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DO THE LEVELS OF FLUORIDE AND VARIOUS ELEMENTS IN BIODENTINE™ INFLUENCE ITS BIOCOMPATIBILITY WITH TOOTH TISSUE?

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ABSTRACT: The aim of the study was to determine the mineral composition of Biodentine™, a new silicate-based material which exhibits odontotropic properties, and check the potential effect of fluoride (F) on the properties of the preparation. For this purpose Biodentine™ was prepared and set under aseptic conditions according to the manufacturer's instructions, and then the ground powder was added to a medium to prepare stock solutions of material. Concentration of fluoride was measured using potentiometric method and other elements using inductively coupled plasma optical emission spectrometry (ICP-OES). The composition and concentrations of the elements (especially F) in Biodentine™, due to their interactions and wide influence on the processes of repair and restoration of tooth tissue, may have a significant impact on the biocompatibility of this material. From a clinical point of view, this may be important in selecting the most effective treatment and determining its predicted prognosis and course.

Keywords: Biocompatibility; Biodentine™; Elements; Fluoride; Tooth tissue.

INTRODUCTION

Bioactive materials have been used for many years in many restorative and regenerative dentistry procedures and have led to considerable progress in endodontic treatment. Divided into bioinert, bioactive and bioresorbable ceramics,[1-4] these products include; Biodentine™, BioAggregate, Theracal LC, Calcium Enriched Matrix (CEM), Endo Sequence Root Repair Material (ERRM), iRoot BP Plus, MTA (Mineral Trioxide Aggregate), NeoMTA plus, MTA Repair HP.[1-3]

Currently, bioceramics are most commonly used for direct pulp capping in the cases of trauma, caries or other mechanical causes.[3,4] In addition, they can be used as root canal sealers, to cover perforations of various locations, internal and external resorption.[5,6] These materials are also used in revitalization, a procedure designed to induce, in contact with the biocompatible material, repair of a calcified barrier in a root with an open apex or uncompleted development. Apexification is also intended for the completion of the apical development process of an incomplete tooth root with a necrotic pulp.[7-9]

Recently, attention has been paid to the development of bioceramics that are capable of not just replacing lost tooth tissue, but also inducing and stimulating their repair and regeneration.[10,11] Some are increasingly being used in revascularization.[7,8,12] In addition, calcium silicate cements used in direct pulp coverage have shown a positive influence on the stages of inflammation.[13,14]

Biodentine™ (Septodont, Saint Maur des Fosses, France) is a new silicate-based material which exhibits odontotropic properties, i.e., it stimulates the formation of reactive and reparative dentine and contributes to the maintenance of vitality of the

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pulp.[1-4] *In vivo* studies have shown that Biodentine™ exhibits low cytotoxicity in osteoblast cultures.[15,16] In pulp, Biodentine™ induces cell proliferation and expression of dentine sialoprotein and osteopontin, and stimulates the formation and mineralization of the tissue barrier.[17,18] From the clinician's point of view, however, every tooth filling material, even with optimal chemical parameters, is a foreign body for the host organism, and so it is very important to know precisely the elemental composition of the preparation – the chemical properties of the elements and interactions between them may significantly affect the biocompatibility of the preparation.[19] The information provided by Biodentine™ manufacturers includes only basic information on the elemental composition, lacking any detailed data on the content of individual elements, especially fluorine, which, being a constituent of the hydroxyfluoroapatite building the tooth tissue, may play a key role in its regeneration.

Therefore, the aim of our study was to determine the mineral composition of Biodentine™ i.e. the concentrations of fluoride (F), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), zinc (Zn), strontium (Sr), manganese (Mn), copper (Cu), sodium (Na) and magnesium (Mg), and the potential effect of F on the properties of the preparation.

MATERIAL AND METHODS

Chemical Properties and Preparation of Biodentine: Biodentine™ (Septodont, Saint Maur des Fosses, France) is available as a powder in capsules with a liquid in ampules. The powder consists mainly of tricalcium and dicalcium silicates ($3CaO*SiO_2$ and $2CaO_8SiO_2$) – the main components of Portland cement, and calcium carbonate ($CaCO_3$). Zirconium dioxide (ZrO_2) is an additive to provide good visibility in X-ray images. The liquid is an aqueous solution of calcium chloride ($CaCl_2*2H_2O$) mixed with polycarboxylate.[1-3] When the powder and liquid are combined, calcium silicate hydrate (so called CSH gel) and calcium hydroxide are obtained. The catalyst for the reaction is calcium chloride while the tricalcium silicate is responsible for the binding reaction. Calcium carbonate is a filler which improves the mechanical properties of the material.[2-4] The setting time of Biodentine™ is approximately 12 minutes after mixing. The material has a low marginal deficiency and after mixing the powder and the liquid, the resulting preparation can be used to fill the entire cavity in the tooth.

Biodentine™ was prepared and set under aseptic conditions according to the manufacturers' instructions, and then ground into powder under cold conditions (–20°C) and sterilized by dry heat. The ground powder was added to a medium to prepare stock solutions (10 mg/mL) of material, and then vortexed. A final concentration of 2 mg/mL was used.[20]

ICP-OES analysis of Biodentine™: Samples (n=6) were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) using an ICAP 7400 Duo (Thermo Scientific) equipped with a concentric nebulizer and cyclonic spray chamber, to determine P, K, Ca, Fe, Zn, Sr, Mn, Cu, Na, Pb and Mg content. Analysis was performed in radial and axial modes.

The samples were first mineralized using a MARS 5 (CEM) microwave digestion system. The weight of the samples for analysis was at least 0.1 g. The samples were

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then transferred to clean polypropylene tubes. Four mL of 65% HNO_3 (Suprapur, Merck) was added to each vial and each sample was allowed 30 minutes pre-reaction time in the clean hood, after which 1mL of non-stabilized 30% H_2O_2 solution (Suprapur, Merck) was added to each vial. The samples were then placed in special Teflon vessels and heated in a microwave digestion system for 35 minutes at 180°C (15 min ramp up to 180°C and held at 180°C for 20 min). At the end of the digestion, all samples were removed from the microwave and allowed to cool to room temperature. In a clean hood, samples were transferred to acid-washed 15 mL polypropylene sample tubes. A further 80-fold and 40-fold dilution was performed prior to ICP-OES measurement. The samples were spiked with an internal standard to provide a final concentration of 0.5 mg/L Ytrium, 1 mL of 1% Triton (Triton X-100, Sigma) and diluted to the final volume of 10 mL with 0.075% nitric acid (Suprapur, Merck|). Samples were stored in a monitored refrigerator at a nominal temperature of 4°C until analysis. Blank samples were prepared by adding concentrated nitric acid to tubes without samples, and subsequently diluted in the same manner described above. Multielement calibration standards (ICP multi-element standard solution IV, Merck, Germany, Phosphorus Standard for ICP, Inorganic Ventures, US) were prepared with different concentrations of inorganic elements in the same manner as the samples and blanks. Samples of reference material (NIST SRM 1486 Bone Meal) (n=3) were prepared in the same manner as the samples. Deionized water (Direct Q UV, Millipore, approximately 18.0 MΩ) was used for the preparation of all solutions.

The wavelengths (nm) for analysis were P 178.284, K 766.490, Ca 315.887, Fe 239.562, Zn 213.856, Sr 421.552, Mn 257.610, Cu 224.700, Na 589.592, Pb 220.353, Mg 285.213.

Fluoride concentration analysis using an ion-selective electrode: To a powdered sample of Biodentine™ (about 10 mg), 1 mL of 2M perchloric acid was added to dissolve it. The sample was then shaken in a Thermomixer for 1 hr at 95ºC to accelerate the dissolution process. After cooling the solution, 0.5 mL of the prepared sample was taken and added to 2 mL of 1M sodium citrate (to complex Ca^{2+} ions) and 1 mL of TISAB II buffer (to lower the pH to 5.5). F content was determined potentiometrically with an ion-selective electrode.[21]

Statistical analysis: Statistical analysis of the obtained results was conducted with Statistica 10 software (Statsoft, Poland). The results were expressed as arithmetical means ± standard deviation (SD).

RESULTS

Table 1 shows the composition of Biodentine™ formulation as reported by the manufacturer and Table 2 shows the elemental content of the formulation as tested by ICP-OES.

Our tests show that, apart from the elements indicated by the manufacturer such as Ca, Si, and Zr, the preparation contained all the elements tested, with some in high concentration, e.g., Fe (421.68 mg/kg), Sr (143.53 mg/kg), and Mg (1109.28 mg/kg).

Table 3 shows the concentration of F in the sample prepared according to the manufacturer's procedure (total) and in its components - powder and activator. Both

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components of the preparation contained F in a high total concentration of 35.71 mg/kg. The table also shows the F concentration in the bone certification material.

Table 1. Composition of Biodentine™ as declared by the manufacturer

Tested material	Manufacturer	Composition as stated by the manufacturer
Biodentine	Septodont France	Powder: Tricalcium ($3CaO*SiO_2$) and dicalcium ($2CaO*SiO_2$) silicate, calcium carbonate ($CaCO_3$), zirconium oxide (ZrO_2) Liquid: 10% calcium chloride ($CaCl_2*2H_2O$), water, polycarboxylate

Table 2. Analysis of Biodentine™ and Bone Meal NIST-SRM 1486 using ICP-OES

Chemical element	Biodentine™ [mg/kg]	NIST-SRM 1486 Certified [mg/kg]	NIST-SRM 1486 Measured [mg/kg]	Recovery (%)
P	55.37 (±1.20)	123000.00	145775.00	118%
K	60.88 (±5.70)	412.00	478.00	116%
Ca	316459.14 (±809.70)	265800.00	251348.00	95%
Fe	421.68 (±1.80)	99.00	105.30	106%
Zn	13.24 (±0.20)	147.00	145.12	98%
Sr	143.53 (±2.60)	264.00	256.14	97%
Mn	5.89 (±0.10)	1.00	0.95	95%
Cu	3.78 (±0.90)	0.80	0.97	121%
At	316.56 (±60.60)	5000.00	4791.65	96%
Mg	1109.28 (±2.50)	4600.00	4257.56	93%

Table 3. Analysis of Biodentine™ and reference material Bone Meal NIST-SRM 1486 using ion-selective electrode

Chemical element	Biodentine™ [mg/kg]	Certified [mg/kg]	Measured [mg/kg]	Recovery (%)
F total	35.710 (±2.05)	45(±1.03)	43(±1.02)	102
F powder	35.565 (±1.95)			
F activator	0.145 (±0.01)			

DISCUSSION

Bioceramics based on tricalcium silicates such as Biodentine™ have a wide range of applications in regenerative endodontics including the treatment of deep caries, covering the pulp after exposure or injury, and in endodontic surgery (treatment of perforations of various locations, retrograde filling and treatment of resorptive lesions).[1-6] Due to many properties that are similar to biological hydroxyapatite, these materials are characterized by very good biocompatibility. During the hydration process, bioceramics form many compounds (e.g. hydroxyapatite) which stimulate regenerative and repairing reactions in the pulp-dentine complex. When in direct contact with bone tissue, the mineral hydroxyapatite exhibits osteoconductive properties, resulting in the production of new bone at the interface. Additionally, bioceramics exhibit intrinsic osteoinductive capacity due to their documented ability to absorb osteoinductive substances at the site of bone healing and repair.[1-4] Furthermore, these materials have antimicrobial properties due to *in situ* precipitation after setting, forming a porous powder containing nanocrystals 1–3 nm in diameter, which inhibit bacterial adhesion.[1,22]

The regeneration of tooth tissue (dentine and root cement) resulting from the use of Biodentine™ may be associated with the presence of F in the preparation. Fluorine is an element whose effect on reducing the incidence of caries has been repeatedly demonstrated in experimental and clinical studies.[23-26] In the microenvironment of the oral cavity, F has a direct reduction on the development of caries by reducing the growth rate and inhibiting the metabolism of bacterial enzymes, inhibiting demineralization and enhancing remineralization (at an appropriate F concentration in saliva above 0.03 ppm).[26-28] F is a component of the apatite crystal, and fluorohydroxyapatite formed during the exchange process showing antimicrobial properties.[1,22,26,27]

Teeth are characterized by a high content of minerals (60% – 80% of dry matter), with the greatest amount of calcium phosphate in the form of hydroxyapatite crystals.[29,30] A unique property of hydroxyapatite is its ability to exchange ions with the environment, where the hydroxyl ion is particularly easily exchanged for F. This form of hydroxyfluoroapatite shows greater resistance to the acids produced in the plaque which constantly surrounds the tooth. F present in hydroxyfluoroapatite reduces the susceptibility of tissues to demineralization and increases the critical pH of demineralization, making the enamel less susceptible to demineralization. Hydroxyapatite has a critical pH of 5.5 and fluoroapatite formed in the presence of F has a critical pH of 4.5. However, in order for fluorohydroxypatite to form, F must be present in the immediate vicinity of the reacting molecules and microelements.[26,27]

The process of F distribution to the enamel is related to the concentration in the environment immediately surrounding the tooth.[27,31] Systematic contact of tooth tissues (hydroxyapatites) with low F concentrations (below 100 ppm) results in the formation of a stable F reserve in the form of hydroxyfluoroapatites. Higher F concentrations (above 100 ppm) would result in the precipitation of insoluble calcium fluoride (Ca_2F) on the enamel surface.[25-27] When the pH of the tooth environment decreases, calcium fluoride accelerates the release of F, which becomes incorporated into the hydroxyapatite crystal lattice. Low ambient F concentrations

(below 50 ppm) and near neutral pH slow down or even stop the process of F uptake by the apatite crystals in the enamel.[25-27]

F is also involved in the process of amelogenesis (during enamel development), (i) acting as a catalyst for the formation of the mineral phase of the enamel, i.e. hydroxyapatite; (ii) replacing hydroxyl ions, to cause the formation of fluoroapatite/ hydroxyapatite; (iii) favoring the correct formation of the enamel crystalline network with optimal physicochemical stability; and (iv) initiating the formation of larger apatite crystals with a lower carbonate content that participate in the removal of water and organic substances from the newly deposited enamel (in the pre-eruptive maturation of the enamel) which makes the enamel less soluble in acids after tooth eruption, favoring the formation of desirable morphological changes in the crowns of teeth.[26,27,32]

A beneficial effect of F has also been observed after tooth eruption (post-eruptive maturation of enamel), which influences (i) the dynamics of re- and demineralization processes; (ii) reduction in bacterial plaque accumulation by interfering with the initial adhesion of bacteria to the acquired membrane through modification of the enamel surface energy; (iii) inhibition of carbohydrate metabolism of bacterial plaque by inhibiting the activity of enolase; and (iv) inhibition of glucose transport into bacterial cells, which leads to a decrease in their production of lactic acid.[25-27]

According to the manufacturer, mixing of the two Biodentine™ components is immediately followed by a reaction of the calcium silicate particles with water to form a high pH solution containing Ca^{2+}, OH^- and silicate ions. Hydration of tricalcium silicate leads to the formation of hydrated calcium silicate (gel) on cement particles and calcium hydroxide nucleates. Subsequently, the CSH gel polymerizes producing a stabilized network, and the alkalinity (pH) of the surrounding medium increases due to the release of calcium hydroxide ions. The CSH gel sets next to the unreacted tricalcium silicate components and, due to its relative impermeability to water, helps to slow down the effects of further reactions.[33] Biodentine™ causes the deposition of an amorphous interfacial calcium phosphate layer with the root dentine. The addition of bioactive glass to Biodentine™ leads to faster apatite formation. Approximately 2 hr after setting, the flexural strength is 34 MPa, with a final hardness of 69 on the Vickers Microhardness Scale after 30 days. These physicochemical parameters are of critical clinical importance for the use of Biodentine™ bioactive dentine substitute.[34,35] It has been shown that when the bioactive glass contains F, a release of fluoroapatite and F is observed.[1,2,22,36]

In this study of Biodentine™, we demonstrated the presence of very high concentrations of Mg, which may also be crucial for its properties, as F has been shown to bind magnesium (forming MgF_2) and thus reduce free Mg ions. F is involved in the formation of hydroxyapatite crystals or participates in exchange reactions with other ions on the surface of the already formed crystal. Mg, Na and carbonate ions are present on the outer surface of the crystal. Their concentrations depend on the properties of the crystal, its degree of mineralization, and the pH. Hydroxyl ions can also be replaced by F, affecting the size and hardness of the hydroxyapatite crystals. In addition, F can react with calcium, resulting in the precipitation of calcium fluoride.[25-27,32,37]

The constant development in dental materials increases the possibilities of treating the effects of dental caries and pulp diseases. There is a wide spectrum of procedures, from the borderline of deep caries treatment and minimally invasive endodontics. Pulp covering procedures (indirect and direct), partial and coronal pulpotomy, pulpectomy, and revascularization (revitalization) are the most commonly performed procedures in regenerative dentistry.

However, without advances in material science, it would not be possible to apply these procedures on the borderline between conservative dentistry and endodontics. It should be remembered that any material applied directly to the pulp should be biocompatible immediately after application and in the long term. Biomaterials have increasingly better biochemical properties that are highly desirable features in the production of dentine substitutes. In spite of the continuous development of new materials and methods, the use of biomaterials is associated with some side effects. One such is the immune response, which may lead to exacerbation of the inflammation and subsequently to treatment failure.

Our previous results used a cell model of human monocytes/macrophages and direct contact bio-material (reflecting the clinical conditions of pulp and periodontium regeneration treatment with Biodentine™) to confirm that Biodentine™ correctly influences the induction of an immune response and modulates the local inflammation without affecting the expression of key enzymes for these processes i.e. cyclooxygenases and metalloproteinases.[13,14] This confirmed the biocompatibility of this material with dental tissue. However, from a clinician's perspective, any material used to fill a tooth – even one exhibiting optimal chemical parameters – is a foreign body introduced into the host (cavity). Therefore, in addition to the chemical parameters, a proven and confirmed biocompatibility of such materials with the pulp-dentine complex is equally important. The continuous development of dental engineering and the creation of new materials both impose an urgent need to monitor and affirm their clinical properties. The clinical application of these materials should only be implemented after a scrupulous examination of their elemental composition, including F content. Detailed studies are necessary to assess the usefulness of these materials in practical clinical use, especially in the treatment of pulp and periapical tissue pathology. The aspect of long-term follow-up is also important here. A key aspect here seems to be the elemental composition of the biomaterials involved in tooth restoration and, moreover, triggering a cascade of biochemical reactions that occur when materials come into direct contact with immune system cells and tooth tissue.

CONCLUSIONS

The composition and concentrations of the elements (especially fluorine) in Biodentine™, due to their interactions and wide influence on the processes of repair and restoration of tooth tissue, may have a significant impact on the biocompatibility of this material. From a clinical point of view, it is necessary for the manufacturer of the preparation to provide a detailed mineral composition. This may be important in selecting the most effective treatment and determining its predicted prognosis and course.

COMPLIANCE WITH ETHICAL STANDARDS

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The authors declare that they have no conflict of interest.

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