An *In-Vitro* Study to Investigate Enamel Cracks in Young and Old Human Teeth Using Methylene Blue Dye and Optical Coherence Tomography

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Introduction

The diagnosis of cracks and their propagation remains a challenge in clinical practice with the current diagnostic tools available. Furthermore, no study has yet shown whether age *per se* contributes to crack formation and propagation.

Optical Coherence Tomography (OCT) is a novel, non-invasive and simple method that provides 3-dimensional characterisation of soft and hard tissues and more recently, enamel cracks. It works by the interference of a low coherence light source with the back scatter generated from the target tissue.

Purpose

1. To compare the use of traditional methylene blue dye (MBD) staining and OCT in the detection of enamel cracks in human teeth.
2. To use OCT to investigate enamel cracks in young and old teeth

Materials and Methods

Fifty-seven unrestored molar teeth were disinfected in ethanol and stored in 4% formaldehyde after extraction. Thirty four were from patients aged 25 and under, while 23 were from patients 50 and over. The mid-buccal point of each was scanned with Swept Source OCT over an area of 6mmx6mm and 2mm depth (Figure 1). The number of cracks were enumerated (Figure 3) and the types of cracks were recorded according to a proposed classification detailed in Table 1.

Teeth were then immersed in MBD for 2 minutes and subsequently washed in distilled water before visual inspection under magnification and good lighting for the presence of cracks.

Ten teeth with most visible stained cracks were selected for further observation. An opaque location reference was placed on either side of a crack (Figure 2) before re-scanning with OCT to create an *en face image.* This would allow the observer to confirm whether or not the cracks that detected by MBD were indeed the same as those that are detected by OCT.

Figure 1. Tooth mounted on scanning table with centre of scanning area indicated with a fine red light

Figure 2. MBD stained toothy with a visible crack referenced on either side with pellets of plasticine

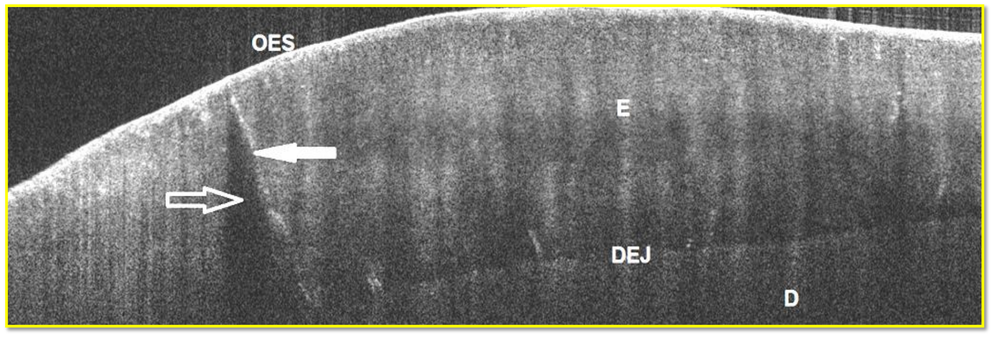
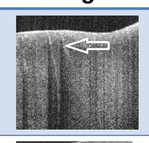
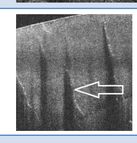
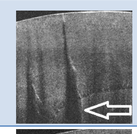
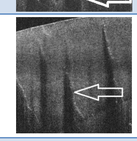
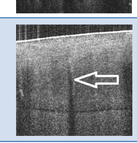


Figure 3: Example of an OCT slice showing an enamel crack (solid arrow) and the artefact "shadow" cast below it (hollow arrow).
OES: outer enamel surface; E: enamel; DEJ: dentino-enamel junction (DEJ) and D: dentine

Table 1. Classification of cracks (arrows) proposed by main researcher

Type	Description of crack	Example OCT Image
1	Extends from outer enamel surface terminating within the enamel.	
2	Extends from outer enamel surface terminating at the DEJ.	
3	Extends from outer enamel surface terminating within the dentine.	
4	Extends from DEJ terminating within the enamel.	
5	Begins and terminates within the enamel.	

Results

OCT generated images of the enamel, DEJ and the superficial dentine with high resolution allowed both the presence and depth of crack penetration to be accurately visualised (Figure 4). OCT detected significantly more cracks than MBD ($p<0.001$). This included the same cracks detected by MBD (Figure 5). Old teeth had significantly more cracks than young teeth ($p<0.001$) (Figure 6). In both age groups, the majority of cracks were of Type 2, spanning from the enamel surface to the DEJ ($P<0.001$) but these were more common in young teeth ($p<0.01$). Type 4 cracks, emanating from the DEJ into the enamel, were greater in old teeth ($p<0.001$).

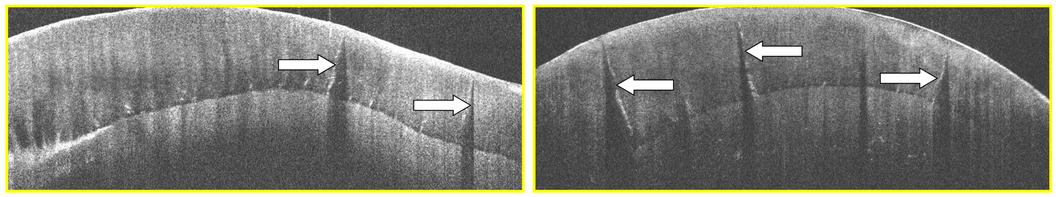


Figure 4: Slices from OCT scans showing cracks (arrowed) in young (left) and old (right) teeth

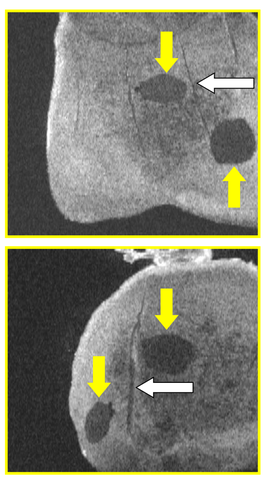


Figure 5. Slices from *en face* OCT scans showing cracks (white arrows) in between location references (yellow arrows)

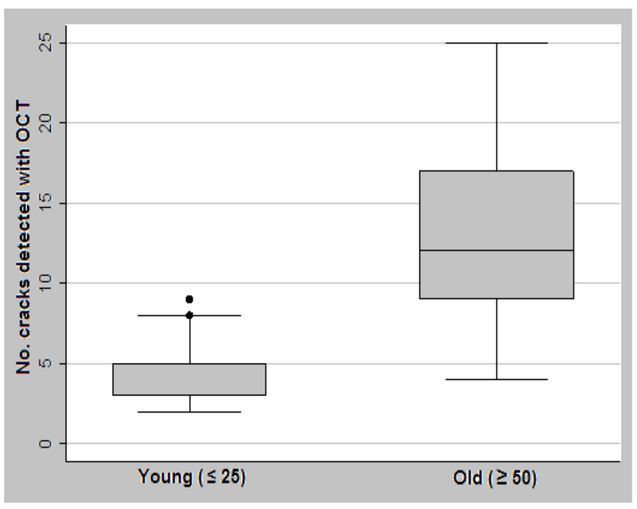


Figure 6. Box and whisker plot showing the number of cracks detected by OCT in young and old teeth

Conclusion

Within the limitations of this study it can be concluded that:

- OCT is a non-invasive tool that provides real time and high resolution imagery of the enamel, DEJ and superficial dentine.
- OCT can detect significantly more enamel cracks than MBD.
- OCT can detect the same cracks that can be stained with MBD, but provide additional information about their depth of penetration.
- In all age groups, the majority of cracks span from the outer enamel surface and terminate at the DEJ.
- Old teeth have significantly more enamel cracks than young teeth.
- Old teeth have significantly more "inside-out" cracks than young teeth, spanning from the DEJ and terminating in the enamel.

References

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