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Toluidine blue staining in the localisation of the resected root fragments during the third molar surgery: An invitro study

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***Abstract*---**Aim: To evaluate the efficiency of Toluidine blue in the staining of the resected root fragments during third molar surgery. Materials And Methods: A sample containing the root and attached bone fragment was used. 0.2 percent of the toluidine blue was used for staining the sample. The stain was washed off from the sample and the intensity of stain colour on the root and bone fragments were observed with a naked eye under daylight . Results: The bone fragment stained in light blue colour whereas the root stained in a dark blue colour Conclusion: In this in vitro study, we conclude that toluidine blue can be used in the localisation of the resected root fragments. However, its safety when used intraorally in proximity to the bone needs to be further evaluated.

***Keywords*---**toluidine blue staining, third molar surgery, localization.

Introduction

Third molars are the most frequently impacted teeth and might fail to erupt into a normal functional position. The prevalence of impacted third molars ranges between 16.7–68.6% across various populations. Impacted mandibular and maxillary third molar in most scenarios causes a lot of discomfort to the individuals (*Peterson's Principles of Oral and Maxillofacial Surgery* 2004). The modality usually practiced to address this problem is the surgical removal of this impacted tooth. Surgical removal of third molars is one of the most common

procedures performed by oral surgeons. These surgeries do not encounter difficulties but at times it can result in complications; a complication rate of 4.6–30.9% following the extraction of third molars is reported in the literature. During the process of surgical removal a common complication encountered by surgeons is the fracture of the root tip during the procedure (Sisk et al. 1986). Safe removal of the roots has been an area of concern for oral and maxillofacial surgeons since a long time. To the best of the authors knowledge this the first study which is conducted to evaluate the efficiency of toluidine blue in the staining of the resected root fragments during third molar surgery.

Material and Methods

Isolation of the Sample

Bifurcated third molar roots with a small piece of bone fragment attached to it was used for the study purpose. The sample was obtained during a third molar surgery and was immediately transported and placed in saline. The blood was washed off using saline and the the sample was placed on the petri dish. Fig 1

Preparation of Toluidine blue

0.01 gram of toluidine blue was mixed with 500 ml of distilled water. A percentage of 0.2% of toluidine blue in 100 ml of distilled water was obtained . This concentration was used in this invitro study.

Staining of the sample

Once the stain was prepared, it was taken using a pipette. Drops of toluidine blue was applied on the sample. After 15 seconds of application the stain was washed off using saline. The observation was recorded under day light using a naked eye.Fig 2

Results

When observed under day light using the naked eye , it was observed that the bone fragments appeared with light basophilic colour whereas the root fragments appeared with strong basophilic colour.Fig 3

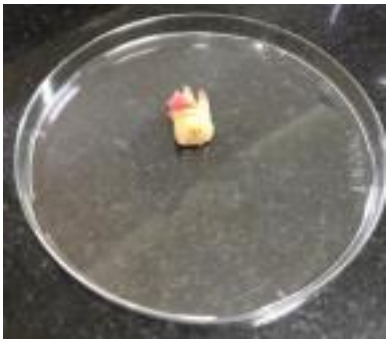

Figure 1

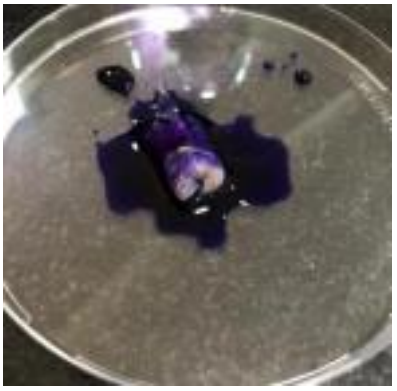

Figure 2

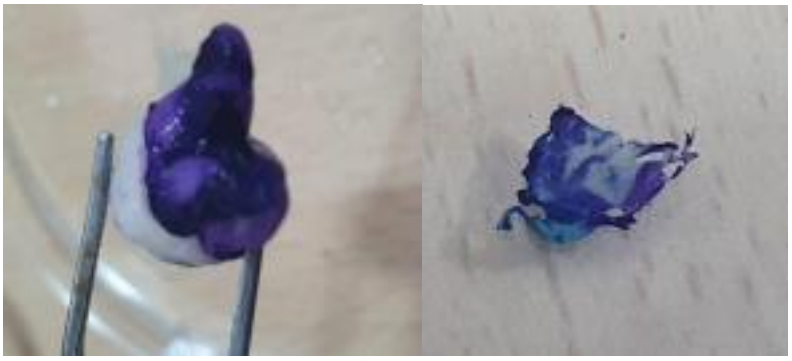

Figure 3

Discussion

Fracture of the root the third molar is one among the many complications observed when performing a third molar surgery (Fang, Hao, and Zheng 2009). A surgeon overcomes this complication with the help of a radiograph which helps to locate the root fragment radiologically (Normando D). Another method practiced is the isolation of the surgical field with copious irrigation and mopping using gauze pads. However this becomes difficult if the surgical area is pooled with blood. 0.2 percentage toluidine blue when applied can help in the easy location of the root fragment by providing a colour contrast. Moreover, the radiation exposure can also be avoided in the patient.

The periodontal ligament which attaches the tooth to the alveolar bone. It is known that it inserts into the root cementum on one side to the alveolar bone on the the other side. The periodontal ligament substance has been estimated to be 70% water (Strydom et al. 2012). It ranges in width from 0.15 to 0.38 mm. The periodontal ligament fibers contains loose connective tissue fibres , blast cells , oxytalan fibers and cell rest of Malassez (Strydom et al. 2012; Jong et al. 2017). In our study we observed a dark blue stain on the root. This could indicate that the toluidine blue stains the periodontal fibers surrounding the tooth which makes it appear in a dark blue stain when compared to the attached bone fragment. However further in vitro studies are required to prove this finding.

Toluidine Blue was first discovered by William Henry Perkin in 1856 . It consists of a basic thiazine metachromatic dye. It has high affinity for acidic tissue components hence it helps to stain the tissues rich in DNA and RNA (Gandolfo et al. 2006). It has found wide applications both as vital staining in living tissues and as a special stain owing to its metachromatic property. Toluidine blue stain can help to highlight components such as mast cell granules , mucins and cartilages (Gandolfo et al. 2006; Pentenero et al. 2005).

There are two techniques of vital staining, namely, intra vital staining in the living body (*in vivo*) and supra vital staining outside the body usually applied to the detached cell. In our study we performed a supra vital staining. It was observed that the 0.2 percentage toluidine stained in different intensity on the specimen. The toluidine blue stained the root and bone fragment dark royal blue and pale royal blue colour respectively which will help in differentiating between the root and the bone fragments during the surgery.

Toluidine blue stain can be used intraorally for the detection of the premalignant lesions. Toluidine blue is generally prepared in 1% concentration for oral application. It can be prepared by using 100 mL of 1% Toluidine of 1 gm toluidine powder, 10 mL of 1% acetic acid, 4.19 mL absolute alcohol, and 86 mL distilled water to make up 100 mL. The observations are based on the interpretation of the colour a dark blue (royal or navy) stain is considered positive, light blue staining is doubtful and when no colour is observed, it is interpreted as negative stain (Gandolfo et al. 2006; Pentenero et al. 2005; Miller, Simms, and Gould 1988). It is also used intraoral in the staining of nucleated scales covering the papillae on the dorsum of the tongue as well as the pores of seromucinous glands in hard palate . Hence the intraoral staining with Toluidine blue is widely practiced (Fang, Hao, and Zheng 2009). However its application when it is in proximity with the bone is questionable. Further invivo and animal studies are required to address this shortcoming.

Conclusion

Toluidine blue can be used for the chair side location of the fractured root fragments during the third molar surgery. However, various invitro and invivo studies are required to establish the safety of this dye when used in a bony socket.

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