Histomorphology and X-Ray Microanalysis of Reparative Dentin in Primary Teeth

A. Robertson,1 and S. Nietzsche2 1. Department of Pedodontics, Institute of Odontology, University of Gothenburg, Göteborg, Sweden. 2. Centre of Electron Microscopy, Friedrich-Schiller-Universität Jena, Germany

INTRODUCTION

All primary and permanent teeth show a layer of secondary dentin around the pulp cavity, which is distinct from the regular primary dentin. It is often rather difficult to distinguish between primary and secondary dentin [1]. Secondary dentin comprises the circumpulpal portion of regular dentin and the primary one produced circumpulpally throughout the later periods of the vital tooth [1]. However, reparative dentin differs in many respects from primary and secondary dentin and is thus easily distinguished from these [1].

The formation of reparative dentin is an important protective function of the dental pulp in response to dentin injury. Calcification of the pulp is common at all ages, but caries and traumatic injuries affecting the pulp are known to increase their incidence [2-4]. Traumatic injuries often give rise to an acceleration of hard tissue formation within the pulp cavity, sometimes leading to complete calcification [7]. Frequent efforts have also been made to study the incidence of reparative dentin as a function of various types of injury [2-4]. Pulpal calcifications have been studied by clinical, radiographical, histological and scanning electron microscopic techniques but information about the nature of pulp calcification in primary teeth is still lacking. Histological methods have not clearly revealed the nature of the micro-environment of the sites of initiation of these forms of pulpal calcification since the earliest sites are not resolved in the light microscope. It has been suggested that dead or inflamed cells, bacteria, matrix fibres or blood vessels and nerves form foci for calcification [2-5]. Limited information is available regarding the prevalence and long-term prognosis of pulp calcification in injured primary teeth [5,6]. Neither are there any studies available of the chemical abundance of Ca and P in reparative dentin.

The purpose of this study was to describe the detailed structure and chemical composition of reparative dentin in primary teeth using light microscopy (LM), scanning electron microscopy (SEM and X-ray microanalysis (XRMA).

MATERIAL AND METHODS

Study Group 1

The material comprised 22 traumatized primary teeth extracted due to trauma immediately or due to complications after traumatic dental injuries. The acute diagnoses included subluxation, extrusive luxation and lateral luxation. Inclusion criteria were: 1. 50% full root length; and 2. Presence of reparative dentin.

ABSTRACT

The purpose of this study was to describe the detailed structure and chemical composition of reparative dentin in primary teeth using light microscopy, scanning electron microscopy and X-ray microanalysis. The study comprised decalcified sections of 22 traumatized and undecalcified sections of 8 primary teeth. The teeth displayed normal pulp tissue morphology, but reduced in size due to the reparative dentin. Five different configurations of reparative dentin were identified on the walls of the pulp cavity. Lower values in reparative dentin compared with normal dentin were found for Ca, P and the ratio Ca/C.

KEYWORDS

light microscopy, histology, scanning electron microscopy, X-ray microanalysis, energy dispersive X-ray spectroscopy, Brown and Brenn staining, demineralized sections, dentin, reparative dentin, primary teeth.
Study Group 2
The material comprised eight exfoliated primary teeth. The inclusion criterion was the presence of reparative dentin.

Light Microscopy
The teeth were fixed and stained immediately after extraction in 10% neutral-buffered formalin. All teeth were decalcified with EDTA, conventionally prepared for paraffin embedding, and then serially sectioned and stained with hematoxylin and eosin (H & E).

The teeth were evaluated by light microscopy and were analyzed according to predetermined parameters:
- Odontoblast layer: 1. Normal structure, reduced or missing. 2. Regular, irregular layer.
- Pulp tissue morphology: Normal, altered tissue.
- Primary and secondary dentin: Regular, irregular dentin.

Scanning Electron Microscopy and X-Ray Microanalysis
All twenty sections from the primary teeth previously examined by LM were analyzed in SEM. The slides were kept in xylene till the cover glass could easily be removed. After mounting on sample holders for the microscope, the sections were sputter coated with gold. They were then examined in a Zeiss (LEO) Gemini IM 1530 field-emission scanning electron microscope at 5 kV and 7 kV.

Six undemineralized primary incisors were sectioned sagittaly in a bucco-lingual direction with a Leitz low speed saw microtome. After examination in an Olympus polarizing microscope employing strain-Free objectives, the sections were mounted on sample holders for the SEM and sputter coated with carbon. For the XRM analysis, a Philips SEM 515 with an EDAX DX 4 ECON detector was used. Measurements of C, O, P and Ca were performed in two locations in reparative and normal dentin, respectively (Figure 1). For all measurements the X-rays were detected by a small window (6.1 x 4.3 µm) at a magnification of 655 x. The relative amounts of C, O, P, and Ca were calculated with the Point Electronic DISS 2 program. All values are to be regarded as semi-quantitative.

In the SEM analysis the following criteria were evaluated: Primary and secondary dentin: Regular, irregular dentin. Interface between the dentin (primary and secondary) and the reparative dentin: Regular, irregular dentin. Reparative dentin: 1. Localization. 2. Regular, irregular dentin.

Statistical Analysis
The data from XRMA were compiled in an Excel spreadsheet and the Mann-Whitney statistical test was used to compare median values for C, O, P, Ca and the ratios Ca/P and Ca/C.

RESULTS
Light Microscopy
Odontoblast layer: The odontoblast layer could be observed in all teeth. The amount of layers varied from one single layer to three or four. The layers were often irregular (Figure 2).

Pulp tissue morphology: The teeth displayed normal pulp tissue morphology, but it was reduced in size due to the reparative dentin. The number of cells, predominately odontoblasts, in the parts of the pulps was nevertheless decreased in the most cases.

Primary and secondary dentin: The primary and secondary dentin look regular and there was no marked border between the layers (Figure 3).

Reparative dentin: Five different configurations of reparative dentin were identified on the walls of the pulp cavity (Figure 4): 1. Hard tissue in the most coronal part of the pulp chamber. 2. Hard tissue in the most coronal part of the pulp chamber in addition to hard tissue along one of the lateral pulp canal walls. 3. Hard tissue in the most coronal part of the pulp chamber in addition to both the lateral pulp canal walls. 4. An isolated formation of new hard tissue on the lateral pulp canal wall. 5. Hard tissue filling up a substantial portion of the coronal pulp chamber.

The reparative dentin had formed in the pulp to a varying extent. The most frequently occurring types of hard tissue formation were type 2 and 4. Interglobular dentin was observed and in some teeth incremental lines with alternating high and low mineral content were seen.

Denticles: In a few teeth free denticles were
observed (Figure 5). The sizes of the denticles in the pulp of the teeth were observed to vary greatly. The size of these was sometimes so small as to be barely perceptible, while others consisted of large conglomerate fused masses. Those denticles were partly lined with an odontoblast-like cell layer. The calcification appeared to consist of discrete smooth-surfaced laminated denticles or irregularly shaped non-laminated denticles. Denticles without laminations often appeared with an irregular outline compared with the laminated denticles. The denticles that consisted of distinct concentrically arranged lamellae did not contain dental tubules. The denticles contained blood and nerves. Diffuse calcifications: All teeth show diffuse calcifications which were distinct and occurred as unorganized masses throughout the pulp (Figure 2). They were often related to blood vessels or nerves.

**Scanning Electron Microscopy**

The primary and secondary dentin. The dentin formed was regular and well-mineralized. The dentin exhibits tubules with a diameter of 3 µm or less (Figure 3b). The interface between the primary and secondary dentin and the reparative dentin: The interface between the secondary dentin and reparative dentin was rough and diffuse. The dentin tubules changed direction passing the interface (Figure 3b).

The reparative dentin: The dentin formed looked with few exceptions irregular and there were a reduced number of tubules. In a few cases the tubules there were sparse and appeared regular and twisted. The number of tubules was reduced on the side of the walls of the pulp while there was no marked reduction in others. In the reparative dentin formed in the horn of the pulp there was no marked reduction in the number of tubules, in some cases. Some tubules could be followed from the primary and secondary dentin into the reparative dentin and a change in the direction of the tubules was often noted. The tubules were either empty or filled with odontoblast processes. They had a variable size and distribution; in addition, occluded tubules with a high mineral content were seen. In some cases the reparative dentin looked more bone-like than dentin-like and there were also intermediate forms.

Denticles: The denticles had the appearance of osteodentin with cell inclusions in a ring-like formation. The bone-like tissue had lacunae-like cavities, free from cells, scattered throughout the tissue.

**X-Ray Microanalysis**

All median and mean values are given in Table 2. There were no differences between C and O in normal and reparative dentin, respectively. The Ca and P values were significantly lower (p<0.05) in reparative dentin compared with normal dentin, however, the ratio Ca/P did not differ between normal and reparative dentin. The ratio Ca/C was significantly lower (p<0.01) in reparative dentin compared with normal dentin.

**DISCUSSION**

This study has shown that the reparative dentin formation, described by comparing light microscopy and scanning electronic microscopy, was irregular and with a reduced number of tubules. The chemical analyses revealed lower values for Ca, P and the ratio Ca/C in reparative dentin compared with normal dentin.

Normal calcification takes place in a pre-formed organic matrix produced by specialized cells e.g. odontoblast and osteoblasts [1]. The original odontoblast layer after having formed the primary dentin may become reduced and in some teeth only a single layer was visible. Hard tissue apposition along the root canal walls is a slow normally occurring physiological aging process. Thus the prevalence of pulp calcification in the primary dentition is expected to be lower than in the permanent dentition. But also in the primary dentin the formation of reparative dentin seems to represent one of the earliest defense mechanisms of the tooth. It is well known that the rate of it may seem to be uncontrolled after dental trauma [3, 7-8]. The onset of reparative dentin formation can be more clearly defined as the first possible moments for its formation to start is when few or larger group of dentinal tubules are exposed to external irritants. Some of the teeth seemed to have been subjected to milder injuries (subluxation) which seem to not affect the underlying odontoblasts as more severe injuries do. If only some of them are destroyed the survivors probably produce the reparative dentin. Reparative dentin differs from primary dentin and secondary dentin and it was thus easily distinguished from these with the light microscope. It seems that the reparative dentin in these cases looked more dentin-like. It was more regular and well-organized. It appears that the milder the injury is to dentin, the lower is the probability that reparative dentin will be seen.

In the cases of moderate injuries (extrusive and lateral luxation) that affect the blood supply and cause cell death, the death of odontoblasts can probably stimulate the underlying cells to produce reparative dentin. The tissue damage may produce localized metabolic changes thereby promoting calcification. The calcifications here seem to be more irregular in pattern and sometimes had a more fibro-dentinal or bone structure. The number of tubules was less than in the primary dentin and the tubules had an irregular pattern. The amount of reparative dentin formed pulpally is probably well in correlation with the amount destroyed in the periphery and is probably also related to the intensity, nature and duration of the external irritant. The different types of reparative dentin are in accordance with earlier studies [3].

The condition of the pulp after dental trauma injuries has been evaluated in differ-
In clinical studies in permanent teeth it has been evaluated by electrical and thermal stimulation [7,8]. In contrast to clinical studies histological studies indicate that pulpal changes often occur [3]. An adequate indication of the vitality of pulp cells has been their ability to continue hard-tissue formation, a criterion widely used for functioning pulp tissue in post-traumatic roentgenologic evaluation [7,8]. However, relatively little experimental evidence is available concerning the incidence and reestablishment of the hard-tissue formation in the pulp after a general trauma. The findings in this study confirm that dentin matrix formation is a reliable determinant of pulpal cell function after a general trauma to the primary dentition. In no cases were inflammatory infiltrates observed and the pulps were in good health. However, in this study there were no teeth with severe injury as intrusive luxations. Andreasen et al. [9] suggest that a moderate injury gives the highest frequency of PCO and that a severe injury more often results in pulp necrosis. The importance of a vital pulp for the development of PCO is also in agreement with a higher frequency of PCO among luxated teeth with open apices than among those with closed apices [9].

In the denticles, interglobular dentin was frequently observed and in some teeth incremental lines with alternating high and low mineral content were seen. Indicating that tertiary dentin like other mineralized tissues is subject to biological rhythms during formation. This is in agreement with earlier studies [2,4]. However, the factors involved in the development of the denticles are largely unknown.

Diffuse calcifications were the most common calcification and it seems to be the simplest calcification. This is in accordance with earlier studies [4]. They occurred in all teeth and it seemed that this type of calcification occur more often than the formation of denticles.

Tooth wear occurs during normal mastication and attrition in the primary dentition. Pathologic wear, including abrasion and erosion may also take place. The reparative dentin formation is an important protective function of the dental pulp. It is also a feature of pulpal cell function after a general trauma to the primary dentition. It is also a feature of pulpal cell function after a general trauma to the primary dentition. It is also a feature of pulpal cell function after a general trauma to the primary dentition. It is also a feature of pulpal cell function after a general trauma to the primary dentition. It is also a feature of pulpal cell function after a general trauma to the primary dentition. It is also a feature of pulpal cell function after a general trauma to the primary dentition. It is also a feature of pulpal cell function after a general trauma to the primary dentition.

The chemical analyses of the reparative dentin showed a less well mineralized dentin which is in line with the differences in the morphological appearance. Even if the material analysed is limited in number, the values for the ratio Ca/C in reparative dentin in particular clearly point to a higher content of organic matter compared with normal dentin. One reason for the differences found could possibly be the activation of already-existing odontoblasts as well as the possible differentiation of new odontoblasts [10].

CONCLUSIONS

The present results show that reparative dentin is formed when pulp in primary teeth is exposed to subluxation and luxation. The varied morphology of the reparative dentin indicates that different stimuli lead to induction of hard tissue forming cells which produce different types of hard tissue. It looks like the reparative dentin formation is an important protective function of the dental pulp. A difference in the chemical composition of reparative and normal dentin was found for Ca, P and the ratio Ca/C with lower values in reparative dentin.

REFERENCES