Introduction

White areas of the enamel surface can be pre- or post-eruptive. Pre-eruptive enamel defects may be localized to a single tooth or affect the whole dentition. They vary in severity from a mild calcification defect to almost complete failure of enamel formation. Many terms have been used to describe these defects, including ‘mottled enamel’, ‘dental fluorosis’, ‘developmental opacities’, ‘internal enamel hypoplasia’ and ‘enamel hypocalcification’ (Small and Murray, 1978).

Post-eruptive changes are usually due to demineralization of enamel. Enamel demineralization or white spot formation can occur during orthodontic treatment (Gorelick et al., 1982; O’Reilly and Featherstone, 1987). The reported prevalence of demineralization occurring during orthodontic treatment ranges from 2 to 96 per cent (Mitchell, 1992a). Unfortunately, there is little consistency in the literature about how these data were recorded. Most in vivo studies of post-orthodontic demineralized white lesions have relied on subjective methods such as visual inspection and simple clinical assessment (Gorelick et al., 1982; Ögaard, 1989). It is possible that many of the researchers who reported higher figures were recording pre- as well as post-treatment white lesions. There are no published studies examining the methodology of distinguishing between the two types of opacity.

Several investigations have shown that the measurement of the size of demineralized white lesions from photographs is valid and reproducible (Benson et al., 2000; Willmot et al., 2000). A method of differentiating developmental white opacities and demineralized white lesions by size using image processing and analysis has been previously reported (Willmot et al., 1998). A method of distinguishing these lesions by characteristics of shape, colour or size would be an appropriate research tool for evaluating new preventive products intra-orally. It has been found that the experience of the clinician contributes to the ability to differentiate clinically between the two different lesions. This aim of this study was to determine whether differences exist in the characteristics of developmental opacities and post-orthodontic white lesions that could be used to distinguish the two lesions using computerized image analysis.

Materials and methods

Pre- and post-orthodontic 35 mm slide photographs in the library of the Orthodontic Department at the Charles Clifford Dental Hospital, Sheffield, UK were selected. The photographs were all taken with a Yashica Dental Eye II camera (Kyocera Yashica UK Limited,
Reading, UK) using a standardized setting of distance, illumination aperture and film (KodaKrome, Kodak UK, London, UK). The slides were examined in a dark room with good back light illumination by an experienced, independent clinician (PEB). The following criteria were used for the selection of images for each category.

Developmental opacities

Pre-treatment clinical photographs were examined for discrete, developmental white opacities on the maxillary anterior teeth (incisors or canines). Slides were rejected if they were of poor quality, there were signs of pre-existing demineralization, especially on the first molars, or there was flash reflection impinging onto the opacity. No more than three lesions were selected from any one patient. In total, 30 images from 21 patients were obtained.

Post-orthodontic lesions

Post-treatment clinical photographs were examined for discrete, demineralized white lesions on maxillary anterior teeth (incisors or canines) that were not present on the pre-treatment photographs. Slides were rejected if they were of poor quality, if there was any evidence of pre-existing demineralization on the pre-treatment photographs or if there was flash reflection impinging onto the opacity. No more than three lesions were selected from any one patient. In total, 30 images from 15 patients were obtained.

Image processing

The slides were captured and converted into digital images using an Epson scanner and software (Perfection 1200 Photo; Epson (UK) Ltd, Hemel Hempstead, Hertfordshire, UK). The images were saved at 2000 dpi as separate tagged image file format (TIFF) files. The images were opened in Adobe Photoshop (version 5.5, Adobe Systems Inc, California, USA). The outline of the buccal surface of the tooth was drawn freehand using the lasso tool and cropped of gingiva and surrounding teeth to disguise whether the tooth was pre- or post-treatment. This was stored as a new TIFF file. Some black and white examples of these cropped images are shown in Figure 1.

Each TIFF file, containing the image of one labial tooth surface, was given a unique computer-generated random code number. These images were analysed by a second individual (KK), who was blind to the aetiology of the lesion. The analysis was carried out in a darkened room, to prevent any reflection on the monitor screen, using the image analysis program Image Pro-Plus™ (v4; Media Cybernetics, Silver Spring, Maryland, USA). The computer monitor screen resolution was set at 1024 × 1024 and colour resolution at 24-bit true colour. Before the measurements were produced, the software was calibrated using a standardized slide taken using standard settings. Imported images were subjected to a 3 × 3 noise reduction filter and contrast enhancement if necessary.

The outline of the labial surface of the tooth was traced using the freehand tool and computer mouse. The images were carefully examined and white lesions identified were traced using the same method. A sample of the surrounding area judged to be sound enamel was identified and traced.

The following characteristics of the lesions were calculated:

White lesion size proportionality (WL%), defined as the white lesion area as a percentage of the total labial surface area of the tooth: WL% = (Area of the white lesion/Area of the tooth) × 100.

Luminance intensity proportionality (LI%), defined as the white lesion grey level as a percentage of the sound enamel grey level, black being 0 and bright white being 255, using the methods described by Pocock (1998) to standardize baseline clinical measurements: LI% = [(Mean grey level of white lesion/Mean grey level of sound enamel)–1] × 100.

Roundness of the white lesion: Roundness = Perimeter²/(4π × Area), where π is a constant with the value of 22/7.

Circularity, or roundness, can be expressed mathematically as a shape descriptor in order to give a numerical indication of lesion shape. There are many possible dimensionless expressions that can describe shape. Roundness is one of the more widely used expressions (Jain, 1988; Russ, 1999; Seul, 2000). Image Pro-Plus™ analysis software calculates this complex mathematical equation where a circular object will have a roundness
of 1. Other shapes will have a roundness greater than 1, this value getting larger the less the shape resembles a true circle.

After 2 weeks the images were placed in a new computer-generated random order and re-analysed.

Statistical analysis

The data were entered into a spreadsheet (Microsoft Excel 2000; Microsoft Corp, Redmond, Washington, USA) and a statistical analysis was carried out in SPSS™ (SPSS for Windows version 11.0, SPSS Inc, Chicago, Illinois, USA). Repeatability was assessed using the intra-class correlation coefficient (ICC) for random error (Shrout and Fleiss, 1979) and one-sample \( t \)-test for systematic error.

To test the hypothesis that there was no difference between the outcome characteristics of developmental white opacities and post-orthodontic white lesions, the mean of the two repeated readings was used. The data were examined using a graph of frequency distribution and the Shapiro–Wilk test to determine whether they were normal. Some of the data were not normally distributed. Therefore, the Mann–Whitney \( U \)-test was used, with a significance level of \( P < 0.05 \).

Results

Repeatability

The results of the intra-examiner repeatability analysis are shown in Table 1. The ICC showed substantial agreement, according to the criteria of Donner and Eliasziw (1987), for all measurements, except developmental white lesion roundness, which was moderate (0.59). There was no evidence of systematic error, except for the repeat readings of white lesion proportionality (\( P = 0.001 \)).

The median values for the three characteristics of size proportionality, luminance proportionality and roundness are given in Table 2.

<table>
<thead>
<tr>
<th>Category</th>
<th>ICC</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportionality (WL%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental</td>
<td>0.72</td>
<td>0.001</td>
</tr>
<tr>
<td>Post-orthodontic</td>
<td>0.68</td>
<td>0.074</td>
</tr>
<tr>
<td>Luminosity (LI%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental</td>
<td>0.76</td>
<td>0.085</td>
</tr>
<tr>
<td>Post-orthodontic</td>
<td>0.75</td>
<td>0.085</td>
</tr>
<tr>
<td>Roundness</td>
<td></td>
<td></td>
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<tr>
<td>Developmental</td>
<td>0.59</td>
<td>0.168</td>
</tr>
<tr>
<td>Post-orthodontic</td>
<td>0.65</td>
<td>0.530</td>
</tr>
</tbody>
</table>

White lesion size proportionality

The median size proportionalities were 7.0 per cent for the developmental white opacities and 9.9 per cent for the post-orthodontic white lesions. The result of the Mann–Whitney \( U \)-test was significant (\( P = 0.042 \)), suggesting that the post-orthodontic lesions were significantly larger as a proportion of the tooth surface than the developmental opacities. However, a difference in size proportionality of 3 per cent is unlikely to be clinically significant and there was considerable overlap between the interquartile ranges (Figure 2). Therefore, size proportionality is unlikely to be a useful distinguishing characteristic.

Luminance intensity proportionality

The median luminance proportionalities were 12.3 per cent for the developmental white opacities and 8.5 per cent for the post-orthodontic white lesions, which were significantly different (\( P = 0.002 \)). The higher luminance proportionality for the developmental opacities suggests that these are significantly whiter than the post-orthodontic lesions. Once again, examination of the interquartile

<table>
<thead>
<tr>
<th>Category</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Interquartile range</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportionality (WL%)</td>
<td></td>
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<tr>
<td>Developmental</td>
<td>7.0</td>
<td>0.9</td>
<td>21.1</td>
<td>4.4–7.0</td>
<td>0.042</td>
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<tr>
<td>Post-orthodontic</td>
<td>9.9</td>
<td>3.1</td>
<td>22.0</td>
<td>5.7–12.7</td>
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</tr>
<tr>
<td>Luminosity (LI%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental</td>
<td>12.3</td>
<td>6.3</td>
<td>41.8</td>
<td>9.3–14.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Post-orthodontic</td>
<td>8.5</td>
<td>2.5</td>
<td>16.4</td>
<td>6.7–11.3</td>
<td></td>
</tr>
<tr>
<td>Roundness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental</td>
<td>2.3</td>
<td>1.3</td>
<td>5.9</td>
<td>1.5–2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-orthodontic</td>
<td>6.6</td>
<td>2.2</td>
<td>12.9</td>
<td>4.9–7.8</td>
<td></td>
</tr>
</tbody>
</table>
ranges (Figure 3) showed considerable overlap and a difference in luminance proportionality of 4 per cent, but this is unlikely to be clinically significant. Therefore, luminance proportionality alone is unlikely to be a useful distinguishing characteristic.

**Roundness**

The median roundness values were 2.3 for the developmental white opacities and 6.6 for the post-orthodontic white lesions. The Mann–Whitney U-test showed that there was a statistically significant difference in the roundness values between the opacities ($P < 0.001$). Examination of the interquartile ranges (Figure 4) showed that there was no overlap. Closer examination of the data indicated that a cut-off point of 3.5 was useful in distinguishing between developmental and post-orthodontic lesions. Only seven of the 60 measurements from the developmental lesions (11.6 per cent) had a roundness value greater than 3.5, whereas only five of the 60 measurements from the post-orthodontic lesions (8.3 per cent) had a roundness value of less than 3.5.

**Discussion**

This study has shown that using the mathematical descriptor of roundness, measured with computerized image analysis, it is possible to distinguish between developmental opacities and post-orthodontic white lesions. A roundness value of 3.5 is a useful cut-off point. Below 3.5 the lesion is probably developmental, above 3.5 the lesion is probably post-orthodontic.

Roundness, as measured with computerized image analysis, is a measure of circularity. A perfect circle has a value of 1. A value greater than 1 indicates decreasing circularity. The developmental white lesions had roundness...
values close to 1. Therefore, the developmental white lesion is nearer to a circular shape compared with post-orthodontic white lesions. Because the perimeters of the developmental opacities are more circular, the outline is more regular and short compared with the post-orthodontic white lesions. This is to be expected. The majority of hypoplastic enamel defects begin with disruption to ameloblast function during the formation of the tooth (Hillson and Bond, 1997). A growth disturbance, such as a childhood illness or dietary deficiency, might cause a group of cells to stop secreting matrix earlier than normal, leading to a linear defect of the enamel. Post-orthodontic white lesions are more likely to be irregular. Their shape will often reflect the margins of the orthodontic bracket and overhanging cement, which attract plaque and are difficult to clean. They frequently assume the form of a rotated ‘c’.

The developmental lesions were found to have a higher luminance intensity proportionality and were therefore, whiter compared with sound enamel than the post-orthodontic white lesions. This difference alone was not found to be useful when differentiating the two types of lesion, as there was greater variation than with the roundness factor. However, in cases where the roundness value was close to 3.5, a luminance intensity proportionality of 11 could be used as a further discriminating factor. Sixty-two per cent of developmental opacities had a luminance intensity proportionality above 11, whereas the same proportion of post-orthodontic lesion measurements was below 11.

The technique of drawing freehand around lesions is likely to introduce random error. Two areas of interest were required to measure white lesion size proportionality. First, an area around the whole labial surface to obtain the size of the tooth, then an area around the white opacity. The reproducibility of measuring in the present study was comparable with that of Mitchell (1992b), who employed a similar calculation utilizing black and white prints of teeth magnified six times. She first traced around the outline of teeth using a digitizer connected to a computer, then around any demineralized lesions. The published data of repeat readings from 10 teeth indicate an ICC of 0.66 for white lesion size proportionality, which is equal to the present study (0.68).

The advantage of using proportions rather than absolute measurements for size and luminance is that it removes the need for a calibration device on the image. The extra step of calibrating the image might introduce further error into the method. In addition, small differences in the photographing and processing techniques can lead to changes in the grey level which would make it very difficult to determine a threshold at which it could be assumed that the enamel is demineralized, without comparison with sound enamel.

The method of calculating luminance intensity proportionality relies on some subjective assessments of what is sound and demineralized enamel. The reproducibility of this study was found to be substantial, but less than that of Willmot et al. (2000), who used the same method for assessing luminance intensity proportionality. They found a coefficient of reliability of 0.88 compared with 0.75 in the present investigation. The difference between the two studies was that Willmot et al. (2000) used demineralized areas on teeth that were developed in vitro and were of a regular shape compared with the irregular post-orthodontic lesions in the current investigation. Error is reduced when there is good contrast between the boundaries of the areas of interest. Unfortunately, there is often not a good contrast between areas of sound and demineralized enamel, the two areas merging into each other without a distinct boundary. This makes accurate tracing difficult and prone to errors, and is probably the reason that the random error was slightly greater for roundness.

Accuracy is further hampered by the reflection of light, usually from the camera flash. Reflections on the tooth surface might lead to failure to identify white lesions or over-estimation of the area of the white lesions. It has been suggested that the camera should be tilted and the flash masked to reduce the number of reflections (Fleming et al., 1989), but neither was carried out in the present study.

A means of reducing tracing error might be to use an optical mouse. The computer mouse utilized in this study employed a ball-tracking device, which has two bodies moving against each other that increase friction and might increase random error. An optical mouse, which uses light to track the movement, may allow more precise movement of the cursor and reduce error.

The location of the opacities or lesions on the labial tooth surface may be another factor in distinguishing between these two types of lesion and is the subject of future research using this imaging technique. The process of converting conventional slides into a digital format is time consuming and may lead to potential errors. The direct use of a digital camera for taking the images of the teeth would eliminate processing- and film-dependent errors. Future research could investigate the use of digital images in the assessment of white enamel lesions. In addition, the use of polarizing filters to reduce flash reflections should be studied.

Conclusions
The following conclusions can be drawn from this study:

1. Roundness is a useful measure when distinguishing developmental and post-orthodontic demineralization using computerized image analysis. Below a roundness value of 3.5, the lesion is probably developmental. Above 3.5, the lesion is probably post-orthodontic.
2. If roundness is not a conclusive method, then a luminance intensity proportionality greater than 11 might indicate that the lesion is a developmental opacity, whereas a level below 11 might indicate that the lesion is post-orthodontic.

3. White lesion size proportionality is not a useful distinguishing characteristic.

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