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Human odontoblast cell numbers after dental injury

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Abstract

Objectives: The purpose of this study was to measure the changes in odontoblast cell numbers in response to cavity restoration variables and patient factors, and the effect these factors have on dental repair by tertiary dentinogenesis. The number of vital odontoblasts is a critical factor for pulpal repair following restorative surgery, and yet little information is available on these cell numbers.

Methods: Class V non-exposed cavities were prepared in the buccal surface of intact first or second premolar teeth of 27 patients, between 9 and 17 years of age. Following tooth extraction (28–163 days) the area of reactionary dentine and the area of the odontoblasts were measured histomorphometrically.

Results: Patient factors, as well as cavity preparation and restoration variables, had little effect on the numbers of odontoblasts per pulpal unit area. However, the age of the patient did appear to have an effect on the reactionary dentine secretory capacity of odontoblasts per unit area, and on the relative number of odontoblasts beneath cut dentinal tubules.

Conclusions: Odontoblast cell numbers were maintained following the preparation of cavities cut into dentine with a 0.5 mm residual dentine thickness. The repair capacity of the pulp–dentine complex would appear to be age dependent, this may explain differences in the success of various restorative treatments between patient

Keywords: Tertiary dentinogenesis; Cavity restoration; Pulp; Odontoblast; Caries; Dentine thickness

1. Introduction

Tooth dentinogenesis is a dynamic process, regulated by a single layer of highly differentiated post-mitotic odontoblast cells. The intrinsic capacity of the dental pulp to repair lost or damaged dentine matrix, and to increase the barrier between itself and the injurious stimuli, is dependent on the vitality of the odontoblast cell layer, following caries, attrition, abrasion, erosion, tissue damage, and trauma [1–5]. Not only must the odontoblasts survive these challenges, but they must also detect and respond to them by synthesising and secreting a reactionary dentine matrix to protect the tooth pulp from further injury. In many cases the most severe challenge to odontoblast survival is not a direct result of these environmental or accidental events, but from the surgical techniques and materials used to restore tooth structure following these events [6–12]. The successful resolutions of restorative treatments are dependent upon harnessing and utilising the natural repair responses of the pulpal cell populations, especially the odontoblasts. The

other pulpal cell populations which occupy the sub-odontoblast layer and the pulp core, are important in supporting dentinogenesis, but do not play a direct role in the secretion of dentine matrix [13]. Dentine matrix can be classified as either primary, secondary or tertiary in origin according to the chronology and circumstances of its secretion by the odontoblasts [14,15]. Primary dentine is secreted at a rate of approximately 4 μm per day during tooth development, until the completion of root formation [16]. Physiological secondary dentine is laid down after completion of root formation in increments of approximately 0.5 μm per day throughout life [17,18]. Unlike primary or secondary dentine that forms along the entire pulp–dentine border, tertiary dentine is focally secreted by odontoblasts in response to primary and secondary dentine injury. The process of tertiary dentine secretion can further be classified as being reactionary or reparative in origin, depending on the severity of the initiating response, and the conditions under which the newly deposited dentine matrix was formed. If these cells survive cavity preparation and restoration surgery, they may secrete a reactionary dentine matrix of repair.

If these cells are irreversibly injured, they must be replaced by a second generation of newly differentiated

odontoblast-like cells, before the secretion of a reparative dentine matrix of repair can take place [19,20]. Reparative dentinogenesis is a much more complex process than reactionary dentinogenesis, and is generally observed following more severe injurious cavity preparation, or a pulp exposure situation. The minimisation of pulpal injury during cavity preparation is clinically advantageous, as it maintains pulp cell function and viability, and reduces the probability of post-operative pulp complications from occurring. The preservation of pulpal cell function and viability following dentine cavity preparation requires the potentially injurious effects of the cavity preparation and restoration procedures to be studied. The choice of different restoration materials and the variables of cavity cutting have often had a highly controversial effect on the dental pulp. For example, the increased traumatic operative procedures involved in cutting deeper cavities, have been claimed to be destructive to the underlying odontoblast cell population [21–23], whilst others dispute this, and claim that factors such as pulpal inflammation caused by microleakage is of greater importance for pulp viability [24]. Also controversial, is the application of zinc oxide eugenol (ZOE) and calcium hydroxide products to lined prepared cavities. The application of calcium hydroxide and ZOE products have been claimed to elicit a persistent stimulating effect on the pulp resulting in its eventual obliteration [25]. Others however, have claimed that the cavity restoration material has had little effect on odontoblast activity [10,26]. Furthermore, the process of tertiary or reactionary dentine formation is a continuing focus of interest, because there is no artificial material that can be placed into a tooth that provides better protection for the pulp than dentine [27]. But the process of pulpal repair appears complex and the mechanisms remain incompletely understood [28]. Hence, the purpose of this study was to examine odontoblast cell survival and reactionary dentine secretion, in response to cavity preparation and restoration factors, in the permanent first or second premolar teeth of patients aged between 9 and 17 years of age.

2. Materials and methods

Class V cavity preparations were placed in the buccal surface, 1 mm above the level of the cemento-enamel junction, of intact first or second noncarious intact premolar teeth, scheduled for extraction for orthodontic reasons. Teeth were polished with pumice paste to remove plaque and calculus, prior to the operative procedure. The tooth surface was isolated with sterile cotton rolls, and saliva was controlled with high-speed evacuation. Cavities were cut into dentine with a Maillefer 129, ISO 010 bur using the least possible pressure at a drill speed of 4000 rpm with water spray coolant.

The cavities were cut into the dentine to a range of cavity residual dentine thicknesses, with the floor of the prepared cavities being maintained curved and parallel to the outer

surface of the tooth. The walls of the cavities were immediately conditioned with a 17% disodium ethylene diamine tetraacetate solution, pH 8 (Roth International, Chicago, USA), for 15 s, flushed with sterile water for 5 s, and dried with an extremely light airstream for 2 s. Teeth were randomly assigned to three experimental groups for cavity restoration. Amalgam (Contour, Kerr, Italy) was used to restore 17 cavities, in conjunction with a calcium hydroxide lining material (Dycal, Dentsply, Milford, DE, USA). A further 7 cavities were restored with Zinc-Oxide Eugenol (ZOE) (Kalzinol, Dentsply, Milford, DE, USA) as well as a further 3 cavity restorations with the Intermediate Restoration Material (IRM) (De Trey Dentsply, Zurich, Switzerland). All materials were prepared and used, strictly according to manufacturer's instructions. The teeth were extracted from 27 patients, aged between 9 and 17 years of age, after a post-operative interval of 28–163 days. The patients had given their informed consent to the tooth extraction, at the Dental Hospital of Marseille, France. The teeth were extracted using a local anesthetic, the roots were clipped and the teeth were then fixed in 10% neutral-buffered formalin for 24 h, demineralised in sodium formate acid (3.4% sodium formate [HCOONa] in 15% formic acid) for 21 days, and then routinely processed and embedded in paraffin wax for histological examination. Sections were cut at 6 μm and routinely stained with hematoxylin and eosin. The area of reactionary dentine was estimated histomorphometrically at 10 $\times$ magnification, using a grid eyepiece graticule [29].

In addition, the number of odontoblasts was counted histomorphometrically per 2112 μm^2 of pulpal unit area using a grid eyepiece graticule [29]. Odontoblasts were counted beneath the dentinal tubules cut by cavity preparation, and also on the opposite side of the pulp chamber, independent of the cavity preparation within each tooth section. The pulpal inflammatory responses were categorised as either slight, moderate or severe, according to the criteria detailed by Baume and Fiore-Donno [30]. The raw data from all the experiments were examined using linear regression analysis of variance (LRANOVA) tests, for raw data containing regressors (STATview software, Cherwell Inc, USA) and analysis of variance (ANOVA) tests for comparing the difference between means (SuperANOVA software, Cherwell Inc, USA). ANOVA post hoc tests [31] were used to compare the differences between variables. These methods have been claimed to be the most versatile and most conservative multiple comparison statistical tests [32]. The measured variables are shown in Table 1.

3. Results

3.1. Histomorphology of reactionary dentine

The preservation of the single layer of odontoblasts could be observed beneath the cavity restorations (Fig. 1). In some

Table 1
Pathohistometric raw data from 27 patients

Age of patient (years)	Time elapsed since surgery (days)	Reactionary dentine area mm ²)	Pulpal inflammatory activity (+++ = severe, ++ = moderate, + = slight)	Cavity restoration product	Patient gender (F = female, M = male)	Tooth position (Mx = maxillary, Md = mandibular)	Cavity residual dentine thickness (mm)	Cavity width (mm)	Cavity depth (mm)	Cavity volume (mm ³)	Cavity floor surface area (mm ²)	Cavity walls surface area (mm ²)	Odontoblast numbers beneath cut dentinal tubules	Odontoblast numbers independent of cut dentinal tubules
17	39	2.79	(++)	Dycal	F	Md	0.63	2.27	1.1	2.32	4.48	11.89	19	13
11	28	0.47	(++)	Dycal	M	Mx	1.06	1.89	0.44	1.23	2.81	5.40	20	23
14	28	0.95	(+)	Dycal	M	Mx	0.88	1.51	0.25	1.80	1.80	3.00	12	18
11	32	0.98	(++)	Dycal	M	Mx	0.73	1.45	0.18	0.29	1.65	2.46	23	11
17	35	0.74	(+)	Dycal	M	Md	0.84	1.77	0.95	2.34	2.45	7.71	16	11
11	33	0.33	(+)	Dycal	M	Md	1.38	1.74	0.63	1.51	2.38	5.83	22	12
11	33	0.00	(+)	Dycal	M	Md	1.69	1.96	0.51	1.53	3.01	6.11	21	26
14	57	1.58	(+)	Dycal	F	Mx	1.10	1.87	0.51	1.39	2.74	5.70	17	14
14	56	1.83	(+)	Dycal	M	Mx	0.88	1.51	0.38	0.69	1.80	3.60	20	11
9	150	0.47	(++)	Dycal	F	Md	1.38	1.58	0.19	0.37	1.95	2.89	23	17
10	85	1.50	(+)	Dycal	M	Mx	1.13	1.96	0.47	1.41	3.01	5.88	15	16
10	56	0.98	(+)	Dycal	F	Mx	1.03	2.15	0.25	0.92	3.62	5.32	23	13
14	74	0.00	(+)	Dycal	F	Md	1.63	1.45	0.76	1.26	1.65	5.11	27	14
11	163	0.00	(+)	Dycal	M	Mx	1.00	1.77	0.69	1.71	2.45	6.30	15	18
10	124	0.79	(+)	Dycal	M	Mx	0.96	1.51	1.14	2.06	1.80	7.21	17	20
11	84	0.54	(+)	Dycal	F	Mx	0.77	1.39	0.48	0.73	1.51	3.61	20	11
10	28	0.00	(++)	IRM	M	Md	1.32	2.02	0.69	2.24	3.20	7.61	22	22
12	77	1.27	(++)	IRM	F	Mx	0.57	2.27	0.38	1.54	4.05	6.76	11	20
12	28	0.23	(++)	IRM	F	Md	1.60	1.26	0.13	0.16	1.25	1.75	26	17
12	123	0.12	(++)	ZOE	M	Mx	1.22	1.66	0.99	2.11	2.17	7.37	13	12
10	127	0.16	(++)	ZOE	F	Md	1.57	1.57	0.88	1.65	1.93	6.25	23	20
10	90	0.03	(+)	ZOE	F	Md	1.62	1.86	0.53	1.41	2.72	5.83	16	23
16	61	0.00	(++)	ZOE	F	Mx	1.45	1.69	0.44	0.96	2.24	4.56	20	20
10	29	0.00	(+)	ZOE	F	Mx	0.95	1.53	0.93	1.66	1.83	6.30	22	20
10	28	0.00	(+)	ZOE	F	Md	0.95	1.73	1.20	2.73	2.34	8.84	16	26
10	28	0.00	(++)	ZOE	M	Md	0.98	1.57	0.97	0.00	1.83	6.71	22	24
10	154	0.35	(+++)	Dycal	F	Md	0.78	1.62	1.48	1.62	2.06	9.58	22	24

Fig. 1. Preservation of the pulp tissue following cavity restoration: 14 year-old female patient with an amalgam/Dycal restored cavity (magnification $320\times$)—74 days had elapsed between cavity restoration and tooth extraction.

cases, a histomorphological evaluation of the extracted premolar specimens revealed the presence of a tertiary dentine matrix beneath dentinal tubules cut by cavity preparation. The tertiary dentine matrix appeared to have a tubular continuity between the secondary dentine matrix and the pre-existing odontoblasts (Fig. 2). These observations were necessary to classify the secreted dentine matrix as being reactionary in origin [33].

3.2. Controls

The RDT of all the cavity restorations was examined within each of the test groups to remove the possible influence of differences in the residual dentine thickness of the cavity preparations. No statistical differences $p = 0.05$ were observed for: age of the patient at treatment (ANOVA $p = 0.661$); cavity restoration material (ANOVA $p = 0.519$); pulpal inflammatory activity (ANOVA $p = 0.513$); gender of patient (ANOVA

Fig. 2. Odontoblast cells associated with areas of reactionary dentinogenesis and adjacent areas of dentine. 14 year-old female patient with an amalgam/Dycal restored cavity (magnification $320\times$)—57 days had elapsed between cavity restoration and tooth extraction.

Table 2

Regression analysis between the number of odontoblasts beneath cut dentinal tubules following cavity preparation and restoration factors

Mean odontoblast cell numbers beneath cut dentinal tubules vs:	<i>P</i> value
Reactionary dentine area	0.0084
Age of patient	0.0122
Mean odontoblast cell numbers independent of cut dentinal tubules	0.167
Cavity residual dentine thickness	0.241
Cavity restoration product	0.271
Cavity volume	0.394
Cavity width	0.510
Premolar position	0.623
Gender of patient	0.689
Cavity depth	0.752
Pulpal inflammatory reaction	0.756
Cavity walls surface area	0.825
Cavity floor surface area	0.901
Time elapsed since surgery	0.951

$p = 0.412$); time elapsed since cavity restoration (ANOVA $p = 0.574$); and mandibular or maxillary premolar tooth position (ANOVA $p = 0.070$).

3.3. Odontoblast pulpal cell position

The number of odontoblasts beneath cut dentinal tubules, was found to be not significantly different to the number of odontoblasts independent of the cavity preparation (Table 2). The box chart (Fig. 3) indicated that cavity preparation and restoration, as well as the secretion of reactionary dentine matrix did not irreversibly injure the underlying odontoblast cells.

3.4. Area of reactionary dentine secreted

The area of reactionary dentine beneath cut dentinal tubules was found to be related to the odontoblast cell number per unit area (Table 2). The regression graph of this relationship showed that the number of odontoblasts per unit area was reducing, as the area of the reactionary dentine was increasing (Fig. 4). The LRANOVA $p = 0.0084$, and the $r^2 = 0.275$; this r^2 has been claimed to show a fair degree of relationship between the regression variables [32]. This regression relationship between the secretion of reactionary dentine, and a corresponding decrease in odontoblast cell number per unit area, suggests that the secretion of reactionary dentine caused an increase in the length of the pulp dentine border.

3.5. Age of the patient

The age of the patient had little effect on the number of odontoblasts per unit area, independent of the cavity preparation (LRANOVA $p = 0.216$). However, beneath

Fig. 3. Bar chart of odontoblast cell numbers per unit of pulp area. The boxes show the standard error of the means, the bars show the standard deviations of the means.

the cavity preparations, the number of odontoblasts was found to decrease per unit area (Table 2) (Fig. 5). The LRANOVA $p = 0.0122$; and the $r^2 = 0.286$; this r^2 has been claimed to show a fair degree of relationship between the regression variables [32]. Furthermore, it was found that the area of reactionary dentine when divided by the odontoblast cell numbers per unit area was found to increase in proportion with the age of the patients (Fig. 6).

The LRANOVA gave $p = 0.0013$ and the $r^2 = 0.344$, this r^2 has been claimed to show a fair degree of relationship between the regression variables [32]. This regression graph indicated that the capacity of each odontoblast to secrete reactionary dentine increased, as the age of the patient increased from 9 years until 17 years of age, irrespective of the cavity preparation or restoration variables.

3.6. Variables with an insignificant correlation to the number of odontoblasts per unit area

The following variables were found to have an insignificant correlation with the number of odontoblasts per unit area; time elapsed since surgery; cavity restoration variables such as the floor surface area, depth, volume, width, and residual dentine thickness; pulpal inflammatory reaction; gender of patient; choice of Amalgam/Dycal, IRM or ZOE cavity restoration products and premolar tooth position (Table 2).

4. Discussion

This study has demonstrated that the odontoblast cell numbers can be preserved following the careful preparation

Fig. 4. Regression graph of the mean reactionary dentine area and the number of odontoblasts per unit area, beneath cut dentinal tubules.

Fig. 5. Regression graph of the age of the patient and odontoblasts per unit area, beneath cut dentinal tubules.

and restoration of dentine cavities. However, cavity preparation and restoration events may injure the pulp, causing necrosis or the activation of apoptosis [34]. The most common method of evaluating pulp tissue reactions to injury is by the qualitative assessment of pulp tissue alterations, however these methods can be subjective [35]. A further problem with these methods, is that although different types of cell death events are easily distinguishable [36], and molecular markers are available for detecting cell death

responses [37], human studies can be limited by the need for chronological information on the post-treatment tissue events. In comparison to qualitative approaches to examine pulpal activity, the morphometric analysis of odontoblast cell numbers has proved, in common with previous morphometric studies [29,35] to be able to provide a relatively uncomplicated method for examining a number of variables of interest.

It is not always possible to determine the exact cause of

Fig. 6. Regression graph of the age of the patient and area of reactionary dentine matrix/odontoblasts per unit area beneath cut dentinal tubules.

death when odontoblasts disappear following restorative intervention, since the survival of these cells can be threatened by a variety of injury sources. The main source of injury caused by cavity cutting is claimed to be frictional heat [38]. The amount of intrapulpal heat generated during cavity preparation and restoration is determined by the drill rotation speed [39–42], size, type and shape of the cutting instrument [43,44], length of the time the instrument is in contact with the dentine [45], the amount of pressure exerted on the handpiece [42,46,47], and the use of cooling techniques [7,48,49]. In addition to heat, rotary cavity cutting also produces injurious audible and ultrasonic vibrations [50].

Sources of pulpal injury associated with restorative procedures have included conditioning of the dentine cavity wall [51], presence of bacteria [52,53], method of placement of the restorative material [54,55] desiccation [9], pulpal inflammation [56,57], chemical irritants [58] and restoration material toxicity [59], and restoration material temperature [60,61]. Odontoblast survival is a multifactorial process, and all of these factors may individually or cumulatively play a role in the demise of odontoblasts. The relative importance of many threats to odontoblast survival during cavity cutting and restoration probably varies from one cavity restoration to another. However, the choice of the restorative material and the parameters of the cut cavity preparation, are among the most controllable and avoidable of the potential sources of injury, and therefore the emphasis of this study has been to focus on these factors.

LRANOVA methods have shown the potential to provide information about the number of odontoblasts per unit area, in response to cavity restoration and patient factor variables. In this present study, little difference was observed between numbers of odontoblasts located beneath cut dentinal tubules, and independent of the cavity preparations (Fig. 3), despite the fact that some secreted a reactionary dentine matrix (Fig. 4). The number of odontoblasts have been reported to fluctuate from birth to 23 years of age, after which they decrease progressively [38] to approximately half their number until 70 years of age is reached [62].

However, graphs of odontoblast cell numbers per unit of pulp area, indicate that odontoblast viability was maintained following the traumatic procedures of cavity preparation and restorative intervention (Figs. 3 and 4). Many studies have reported losses in odontoblast survival, with cavity preparation variables such as a decreasing cavity residual dentine thickness [22,23,25,26]. Whilst no such effect was observable in this present study (Table 2), nevertheless, it must be accepted that the cavity residual dentine thickness (RDT) could become more critical as it is reduced, allowing the greater diffusion of potentially injurious cytotoxins in a pulpward direction [63]. Because of the buffering properties of dentine, and the ways in which this can modify the possible cytotoxic properties of dental materials [64] there can be large differences between the effect of a material when it is used to restore exposed and non-exposed cavity

preparations. Stanley et al. [65] found that a RDT of 2 mm would protect the pulp from most restorative procedures, and more recently Pameijer et al. [66] showed that a RDT of 1 mm or more would be effective at protecting the pulp tissue from the cytotoxic effects of zinc phosphate and glass ionomer cements during luting procedures. The work presented here would suggest that these are cautious estimates, and deeper cavity preparations down to 0.5 mm appeared to have little effect on odontoblast cell numbers per unit area (Table 2). Furthermore, the restoration of cavity preparations with amalgam/Dycal, IRM or ZOE, did not appear to influence the number of odontoblasts per unit area. This may be attributed to the effect of dentine buffering, which protected the pulp from the possible cytotoxic effect of the restorative products [63].

The quantity of the reactionary dentine secreted by each odontoblast in permanent premolar teeth appeared to increase as the age of the patient increased (Fig. 6). The change in dentinogenic activity of the restored teeth of patients with different ages, can be attributed to maturation related changes within the dental pulp [67–69]. Although the regression graph (Fig. 6) showed that the area of the reactionary dentine secretory activity of each odontoblast increased, this could not be expected to be representative of the tertiary dentinogenic activity of a tooth into old age.

The dentinogenic response potential of teeth could be expected to reach a maturation plateau and thereafter diminish into old age, as a reduction in the rate of dentinogenesis has been observed in the elderly [70,71], and with animal models [72]. Moreover, age has been observed to be a factor in the secretion of reactionary, physiologic sclerotic and reparative dentine [73]. These changes in odontoblast activity can correspond to changes in odontoblasts observed at the molecular level with the ageing process [74]. Changes in dentine due to ageing and disease processes introduce clinically significant alterations in pulpal structure and properties, and our understanding of these alterations is only in its infancy. The impact of these alterations on current and future treatments is expected to be profound [75]. The secretion of reactionary dentine matrix has been observed to be accomplished rapidly and is highly regulated [76], and in this study, reactionary dentine matrix was not uniformly secreted by the odontoblasts in response to cavity restorations. The mechanisms which transduce dentine damage to the odontoblasts and upregulate their synthetic and secretory activity remain incompletely understood [28]. Nevertheless, this study has established that the secretion of reactionary dentine is a more complex process, than an automatic response of the odontoblasts to cavity restoration. The factors which mediate the secretion of reactionary dentine are not known, but they may include the following; the cavity residual dentine thickness [10,77,78], damage to the odontoblast cell process [79], injury to the intratubular elements [80], nerve damage [81–83] and the presence of growth factors [84] solubilised from the dentine matrix [85,86]. Despite these investigations, much remains to be

accomplished in order to understand the mediators of dentinogenesis and the role played by the odontoblast. However, this study has established that carefully cut, deep dentine cavities, do not necessarily irreversibly injure the underlying odontoblast cells. The repair capacity of teeth would appear to be age dependent, this may explain differences in the success of various restorative treatments between patients.

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