

## Assessment of dentin reaction after Biodentine application

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### ABSTRACT

Recently it has been shown that very deep lesions, extending through the enamel into the dentin, can still be remineralized when brought into contact with a mineralizing agent. Biodentine is a calcium silicate based dentin substitute recommended to use in dentistry for direct and indirect pulp capping, perforations, apexification and retrograde root filling. The aim of the study was to examine the reaction between Biodentine and dentin comparing chemical composition of both of them in microspectral analysis. The human molars dentinal samples with Biodentine were analyzed by scanning electron microscopy (SEM) and electron probe microanalyser (EDS) with image observation function. Additionally Raman microscope was used to identify Biodentine/dentine interface. The results showed that Biodentine released some of its components into dentin specimens and caused the uptake of Ca and Si in the adjacent dentine. The formation of an interfacial layer at the Biodentine/dentin border was identified as a “Transition Zone”. On the basis of performed experiment it can be concluded that Biodentine is an active dental material and microanalytic methods used in the study are suitable to investigate it. The obtained outcomes can open up possibilities for a non-operative approach to deep caries cavities.

**Keywords:** dentin, Biodentine, microspectral analysis

### INTRODUCTION

Several new calcium silicate based materials have been recently developed [5]. Biodentine (Septodont, France) – “bioactive dentin substitute” is a one among them. This is a modern dentine replacement based on Portland cement. The effect of “bioactive dentin substitute” consists in originating mineralized microconnections between the material and dentin tubules. Bioactive nature of Biodentine is based on odontotropic features causing formation of tertiary dentin and dentin bridges. This is the reason why it is recommended in direct and indirect pulp capping, perforations, apexification, resorptions, pulp exposures and retrograde root filling. Its wide use and very good physical properties are the best assets [9,11,12,14].

The main core of the powder mixture are silicates: tricalcium silicate and di-calcium silicate. It also contains calcium carbonate and oxide as filler, iron oxide responsi-

ble for shade and zirconium oxide as radiopacifier. Adding calcium chloride to the liquid part accelerates the system and hydrosoluble polymer component reduces water in the mixture. The ability of calcium silicate to interact with water provide setting and hardening of the cement. This is a hydration of the tricalcium silicate, which produces a hydrated calcium silicate gel and calcium hydroxide. This dissolution process occurs on the surface of each grain of calcium silicate. The hydrated calcium silicate gel and the excess of calcium hydroxide tend to precipitate at the surface of the particles and in the pores of the powder, due to saturation of the medium. The precipitation process is reinforced in systems with low water content. The unreacted tricalcium silicate grains are surrounded by layers of calcium silicate hydrated gel, which are relatively impermeable to water, thereby slowing down the effects of further reactions. The C-S-H gel formation is due to the permanent hydration of the tricalcium silicate, which gradually fills in the spaces between the tricalcium silicate grains. The hardening process results from the formation of crystals that are deposited in a supersaturated solution [3,13].

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The natural example for Biodentine's developers was human hard tissue – dentin. By weight, approximately seventy percentage of dentin consists of the mineral hydroxylapatite, twenty percentage is organic material and ten percentage is water. It gives the dentin unique features like flexibility and resilience, which prevent mechanical traumas. Hydroxylapatite with a small amount of mineral salts underlie inorganic part of dentin. The organic material consists of 50% collagen fibrils and ground substances (mucopolysaccharides). Dentin is bone like matrix characterized by multiple closely packed dentinal tubules that traverse its entire thickness and contain the cytoplasmic extensions of odontoblasts that once formed the dentine and maintain it, peritubular dentin and intertubular dentin. The cell bodies of the odontoblasts are aligned along the inner aspect of dentine against a layer of predentin where they also form the peripheral boundary of the dental pulp [6].

The aim of the study was to present the reaction between Biodentine and dentin comparing their chemical composition and to check the created interface between them by microspectral analysis.

## MATERIAL AND METHODS

Human extracted molar teeth without caries (collected in written agreement with every patient) were included in the study. Inside the teeth class 1 cavities according to Black were prepared with high rotation handpiece and diamond bur under water coolant to the dentin level. Then the Biodentine material was prepared by pouring 5 drops of liquid into the capsule with a powder and mixing for 30 seconds in a triturator. Application was performed at once in all cavities without any conditioning treatment of dentin. Biodentine set in 12 minutes from the start of mixing. Later, every sample was cut perpendicular of 1.0 mm thick by using Diamond Cutter Micracut 175 (Metkon) with water cooling and dried. The samples were stored in a freezer at 4°C. Before analysis, the teeth samples were not powdered with any material.

Three methods were used in this research to assess chemical reactions, all belong to micro-analytic methods. SEM - Scanning Electron Microscope method uses a focused beam of high-energy electrons to generate a variety signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition and crystalline structure. Selected areas of the sample were magnified from 416x to 5.29kx. Energy Dispersive X-Ray Spectroscopy (EDS or EDX) is a chemical microanalysis technique used in conjunction with scanning electron microscopy (SEM). The EDS technique detects X-rays emitted from the sample during bombardment by an electron beam to characterize

the elemental composition of the analyzed volume. It can be used for the elemental analysis and chemical characterization of the sample. The last used method is Raman Spectroscopy, which is a spectroscopic technique based on inelastic scattering of monochromatic light, usually from a laser source. Inelastic scattering means that the frequency of photons in monochromatic light changes upon interaction with a sample. Photons of the laser light are absorbed by the sample and then reemitted. Frequency of the reemitted photons is shifted up or down in comparison with original monochromatic frequency, which is called the Raman effect. This shift provides information about vibrational, rotational and other low frequency transitions in molecules. Raman spectroscopy can be used to study solid, liquid and gaseous samples. It became a popular technique for biomedical applications such as identification or characterization of biological materials. This technique is very useful in analyzing chemical function groups [10, 15].

## RESULTS

The obtained results are presented on Figures 1–7. Performed examination revealed that early effects of Biodentine – dentin reaction indicated three zones of the examined interface: Biodentine, Biodentine Transition (BT) and dentin. The graph the background of which is composed by optical signal (Fig. 1), showing the level of phosphorus and silicon in the Biodentine Transition zone, contains more phosphorus than Biodentine and dentin. Level of silicon is lower than in Biodentine but slightly higher than in dentin.

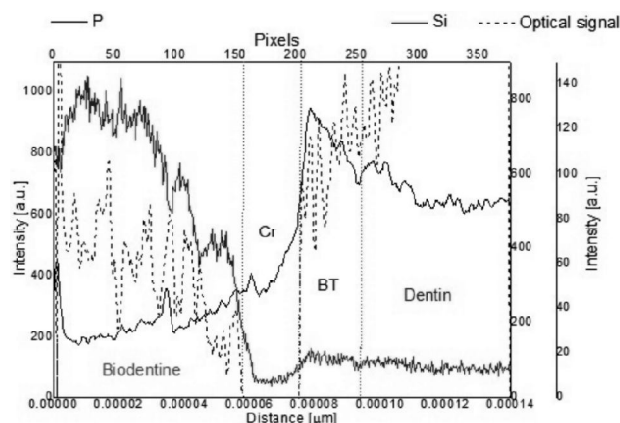


Fig. 1. Comparative evaluation of phosphate and silicon ions in Biodentine, Biodentine Transition (BT) zone and dentin

Spectra of Biodentine Transition zone (Fig. 2) indicated the presence of ions as C, Si, Mg, Cl, Ca, O, P on the border between Biodentine and dentin where the concentration of calcium, phosphorus and oxygen was the highest. The diffusion of ions took place there. SEM im-

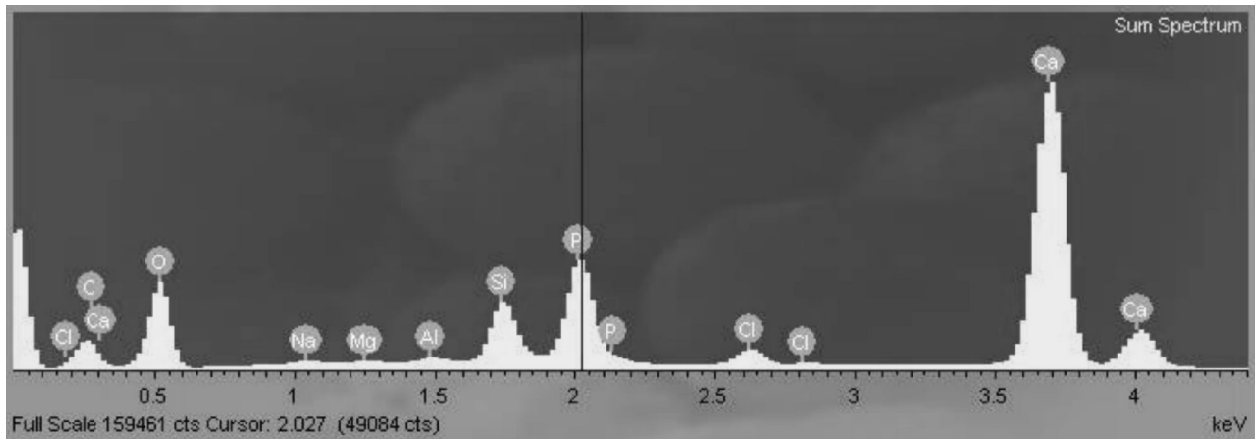


Fig. 2. The concentration of ions in Biodentine Transition zone presented on linear spectrum

age also revealed Biodentine Transition zone (Fig. 3). Linear spectrum of dentin and Biodentine Transition zone showed the differences between them in calcium and phosphorus ions. The growth of calcium and silicon ions presented on Fig. 4 confirmed existing of Biodentine Transition zone. The length of the BT zone (Fig. 5) was checked twice, firstly after one week and secondly after one month. The results were respectively: 18.44  $\mu\text{m}$  after one week and 56.43  $\mu\text{m}$  after one month. With the use of Raman spectroscopy method, spectra for functional groups characteristic for dentin, Biodentine and Biodentine Transition zone were performed. Linear spectra of silicate, phosphate and carbonate ions showed higher concentration in Biodentine Transition zone than in dentin (Fig. 6). Checking the layout and depth of penetration carbonate and phosphate ions was also affirmed in the next trial (Fig. 7).

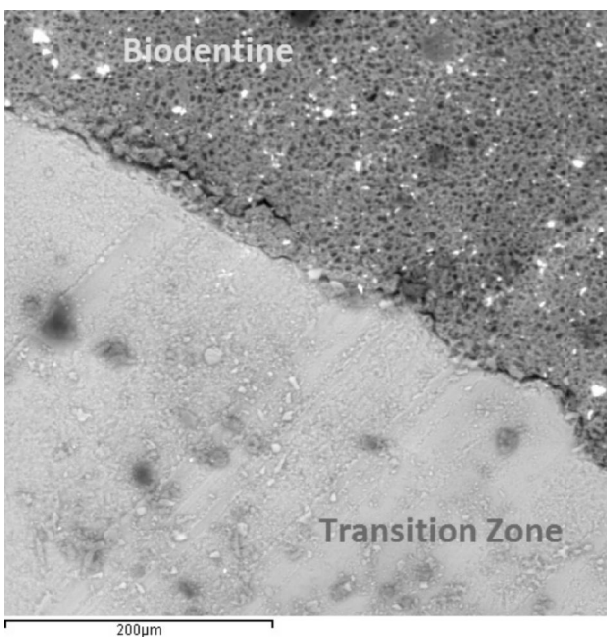


Fig. 3. SEM picture of the border between Biodentine and Biodentine Transition zone

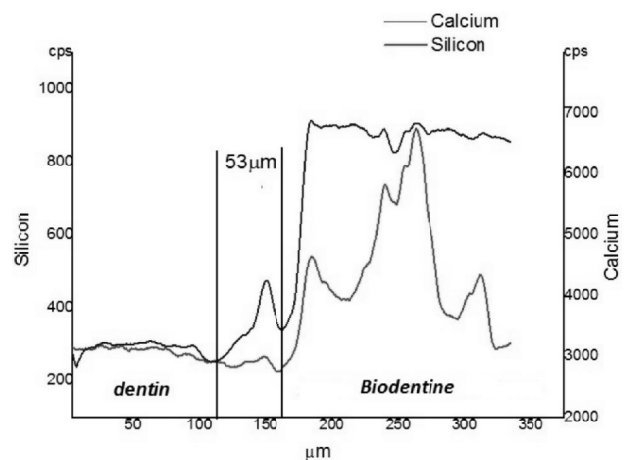


Fig. 4. Comparative level of calcium and silicon ions in dentin, Biodentine Transition zone and Biodentine

## DISCUSSION

Structure and composition of Biodentine were examined by many authors [1,2,4,5,7,8]. In all of them it was visible as no homologous material. After mixing of two components, the formation of crystals is deposited in supersaturated solution. The final effect is a crystalline structure with different parts visible as dark and light crystals - what was checked in previous study [11]. This study was performed to identify and assess the border between material and dentin. Like in Han and Okiji research [4], the presence of an "interfacial layer" (in this research named Biodentine Transition zone) was revealed. It was identified as "brighter area" under the back-scattered structure within dental tubules along the dentin-material border.

Studies on the ultrastructure of the Biodentine/dentine interface have demonstrated the formation of mineral-rich interfacial layer and a tag-like structure extending to the dental tubules [1–5,13]. Own SEM examination revealed high level of calcium, silicon and phosphorus ions in the

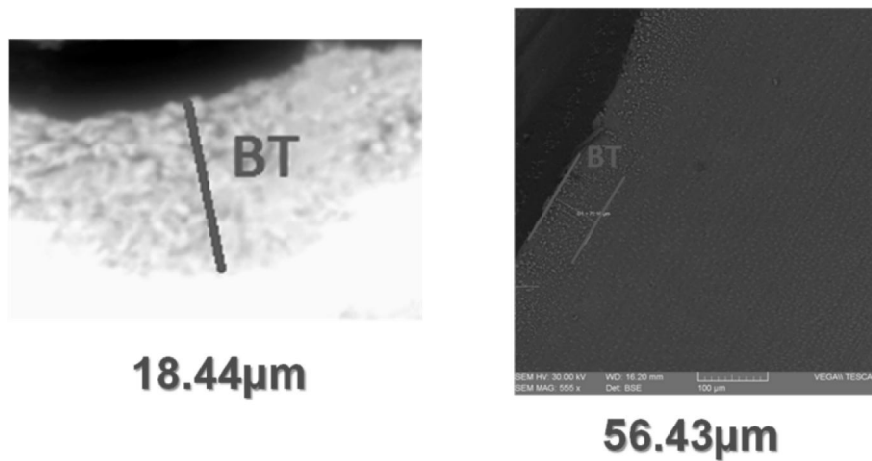


Fig. 5. Biodentine Transition zone

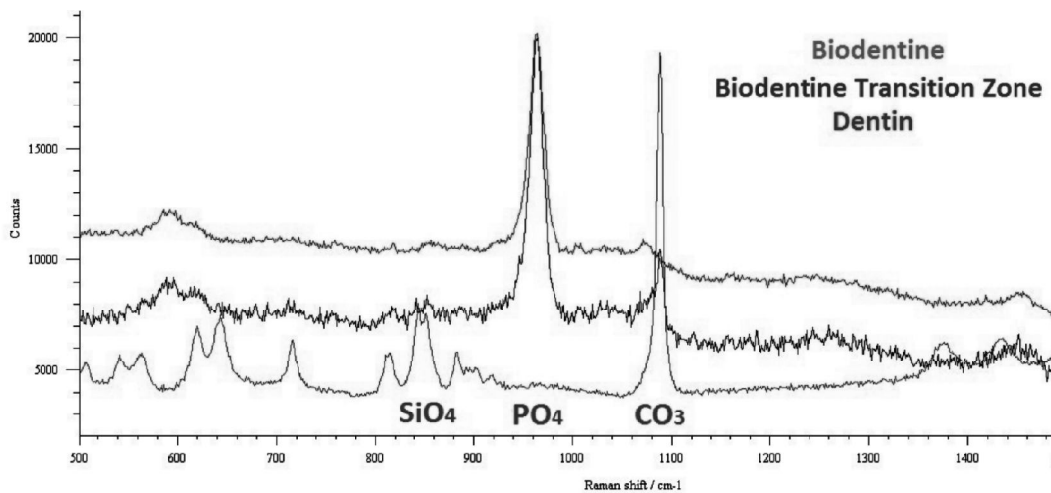


Fig. 6. Comparison of Raman spectra of dentin, Biodentine Transition zone and Biodentine

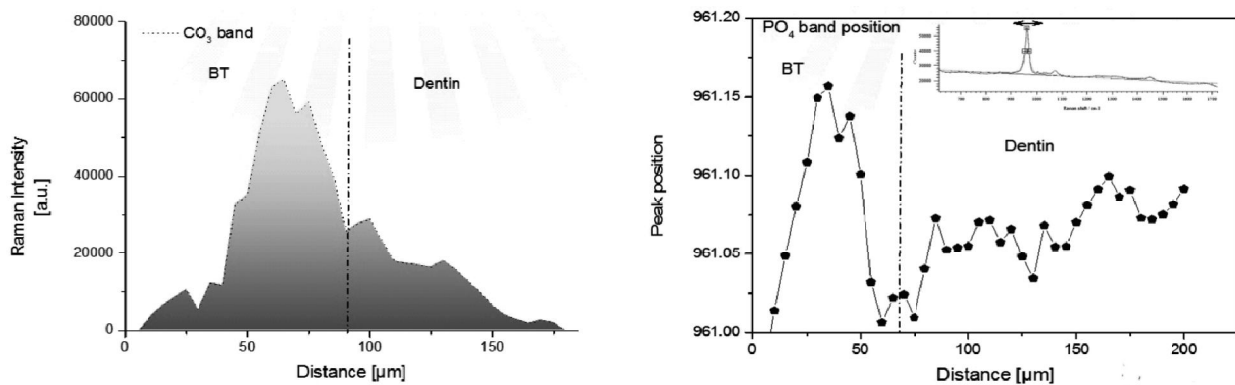


Fig. 7. Layout and depth of penetration  $\text{CO}_3^{2-}$  and  $\text{PO}_4^{3-}$  ions

Biodentine Transition zone. Existence of C, Mg, Cl ions was also confirmed. The comparison of Raman spectra of Biodentine, Biodentine Transition zone and dentin showed that the level of phosphate, silicate and carbonate ions is higher in Biodentine Transition zone than in dentin.

It was also noticed that the length of Biodentine Transition zone is increasing with time. This is in agreement with other researchers' results where the thickness of reactionary dentin after 8 days was from 20 to 40  $\mu\text{m}$  and

after one month from 150 to 180  $\mu\text{m}$  [13]. In rats model on biocompatibility and bioactivity of Biodentine the length of reactionary dentin zone after 15 days was 40 to 80  $\mu\text{m}$  and after one month around 160  $\mu\text{m}$  [1].

This study demonstrated the formation of Si-rich layer in dentine in contact with Biodentine. The Si-rich layer was constantly narrower than the Ca-rich layer, most likely because the amount of Si released from Biodentine is much smaller than Ca what was observed by others too

[2,5]. It can be suspected that obtained results would indicate biological response at the interface of the material and the bond between Biodentine and tissue. Biocompatibility and bioactivity of Biodentine led to use it in constructing scaffolds for the dentin. Tricalcium silicate ions remained in dentin tubules and catalyze chemical reaction. The present results demonstrated the uptake of Ca, P and Si ions in the dentine in contact with Biodentine. This may represent the mineralisation ability of this calcium-silicate material. Ca and Si uptake most probably causes chemical and structural modification of dentin, which may result in higher acid resistance and physical strength. These findings suggest that the release of Si from calcium silicate-based materials may confer additional in vivo bioactivity of these materials [4,5].

## CONCLUSIONS

The performed study demonstrated that Biodentine is an active calcium silicate-based dental material and microspectral methods used in the experiment are suitable techniques to investigate it and its reactions. The chemical process resulting in the formation of the interfacial layer at the Biodentine – dentin border (Biodentine Transition zone) can open up possibility for non-operative approach to deep caries cavities. Longer time of observation and other analytical techniques are necessary to observe bioactivity of Biodentine and compare it with other materials.

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