Histometric evaluation of odontoblast responses to Nobetec® and Super Syntrex®

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Abstract – A reproducible method to determine reduction in odontoblast number and variation in dentine thickness was devised. Using this method the pulp reaction patterns to Nobetec® (a modified zinc oxide-eugenol cement) and Super Syntrex® (a silicate cement) in vervet monkeys were quantitated. This investigation showed that statistically significant reductions in odontoblast numbers were seen under cut dentinal tubules compared to areas under a cavity with no cut tubules. Reparative dentine thickness was similar under both materials and the mean thickness doubled between 28 and 56 days after cavity preparation. Both materials were found to be irritant. It is suggested that quantitation of odontoblasts and reparative dentine thickness under cut dentinal tubules may be used as the main criteria in pulpal studies in order to discriminate between test materials.

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It is difficult to standardize conditions when pulpal reactions to restorative materials are assessed (1–4). Techniques such as cytotoxicity tests and implantation tests (5) have been tried, but a recent comparative investigation has concluded that teeth remain the site of choice for the final biological testing of dental materials used in cavities (6).

Generally, a buccal restoration is the basis for an evaluation (2, 7–9), followed by examination of demineralized, stained sections (2, 10, 11). Criteria for histological assessment have been recommended by the American Dental Association (9) and the Fédération Dentaire Internationale (8), as well as by many individual researchers. However, there is no complete agreement on all the criteria. Even between the ADA and FDI there is a lack of common ground (4). These two organizations have adopted a semiquantitative approach to the evaluation of pulp reactions, using arbitrary scores of degree of response (12–15) and similar or modified semi-quantitative systems have been used by other researchers (1, 2, 11, 16–18). Since the allocation of scores is subjective, variations can occur between observers which may introduce bias into their conclusions. A further point is that there is a lack of agreement over which criteria are most valuable (3, 7, 19, 20).

The aims of this study were to investigate reduction in odontoblast number and thickness of reparative dentine as possible methods of assessing pulpal response.

Material and methods

The histological sections examined were on file in the University of the Witwatersrand, Dental Research Institute, from a series of 6 pulpal response studies carried out over a 5-year period, using the vervet monkey (Cercopithecus aethiops pygerythrus), and a standardized operative technique (4). Information on the sections is stored in a data base, which enables selection to be made without an investigator knowing the details of a specific section. In these studies, the material termed the "negative control" was Nobetec® (Bofors, Nobel, Pharma, Sweden), a zinc oxide-eugenol cement, and that termed the "positive control" was Super Syntrex® (Amalgamated Dental, England), a silicate cement placed in unlined cavities. Sections of teeth containing these materials were selected using the following criteria:

1. There must be no separation of odontoblasts from predentine, nor tissue tears, staining artefacts or folding.

2. The sections must be cut along the dentinal tubules (1), tangential sections must be excluded (10).
3. The pulp chamber must extend at least 1 mm beyond the incisal and cervical margins of the cavity.
4. The cavity floor must be parallel to the pulp wall beneath it.

From 251 sections examined, 79 were found to satisfy all the criteria. These were examined in a Univar research microscope fitted with measuring graticule (consisting of numbered squares with sides of 110 μm at × 25 magnification (Reichert, Vienna, Austria)). Odontoblast nuclei were counted in four zones, each consisting of two adjacent squares (110 μm × 220 μm). These nuclei had to be oval, close to the predentine layer and towards the base of the cell. If a round nucleus appeared to be part of an odontoblast it was included.

The four zones examined were chosen to include the following (Fig. 1):

1. An area of uncut dentinal tubules incisal to the cavity wall (Zone 1).
2. An area of uncut dentinal tubules below the floor of the cavity (Zone 2).
3. An area of cut dentinal tubules below the floor of the cavity (Zone 3).
4. An area of cut dentinal tubules cervical to the gingival margin of the cavity (Zone 4).

The zones were demarcated by placing the horizontal lines of the graticule parallel to the pulpodentinal junction. Zones 1 and 2 were separated by placing a vertical line through the junction of cavity floor and incisal wall (Fig. 2); Zones 3 and 4 were similarly separated using the junction of cavity floor and cervical wall.

The following linear measurements were made using the same graticule, estimated to the nearest 1/10th of a square (11 μm).

1. Original dentine thickness along lines perpendicular to the pulpo-dentinal wall at the deepest parts of the incisal (LD) and cervical (MG) cavity margins.

2. Remaining dentine thickness at the deepest parts of the incisal (CD) and cervical (FG) cavity margins.

3. Cut dentinal tubule length at the deepest parts of the incisal (CE) and cervical (FH) cavity margins.

4. Incisogingival height of the cavity between deepest points of the axioincisal (A) and axiocervical (B) line angles.

5. Maximum reparative dentine thickness along a line perpendicular to the pulpodentinal wall (NO).

To check reproducibility of these measurements, 10 sections were examined by the evaluator (N.B.) on two occasions 6 wk apart. In order to determine that the cavities and teeth were comparable, the original dentinal thickness, remaining dentinal thickness and cut dentinal tubule length for the Nobetec and Syntrex groups at the 3 periods were compared. The controls in this study were sections of 6 teeth without cavities. These sections were oriented so that the axio-pulpal wall was parallel to

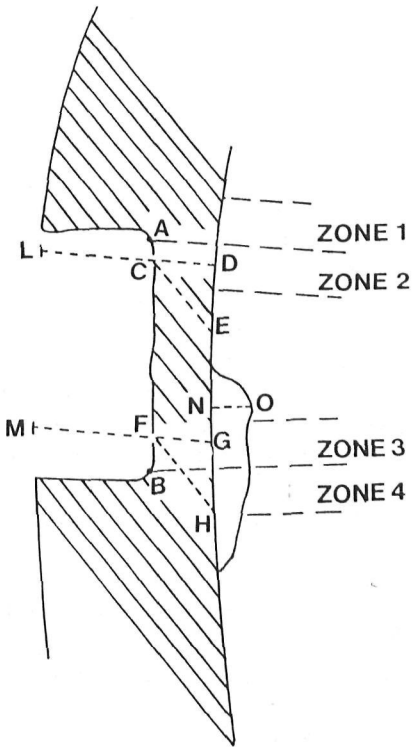

Fig. 1. Schematic layout of areas measured. The landmarks are described in the text.

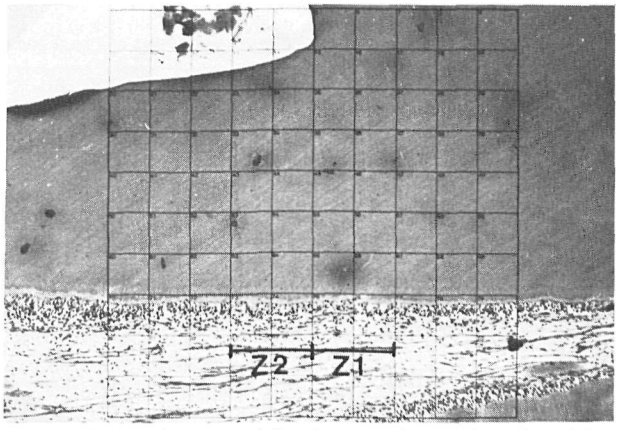

Fig. 2. Slide table oriented for the counting of the odontoblast nuclei in Zone 1 and Zone 2. Zone 1 occupies squares 75 and 76 (220 μm). Zone 2 occupies squares 73 and 74 (220 μm). H & E × 70.

the horizontal graticule lines. The mean cavity height for the experimental series was 1.3 mm. The outer edges of the graticule squares separated by approximately this height, in approximately the same portion of the crown, were considered to be the equivalent of the cavity margins. Odontoblast nuclei were counted in four zones oriented around the specified points.

All results were transferred to computer cards and analyzed using SAS (22) and SPSS (23). Coding of the sections was broken after all measurements had been completed. Statistical tests used were the one-way analysis of variance and Student's t test for independent samples (21), while the minimum critical level of statistical significance chosen was $P<0.05$.

Results

Application of Student's t test for related samples (21) showed no statistically significant differences either for cell counts or linear measurements. One way analyses of variance of the results of the dentinal thickness and cut dentinal tubule length (Table 1) showed that there was a statistically significant difference between the cut dentinal tubules at the incisal and cervical margins of the cavities. The absence of any other statistically significant differences indicates that the cavities in all groups were comparable.

Table 2 lists the mean odontoblast nuclei counts. The mean counts in Zones 1 and 2 did not differ significantly from each other. Similarly, mean counts in Zones 3 and 4 did not differ significantly from each other. The mean nuclei counts in Zones 3 and 4, that is the zones in which the dentinal tubules had been cut during cavity preparation, were approximately 35% less than in Zones 1 and 2 (uncut tubules), a statistically significant result.

These observations were noted for both Nobetec® and Syntrex® and at the 3 time periods.

Variations in mean nuclei counts according to test material and time are illustrated in Table 2. In the zones without cut dentinal tubules (Zones 1 and 2), at 2 d the mean nuclei counts were higher for Syntrex®, at 28 d they were similar and at 56 d the Syntrex® results were lower. In the zones with cut dentinal tubules (Zones 3 and 4) at 2 d a variable pattern was seen, at 28 d the results for both materials were similar and at 56 d the mean nuclei counts for Syntrex® were lower than for the Nobetec® specimens. Apart from one result at 56 d, the differences were slight and none was statistically significant. Mean nuclei counts in comparable zones in the control specimens did not show a reduction when Zones 1 and 2 were compared to Zones 3 and 4.

Reparative dentine thickness

No reparative dentine was seen at the 2-d observation time in any group. At 28 d the mean thicknesses in mm for the materials were: Nobetec® 0.10 (sd 0.15), Syntrex® 0.09 (sd 0.36) and the mean values at 56 d were Nobetec® 0.20 (sd 0.71), Syntrex® 0.18 (sd 0.70). At both these time intervals reactions to Nobetec® and Syntrex® were similar, and for both materials, as the observation time doubled, so did the mean reparative dentine thickness.

Discussion

The specimens examined were comparable except for the difference in cut dentinal tubule length at the incisal and cervical cavity line angles. This is

Table 1. Dentine thickness and remaining dentine thickness at incisal end and cervical end of cavity in mm, by material by time

		2 d		Post-operative time 28 d		56 d	
		Nobetec®	Syntrex®	Nobetec®	Syntrex®	Nobetex®	Syntrex®
Original dentine thickness (Incisal) – LD	n	13	13	12	15	12	14
	mean	1.20	1.21	1.22	1.20	1.20	1.18
	SD	2.22	1.45	1.60	1.21	1.09	1.23
Original dentine thickness (Cervical) – MG	mean	1.21	1.28	1.29	1.27	1.28	1.28
	SD	1.67	1.90	1.11	1.44	0.98	1.48
Remaining dentine thickness (Incisal) – CD	mean	0.59	0.61	0.59	0.63	0.57	0.66
	SD	3.12	1.43	2.48	1.07	1.70	2.14
Remaining dentine thickness (Cervical) – FG	mean	0.48	0.57	0.54	0.60	0.59	0.59
	SD	1.79	1.67	1.99	1.43	2.46	1.84
Cut dentinal tubule (Incisal) – CE	mean	0.92	1.10	1.02	1.20	1.02	1.20
	SD	5.10	2.94	3.92	2.34	3.52	4.56
Cut dentinal tubule (Cervical) – FH	mean	0.75	0.89	0.84	1.01	0.90	0.96
	SD	3.23	2.82	3.66	2.11	3.91	3.55

Table 2. Details of odontoblast nuclei counts

Material	Post-operative time (days)	n	Zone 1		Zone 2		Zone 3		Zone 4	
			mean	SD	mean	SD	mean	SD	mean	SD
Nobetec®	2	13	84.2	19.3	83.8	20.2	49.8	36.5	50.6	36.5
	28	12	82.9	23.1	79.3	31.4	61.2	38.6	53.2	29.0
	56	12	88.4	18.1	71.3	22.2	50.2	29.8	44.0	23.6
Syntrex®	2	13	89.7	24.6	90.8	19.4	55.5	38.4	47.1	35.7
	28	15	80.5	26.6	80.7	27.1	57.6	31.1	53.1	22.6
	56	14	66.7	25.0	58.2	25.1	37.6	20.7	38.6	16.6
Controls		6	81.7	8.7	77.3	8.9	78.7	2.9	78.7	10.0

due to a variation in orientation of the dentinal tubules.

The counting of odontoblast nuclei was easily achieved and reproducible with the method used, which is more objective than the technique mentioned by Plant & Jones (17).

The vervet monkey has been shown in earlier studies to be a suitable non-human primate for pulpal response studies (4, 24). A characteristic of pulpal response studies in monkeys, however, is that reparative dentine is formed sooner and in greater amounts than in man (13).

The present investigation showed that the odontoblasts under a cavity were reduced in number only in areas in which the dentinal tubules had been cut. This was probably due to the operative trauma, material toxicity and possible microleakage.

Both materials tested produced the same type of results, yet Nobetec® has been regarded as a bland temporary restorative material and negative control in pulpal response studies (4, 24) although it has been recorded that the material is irritant (4, 25). The present investigation confirmed that it was as irritant to the pulp as the unlined silicate Syntrex®, probably due to the additives or their modification of the properties of zinc oxide-eugenol. This finding was further confirmation that a zinc oxide-eugenol mixture without additives should be used as a negative control (8, 9).

It is suggested that the quantitation described could be used as a sensitive method to discriminate between test materials. The investigation also suggested that areas with cut dentinal tubules may be the site of choice in assessing pulpal reactions.

Further investigations are necessary to determine whether the rate of reparative dentine formation continues to be directly proportional to the post-operative time.

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