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Case Report

Histopathologic and Microbiologic Investigations for Thirteen-Years Intentionally re-Implanted Maxillary Second Molar, A Case Report.

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ABSTRACT

Background:
A re-implanted maxillary 2nd molar with amalgam retrograde filling material was retained for 13 years. Histopathologic and Microbiologic investigations to reveal reasons for retaining and subsequent failure.

Material and methods:
The tooth was intentionally re-implanted 13 years ago with a retrograde amalgam filling material. After extraction of the tooth serial sections were done and stained with Hematoxylin and eosin stain (H&E) and Gram stains.

Results:
Histopathologic sections revealed cellular cementum deposition at the apex of the tooth. Sharpey's fibers were attached to the cementum. Granulation tissue was obvious around the apex of the tooth. Gram stain revealed accumulation of micro-organisms inside dentinal tubules, especially at the apex.

Conclusion:
The amalgam used as retrograde filling material, in this case, was retained for thirteen years and subsequent failure might be attributed to the invasion of a microorganism after a crown fracture.

1. INTRODUCTION

Conventional root canal treatment is the treatment of choice for managing pulpal and periapical inflammation [1]. However, anatomical complexity and inaccessibility may interfere with conventional root canal treatment such as (anastomosis, delta, missed canal, calcification) or proximity to a vital structure, i.e., mandibular and mental nerves [2-4]. Intentional replantation (IR) is considered as the last treatment option as an alternative to conventional endodontic treatment [5]. The concept of IR was defined by Grossman 1966, Jain A. et al 2018, and by Peer M 2004, as an endodontic treatment procedure in which a tooth atraumatic extracted and treated extra-orally and then re-inserted into its socket [6-8].

The success of IR was proven in many studies, and the factors that influence the success rate are related to periodontal ligament vitality, storage conditions, and extraoral time [9-11].

A reduction of extraoral time is essential in prevention of ankylosis and root resorption and promotion of the peri-radicular healing process by preventing periodontal cell damage and dehydration [12]. Favorable healing and regeneration of the periodontal ligament takes about 7 to 10 days [13].

Root end filling material also plays a definitive role in the regeneration of periodontal attachment apparatus of

the reimplanted tooth, including cementum overlying the resected root-end surface, periodontal ligament (PDL), and alveolar bone all together provide an apical seal to prevent the peri-radicular leakage of residual irritants from the root canal into the periapical tissues [14].

Amalgam was the most widely used material for root-end fillings for a long time because amalgam has a good radio-opacity and it is non-resorbable in tissue fluids [15]. With time amalgam has an advantage in improving the apical seal by its corrosion products [16], on the other hand, this may lead to porosity and this porosity may enhance bacteria stagnation that might lead to periapical pathosis and causes failure of the replantation [17].

These potential disadvantages of the placing amalgam engorg development of new materials that have ideal properties of endodontic repair material which are non-toxic, non-carcinoginc, insoluble in tissues, and dimensionally stable. Nowadays the most common alternative root-end filling materials used is bioactive material with multiple physical and biological properties. These materials are based on radiopacifier (tricalcium silicate) which are biocompatible and bioactive materials. For instance, Biodentine, EndoSequence Root Repair Material Putty, and iRoot BP Plus Root Repair Material (BP-RRM) are products of the new calcium silicate cements [18,19].

Various complications of intentional replantation can occur depending on the degree of root surface damage during the replantation procedure [15]. If the damaged area is small, cells which have the potential to form new cementum and periodontal ligament are capable of covering the damaged root surface. This type of healing is termed surface resorption [20].

Over time, physiologic bone turnover takes place in the cementum on the root surface, which is resorbed and replaced with bone. This process is called replacement resorption. If there is a persisted inflammatory stimulus, healing cannot occur and inflammatory resorption takes place [20].

Ankylosis is defined as the fusion of the alveolar bone and the cementum on the root surface; consequently, the tooth is fixed with no mobility and gives a high percussion tone [21].

Ankylosis is one of the complications of the replantation procedure, inflammatory resorption may also occur which lead to mobility and extrusion of the replanted tooth [22]. Other complications the replantation procedure may appear including paresthesia, maxillary sinus pain, swelling, and periodontal pocket formation which has a greater chance to create root or crown fracture [9].

This case report aimed to evaluate the histopathology of extracted tooth that was replanted with amalgam retrograde filling material and retained for 13 years using histopathology and microbiology investigations of the peri-radicular area of the replanted tooth.

2. MATERIALS AND METHODS

Case report: A healthy 35-years-old Syrian female patient presented at Umm Al-Qura University Dental Hospital (UQUDENT) for dental treatment-seeking comprehensive dental treatment. After routine panoramic radiograph examination, ill-defined radiolucency and localized radio-opacity at root ends were noticed in the apical third of tooth #27. The root-end filling (amalgam) was determined radiographically (Figure1&2). As the patient explained, the tooth has been treated 13 years ago by intentional replantation after atraumatic extraction extra-oral root canal treatment (RCT), and amalgam retrograde filling material was performed on both root's ends. By asking the patient, she claimed that the tooth was not protected by a full-coverage crown and that causes loss of coronal tooth structure and its subsequent fracture one year earlier. Deep coronal caries lesion beyond the alveolar bone crest was detected both clinically and radiographically. Clinical deep pocket all around the remaining tooth was determined. From periodontal and prosthodontic view, the tooth was indicated for extraction.

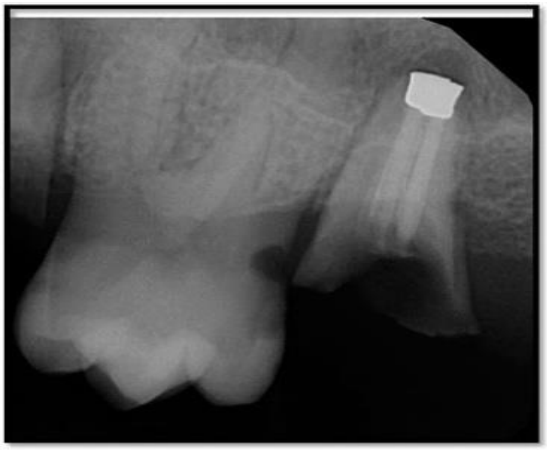

Figure 1:A peri-apical x-ray film shows tooth#27 with the widening of lamina dura and presence of periapical pathosis. (Amalgam root-end filling)

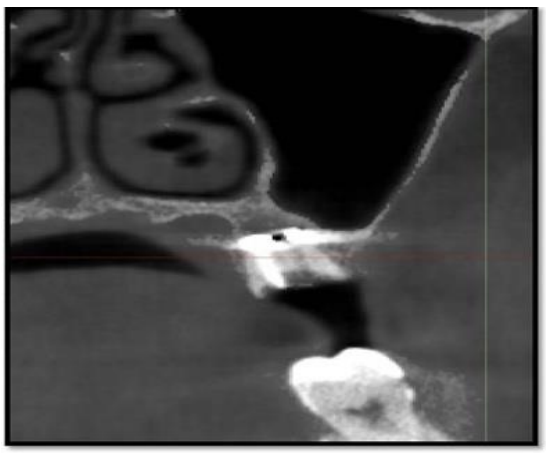

Figure 2:A preoperative CBCT scan shows the proximity of the buccal root to the maxillary sinus.

A CBCT scan with a three-dimensional analysis was performed on the tooth to determine the relationship between the roots and the maxillary sinus (this was very beneficial to limit the destruction of bone during extraction). CBCT scan showed the relation of retrograde amalgam filing to the maxillary sinus floor and the proximity of the buccal root to the maxillary sinus floor. (Figure 3)

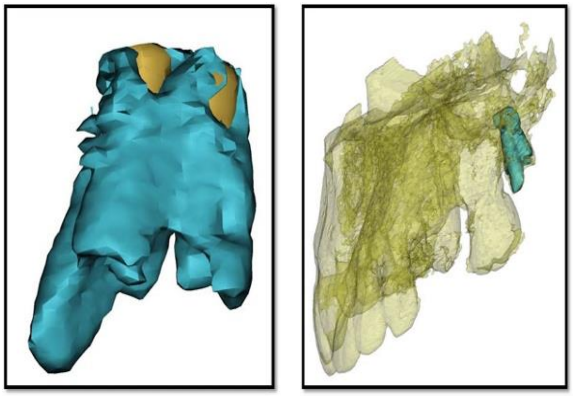

Figure 3:Pre-operative CBCT scan with a 3D view showing the relation of retrograde amalgam filing to the maxillary sinus floor. The proximity of the buccal root to the sinus floor was indicated.

2.1 Extraction procedure

After the patient signed informed consent, atraumatic extraction was done carefully without damaging the root surface. The tooth was extracted under local anesthesia with 4% articaine with 1:100.000 epinephrine with the aid of loop magnification.

Granulation tissue was removed from the peri-apical area for histopathological examination. Although the tooth was very close to the sinus, it was a simple extraction. This might be due to long-standing chronic peri-apical inflammation which caused alveolar bone resorption around the roots; also the tooth had fused roots.

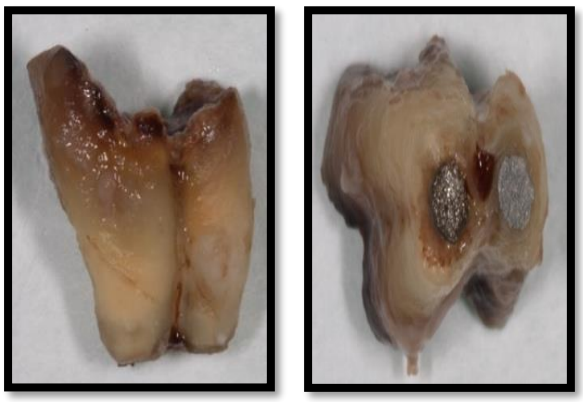

Figure 4: Photographs show the extracted tooth with the retrograde amalgam fillings

2.2 Biopsy preparation

The biopsy specimen was immediately immersed in 10% neutral buffered formalin and fixed for 48 hours. The extracted tooth was immersed in an EDTA aqueous solution for decalcification. At the end of the demineralization process, amalgam was carefully removed from the apical seat. The microtome -set at 4 microns was used for meticulous longitudinal serial sections. The tissue was stained with Hematoxylin & Eosin for assessment of inflammation and Gram stain for staining bacteria. Slides were examined under a light microscope.

3. RESULTS

The microstructure examination revealed cementum deposition of cellular cementum at the apex of the tooth that was represented by incremental lines of Salter (Figure 6). Sharpey's fibers from the periodontal ligament were attached to the cementum (Figure 5,8) On examination of Gram-stained sections revealed micro-organisms in the dentinal tubules of dentine at the apex of the re-implanted tooth (beaded dentine) (Figure 7). Granulation tissue with infiltrated chronic inflammatory cells (lymphocytes and plasma cells) and dilated blood vessels appeared at the peri-apical area of the tooth (Figure 6).

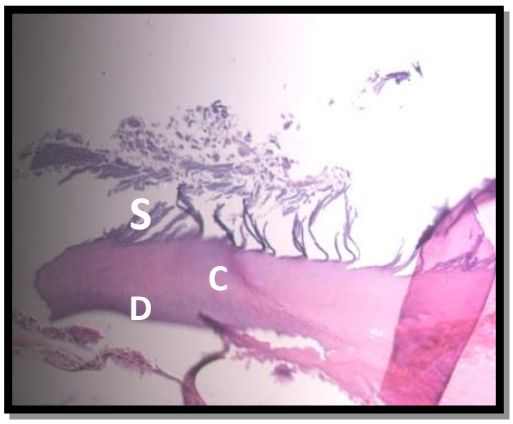

Figure 5: A photomicrograph shows Sharpey's fibers attached to the cementum at the apex of the re-implanted tooth (H&E mag.X100)

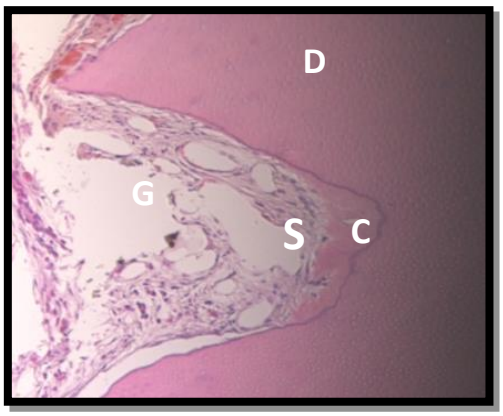

Figure 6: A photomicrograph shows cementum deposition with the attachment of Sharpey's fibers and granulation tissues (H&E mag.X100)

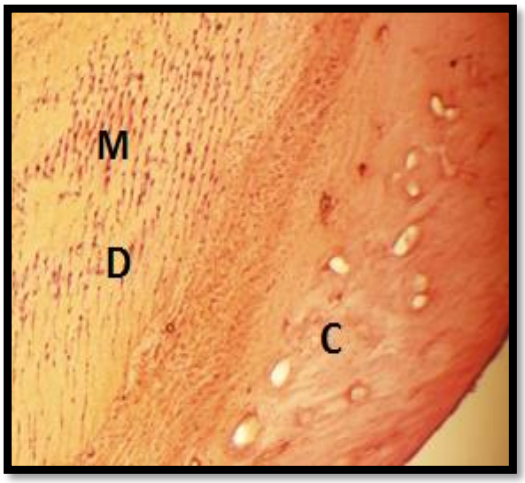

Figure 7: A photomicrograph shows Gram-stained micro-organisms in dentinal tubules and hypercementosis of cellular cementum (Gram mag.X100)

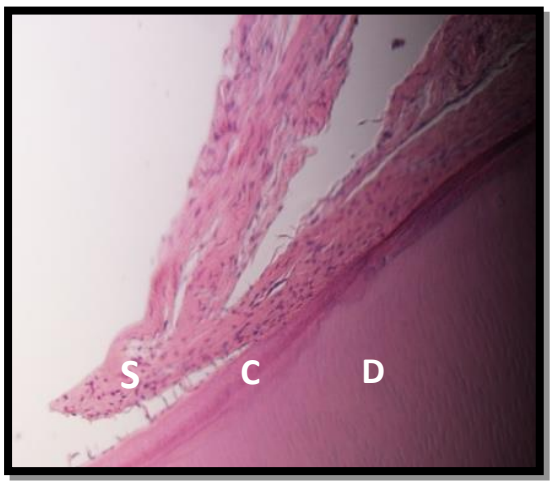

Figure 8:A photomicrograph shows Sharpey's fibers attached to the cementum deposition at the apex of re-implanted tooth (H&E mag.X200)

D=Dentine, C=Cementum, S=Sharpey's fibers,
G= Granulation tissue M= Micro-organisms

4. DISCUSSION

This study evaluated the retention of internationally replanted right maxillary second molar by clinical examination, radiographic examination as well as histopathological and microbiological examinations. Although there are many reports of success rates after Intentional replantation (IR) procedures, this is the first report that performed the histopathological examination of 13 years intentionally replanted tooth.

On other hand, many studies report the success of cases of intentional replantation, W. lee et al, 2012 they report a higher success rate of IR mandibular molar[1]. In a case series report by Asgary, S. et al, 2014 they found IR can have a high success rate with bio- regenerative material[10]. Whereas the study was done by Bender, I. B., & Rossman, L. E. 1993 reported 31 cases of intentional replantation with an overall success rate of 80.6% with survival times up to 22 years [9].

Jong T. et al, 2017 explained the success of long-term re-implanted tooth through histopathological examinations which show the presence of insertion of Sharpey's fibers from periodontal ligament inside several layers of secondary cementum (cementum bound Sharpey's fibers) as noticed in (Figures 5,8) [23]. The role of new cementum deposition in the strong anchorage of reimplanted human teeth are reported previously by Fiane et al, 2014 [24]. That anchorage depends on manipulation procedures and the factors affecting the periodontal ligament vitality as extraoral time and storage conditions [11]. Cho S. et al., Jang Y. et. al., and Nizam N. et. al 2016, all agreed that when the extraoral time is greater than 15 minutes that increases the risk of complications and the occurrence of ankylosis as well as root resorption, thus reducing the survival rate of IR. [2, 25, 26]. It was recommended to place a root-end filling material that provides the good sealing ability of the apices of the roots [27].

Mineral trioxide aggregate (MTA) is considered an alternative root-end filling material because it has better sealing ability than amalgam[17].

Mineral trioxide aggregate (MTA) was found to be the most preferred root-end filling material "gold standard" [14, 28, 29]. Although MTA has drawbacks like the presence of toxic material, difficult handling property, high cost, and discoloration these drawbacks encouraged scientists to do improvements of a numerous new calcium silicate-based materials [30, 31].

Bio-dentine is one of the alternative root-end filing materials, bio-dentine is biocompatible and it has the ability of differentiation and mineralization of odontoblast[32]. Additionally, 4-META/MMA-TBB (4-methacryloxyethyl trimellitate anhydride in methyl methacrylate initiated by tri-n-butyl borane) resin has also been recently used for root-end retrograde filling and the adhesion of fractured teeth before replantation [33-36]. The use of 4-META/MMA-TBB resin root ends retrograde filling material shows well-marked alveolar bone regeneration [33]. In a case series report by Asgary, s. et al, 2014 they found IR can have a high success rate with bio-regenerative material [10].

The failure of the present intentionally reimplanted tooth can be referred to the ill-defined periapical radiolucency which was noticed in x-ray examination, (Figure 1), and to the presence of granulation tissue in histopathological examination (Figure 6). The presence of periapical radiolucency and granulation tissue peri-apically may be explained as bacteria managed to pass through the micro-leakages of amalgam retrograde filling material and that is supported by Mazumdar, P., et al. [37]. Mazumdar, P., et al. evaluated the apical sealing

properties of biosilicate material (biodentine) in comparison with grey mineral trioxide aggregate, and white mineral trioxide aggregate, and light cure glass ionomer cement and silver amalgam by using the dye penetration method, they found the leak-proof seal of biosilicate material showed minimal leakages 10%, while 55% of micro-leakages were presented in the retrograde silver amalgam filling through which dye passed to reach the periapical area. Mazumdar, P., et al results are inconsistent with this study[37]. This may be explained as amalgam, by the time of exposure to the oral environment, was subjected to corrosion, corrosion products are deposited in the gap resulting in decrease the microleakage [16]. On other hand Omezli M. and Torul D, 2015 suggested that corrosion products may cause peri-apical foreign body reactions of long-standing retrograde amalgam filling material [38].

Loss of coronal structure is one of the reasons for the failure of this case which the patient suffered from crown fracture one year before her first visit to Umm Al-Qura University Dental Hospital (UQUDENT).

Deep coronal caries lesion beyond the alveolar bone crest was detected both clinically and radiographically. Deep pockets, detected by clinical examination, all around the remaining roots may suggest a pathway by which microorganisms have been approached to the peri-apical region as described by Laresen and Fiehn 2017, who emphasized the role of biofilms in the teeth and their supporting tissues [39]. The supragingival biofilm spread along the root surface into periodontium and form a periodontal pocket, and a subgingival biofilm is formed and may cause periodontal diseases and eventually cause chronic peri-apical inflammation which may explain the results of the present case.

5. CONCLUSION

In this case, intentionally replanted tooth was retained functioning for the last 13 years. This conclusion was based on histopathological results which showed a new periodontal attachment between the root and alveolar bone was observed. Amalgam was good material at that time, but failure was due to bacterial invasion through crown fracture.

CONFLICT OF INTEREST

The authors state there are no conflicts of interest.

ETHICAL STATEMENT

Ethical approval was obtained from the college ethical committee (IRB number 118-18).

Consent form was obtained from the patient to perform this study and for publication.

This case report has not been published elsewhere, and it has not been submitted simultaneously for publication elsewhere.

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