals as compared to calcium hydroxide. Its micro mechanical adhesion is caused by alkaline effect during setting reaction which causes organic tissues to dissolve out of dentinal tubules. This material is actually formulated using the MTA based cement technology and the improvement of some properties of these types of cements, such as physical qualities and handling. The setting period of this material is an improvement as compared to other calcium silicate materials it is as short as 9 – 12 minutes. According to Grech et al. the final setting time of biodentine is 45minutes, however, 9 – 12 minutes indicated in the product sheet is the initial setting time. In his study the author described that this methodology uses a vicat apparatus with a needle of specific mass. And the setting time of mixture calculated as the time taken from the start of mixing until the indentor fails to leave a mark on the set material surface. He also demonstrated the washout resistance of Biodentine. Washout of a material is defined as the tendency of freshly prepared cement paste to disintegrate upon early contact with fluids such as blood or other fluids. In his study the Biodentine did not reveal favourable results as the material demonstrated a high washout with every drop used in the methodology. The author attributed this results due to the added additives in the liquid portion that is the surfactant effect water soluble polymer added to the material to reduce the water/cement ratio. [7,8,9]

Adaptation of restorative materials to tooth cavity walls and absence of gaps between restorative and lining materials is cruicial for the longevity of the restorations. Marginal gap formation is related to discomfort in conjunction with occlusal forces, which may be attributed to fluid accumulation within the gap and subsequent fluid movement within the tubules. Gaps between various interfaces are influenced by several factors such as internal adaptation of restorative material to the cavity, bond strength and ability of the bond to resist strain and inco-operation of nano-filler particles. The advantages of nano filler particles is that they improve thermo mechanical properties and also reduce the degradation of restorative material due to their large surface area. [9] Nanotechnology started with the beginning of the era of microfills. Nanotechnology mainly consist of the processing of, separation, consolidation and deformation of materials by one atom or one molecule. Nano sized silver metallic particles are unique, it size ranges from 1-100 nm and can considerably change physical, chemical and biologic properties due to their surface to volume ratio. The silver nanoparticles can be synthesize using several approaches including physical, chemical and biological. In this study silver nanoparticles were synthesized using a chemical approach to obtain the particle sizes ranging from 40 to 60 nm. Because of their small size, silver nanoparticles possess chemical, physical and biologic properties distinctive from those presented by traditional bulk materials.[11] Their small particles and larger surface area provide potent antibacterial effects and mechanical strength at a low filler level, diminishing silver particle concentration necessary for its efficacy and avoiding negative influence on mechanical properties. So in this study, we have checked at what percentage the adaptability is better between pulpal floor and restorative material. Silver can able to penetrate easily the bacterial cell membrane resulting in rapid bacteriocidal activity. Silver can interfere with DNA and proteins by interfere with SH- Groups, and also the base pairing, DNA unwinding, cell wall synthesis and respiratory processes resulting in bacterial death. [12]

The effect of silver additives on properties of biomineralizing pulp protecting agents are not yet discussed till now. Even though the bio-mineralizing pulp protecting material proved its clinical efficacy, the time period between the liner placement and bio-mineralization, it takes about 30 days and the prolonged setting time of these material which is cruicial especially the situations like deep carious lesions with infected dentin, the practitioner removes most but not all of the infected dentin when there is less remaining dentin thickness, seals the cavity and proceeds with restoration. Cariogenic bacteria which are present under the restoration proliferate and leads to secondary caries.[13] The space which are present between the restoration and pulpal floor which serve the zone for the multiplication of cariogenic bacterias. So in this study nano-sized silver particles were synthesized and incooperated onto the pulp protecting material to check its adaptability between pulp protecting material and dentin. In a study by saghiri et al 2015 evaluated the effect of particle size on calcium release and elevation of PH of endodontic cements, he said that nano modification of MTA remarkably increased the calcium ion release at all time intervals of post setting, which can significantly influence the osteogenic properties of human dental pulp cells and as a consequence enhanced mineralized matrix nodule formation to achieve desirable clinical outcome.[14] Another study by Bernardi et al 2015, evaluated the effects of the addition of nanoparticulate calcium carbonate on setting time, dimensional change, compressive strength, solubility and PH of MTA. He observed the addition of nanoparticulate calcium carbonate to MTA accelerated the setting time. [15] Several other studies using nanoparticles as an additive for Portland cement found similar results in the decreasing setting time (sato & Diallo 2010, Sato & Beaudoin 2011, camiletti et al 2013). According to these studies, the setting accelerator effect is due to the action of nanoparticle sowing, that is, it acts as a seed (site/point), which stimulate growth (nucleation) of calcium silicate hydrate and accelerates the process of Portland cement hydration. Furthermore, the addition of nanoparticles to Portland cement provides a more effective cement/water interaction and induces the formation of a solid microstructure by increasing the contact points due to larger proportion of hydrated cement (camilleri et al 2013). [16,17]

Based on the results of this study, the added nanoparticle to MTA and Biodentine improved the internal cavity adaptation.

Conclusion

Based on the present study, it can be concluded that:

A minimum of 5 to 10 wt% of silver nanoparticle has a significant role to improve the physicochemical behaviour of pulp protecting bio-mineralizing materials for the better cavity adaptation.

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17. Seeding effect of nano calcium carbonate on the hydration of Tri calcium silicare; Sato T, Diallo F et al; Journal of Transportation Research Board; 2010.

Legends Figures

Figure 1 a

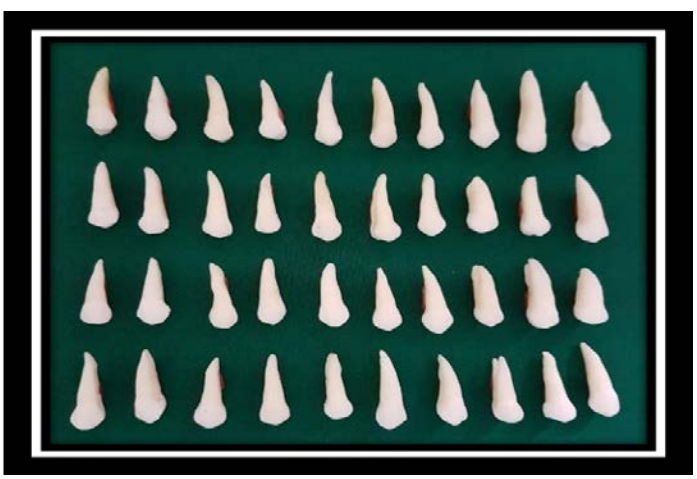


Figure 1 b

Fig 1a and b: Intact extracted Human maxillary premolars

Figure 2: Class 1 cavities were prepared

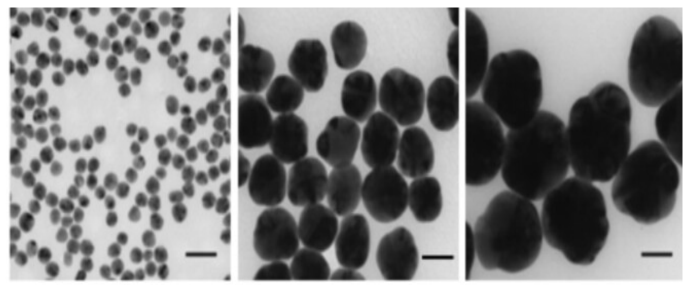


Figure 3 a

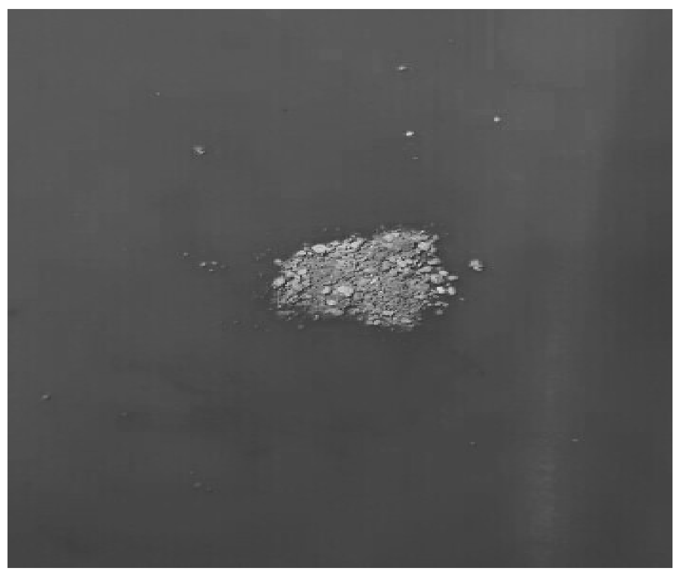


Figure 3 b

Fig 3a and b : Silver nanoparticles were synthesized by NaBH4 reduction of AgNO3 and PEG 2000 as a capping agent

Figure 4 a

Figure 4 b

Figure 4a and b: The Ag Nanoparticles divided into different concentrations (0,2,5,10 wt %) with the use of weighing machine

Figure 5 a

Figure 5 b

Figure 5: a and b MTA and Biodentine

a b c

Figure 6a, b and c : Incooperation of silver nanoparticles into MTA and Biodentine

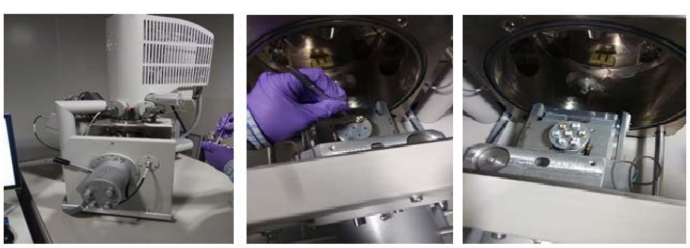
Figure 7: 1mm of Liner applied and rechecking the Cavity depth with Williams's periodontal probe

Figure 8: Cavity is restored with composite resin

Figure 9: the specimens were thermocycled for 24Hrs. Each cycles consist of 15s of immersion in 15 degree celcius water bath, 10 S Dwell time 15s immersion in 55 degree celcius water bath.

a b

Figure 10 a and b: Teeth were sectioned through center of restoration in bucco-palatal direction



a b c

Figure 11 a,b and c : Scanning Electron Microscopy for evaluation of internal cavity adaptation of dentin with Liner material.

SEM images

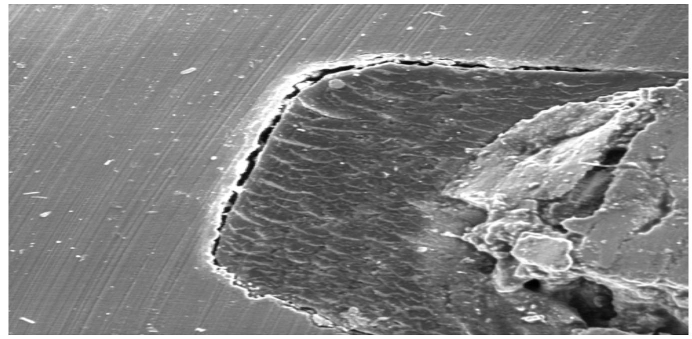


Figure 12: MTA 0wt% AgNP

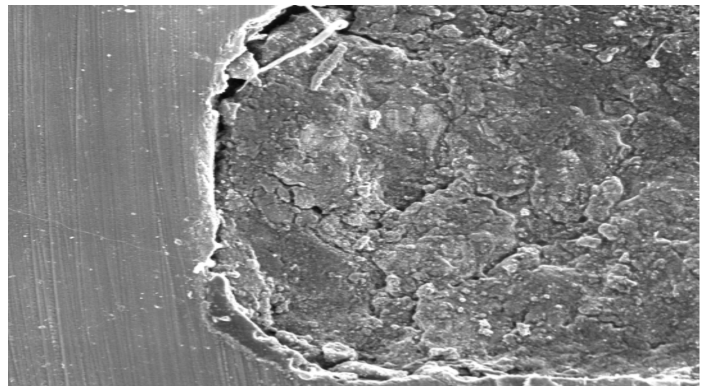


Figure 13: MTA 2wt% AgNP

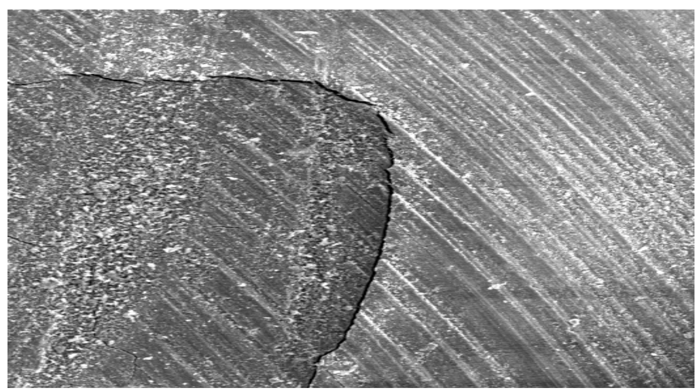


Figure 14: MTA 5wt% AgNP

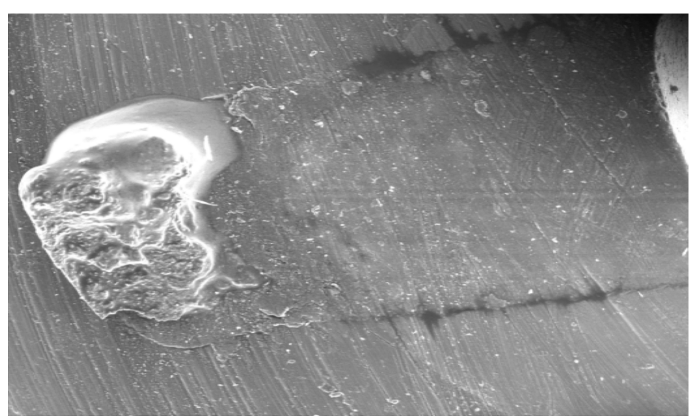


Figure 15 : MTA 10wt% AgNP

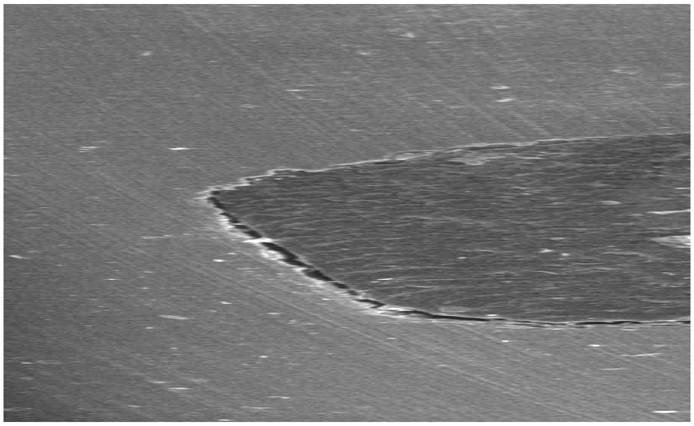


Figure 16 : Biodentine 0wt% AgNP

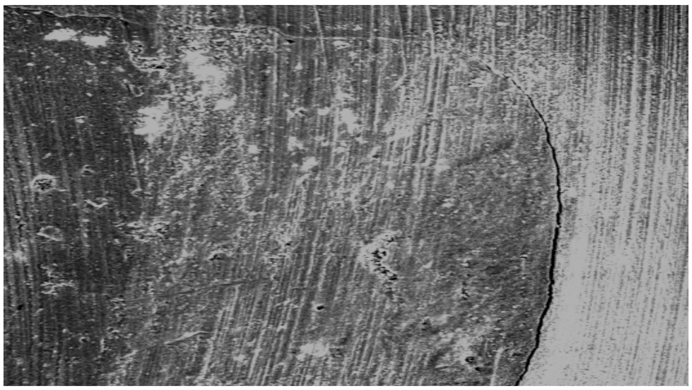


Figure 17 : Biodentine 2wt% AgNP

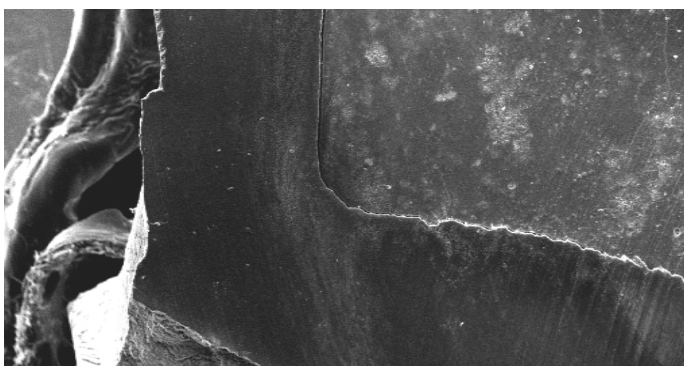


Figure 18: Biodentine 5wt% AgNP

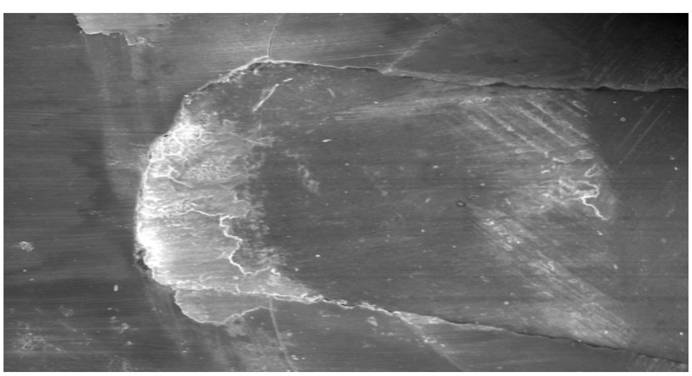


Figure 19 : Biodentine 10wt% AgNP

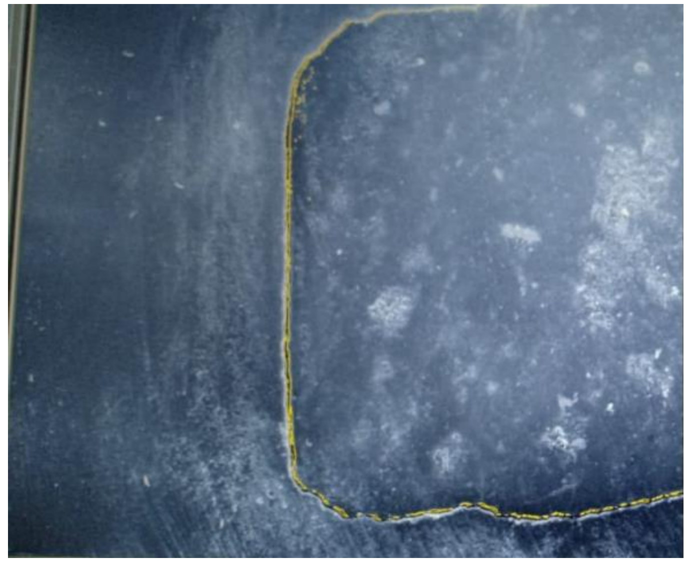


Figure 20: Using Image J software Gap volume was assessed and analysed

SEM Analysis report

It showed that, the Silver Nanoparticle additives to the biomineralizing pulp protecting material in comparison with conventional types, showed a better cavity adaptation at 5 to 10wt % concentration of silver nanoparticles.

Table 1: summarizes the values obtained by Image J software.

0 wt% Ag NP Biodentine	2wt% Ag NP Biodentine	5wt% Ag NP Biodentine	10wt% Ag NP Biodentine	0 wt% Ag NP MTA	2 wt% Ag NP MTA	05wt% Ag NP MTA	10wt% Ag NP MTA
900.039	609.871	200.012	201.013	1209.451	970.021	250.371	250.721
855.60	559.021	195.981	190.001	1200.451	982.021	252.341	251.821
848.32	568.71	210.112	209.981	1199.022	985.981	244.562	243.900
943.71	622.82	208.008	208.999	1192.990	972.982	249.670	246.800
925.72	584.05	190.781	190.001	1211.980	975.990	252.502	251.800
856.70	622.82	210.112	209.008	1204.011	979.002	256.908	255.001
823.23	584.05	198.721	198.018	1208.971	980.567	250.273	248.081
811.10	601.92	192.791	190.011	1191.089	983.900	259.900	257.072
950.05	592.18	202.203	201.001	1195.665	974.456	249.298	246.999
920.25	600.08	208.001	207.891	1200.995	976.687	249.999	246.345