UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA", BUCURESTI
DOCTORAL SCHOOL
THE FIELD OF DENTISTRY

ABSTRACT OF DOCTORAL THESIS

IN VITRO AND *IN VIVO* EVALUATION OF PULP CAPPING MATERIALS

PhD supervisor:

PROF. UNIV. DR. DIDILESCU ANDREEA

PhD student:

ANDREI MIHAI

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CHAPTER 3 - GENERAL OBJECTIVE, SPECIFIC OBJECTIVES AND WORKING HYPOTHESIS

General objective

The overall objective was to compare *in vitro* and *in vivo* two tricalcium silicate-based pulp capping biomaterials with different mechanisms of the setting reaction.

Specific objectives

1. Highlighting the nature of the component phases of materials by complex thermal analysis and identifying the mineral phases of materials by X-ray diffraction
2. Characterization of material surfaces by scanning electron microscopy coupled with X-ray energy dispersive microprobe, topography by atomic force microscopy measurements and determination of hydrophobic-hydrophilic balance by contact angle measurements.
3. Measurement of material strength by determining Vickers microhardness.
4. *In vitro* characterization of biomineralization of materials by scanning electron microscopy coupled with energy dispersive X-ray microprobe and Fourier-transform infrared spectroscopy
5. *In vivo* evaluation of the two materials after performing the clinical direct pulp capping procedure directly on the animal model by histological interpretation of the results obtained.

3.3. Working hypothesis

We started from the hypothesis that there are differences in the effect of the two materials tested on the physiology of the pulpo-dentinal complex, one of the materials is currently considered the gold standard and the second material is a much newer, hybrid material modified to improve the physicochemical and biological properties.

CHAPTER 4. IN VITRO CHARACTERISATION OF TWO CALCIUM SILICATE-BASED MATERIALS

Introduction

MTA cement is a biomaterial used in the endodontic space, having been introduced into dental practice in the early 1990s, and is used in clinical vital pulp therapy procedures, in regenerative endodontic manoeuvres as an apical barrier in teeth with necrotic pulps and open apices, for repairing root perforations or pulp chamber floor perforations, as a root filling material. MTA cements can now be seen as the new gold standard in vital therapy manoeuvres such as direct or indirect pulp capping.

In recent years, attempts have been made to develop new, modified or hybrid MTA-derived cements, to compensate for certain disadvantages and to improve physico-chemical, antibacterial and biological properties. One such material is TheraCal LC cement, a tricalcium silicate-based material modified with light-cured resin. TheraCal LC is used clinically as a base filling or liner material as well as a pulp capping agent, acting as an isolator or barrier with a protective role for the dental pulp.

Both materials are bioactive and capable of inducing remineralisation through their ability to form apatite using calcium silicates and/or calcium aluminates. This results in the formation of dentin bridges. Although TheraCal LC has shown some favourable results compared to MTA, this material has been under-investigated.

The results of this study offer new insights into the field of dental materials based on calcium silicates. The specific aims were to evaluate and compare *in vitro*: (i) chemical composition, (ii) surface and microstructural characteristics and (iii) strength testing of two commercial dental pulp capping materials with different mechanisms of the setting reaction: TheraCal LC and BIO MTA+.

Materials and methods

Material

The two materials included in the study were:

- Theracal LC (*Bisco*, Schaumburg, IL, USA)
- BIO MTA+ (*Cerkamed*, Stalowa, Poland) Methods

The materials were analyzed (i) mineralogically by X-ray diffraction (XRD), complex thermal analysis (DTA-TG), (ii) microstructurally and surface properties by scanning electron microscopy (SEM) coupled with energy dispersive X-ray (EDX), atomic force microscopy (AFM), contact angle (CA) measurements and determination of mechanical microhardness by Vickers microhardness tests.

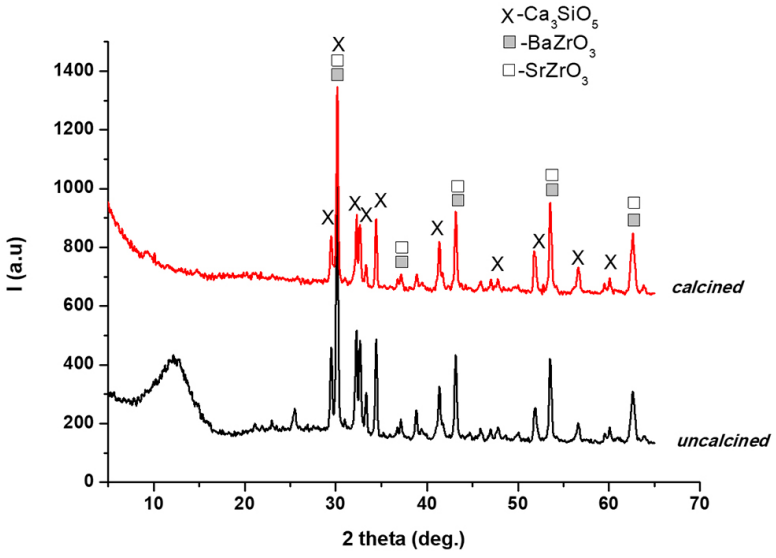
Results

4.3.1. Complex thermal analysis

Complex thermal analysis was performed on the reinforced TheraCal LC to highlight the nature of the material's component phases. Three exothermic mass-loss phases can be seen on the DTA diagram, with a stronger effect at 426ºC. The mass loss can be attributed to thermal decomposition and combustion of the organic component of the TheraCal LC sample.

4.3.2. X-ray diffraction

XRD plots for TheraCal LC suggested that the mineral phase was mainly formed of tricalcium silicate ($Ca_3\ SiO_5$, PCDFWIN [042-0551]) and two Ba- and Sr-rich phases ($BaZrO_3$ -PCDFWIN [006-0399] and $SrZrO_3$ -PCDFWIN [023-0651]). Haloes in the low-angle range (5º-20º) for the uncalcined sample is due to the presence of the organic phase.

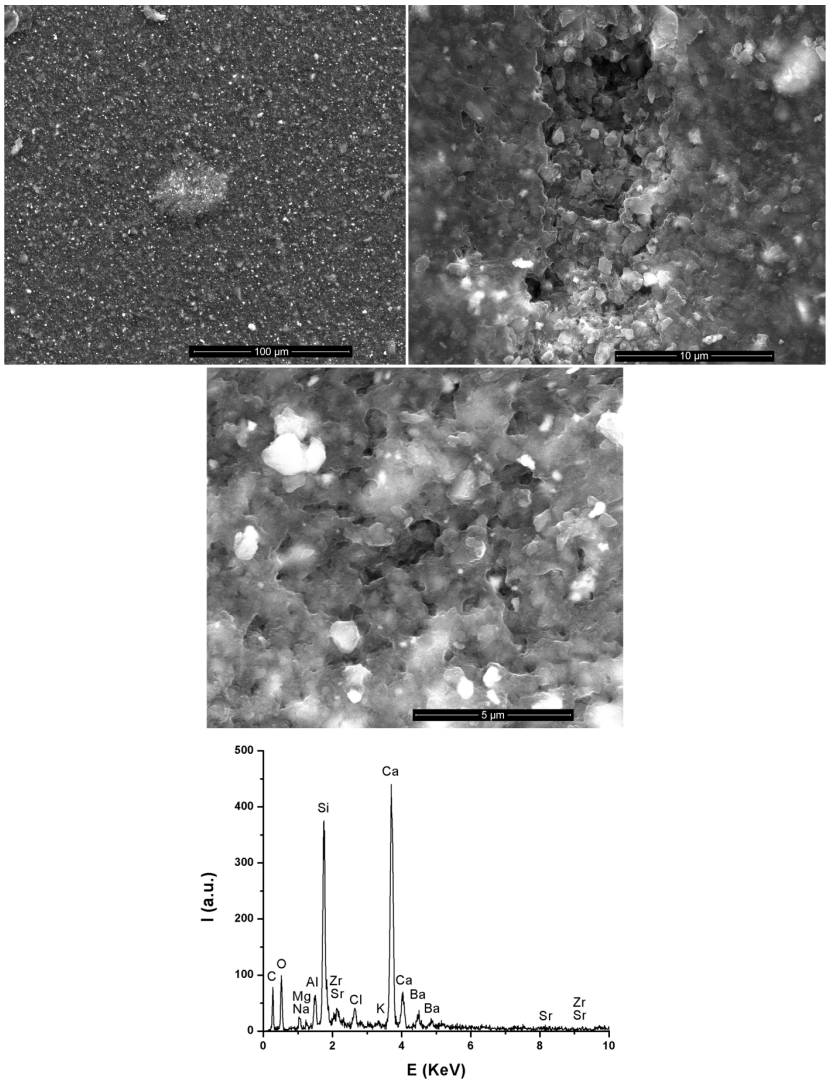


XRD diagram for hardened, calcined and uncalcined TheraCal

The XRD diagram of BIO MTA+ anhydrous powder showed the main mineralogical phases: tricalcium silicate ($Ca_3\ SiO_5$, PCDFWIN [042-0551]), dicalcium silicate ($Ca_2\ SiO_4$, PCDFWIN [036-0642]) and bismuth oxide ($Bi_2\ O_3$ - PCDFWIN [071-0466]).

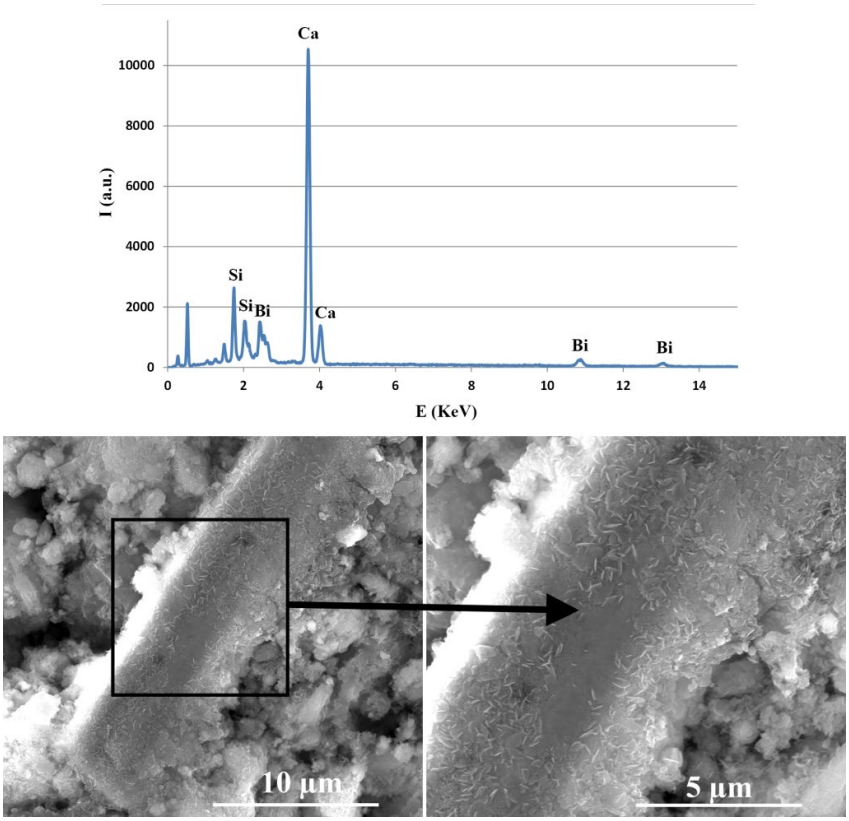
4.3.3. Scanning electron microscopy coupled with EDX microprobe

Scanning electron microscopy was initially performed on both samples of hardened material. The TheraCal LC hardened sample showed mineral particles uniformly distributed in the organic matrix, characterized by the presence of micro-porosity. The EDX spectra correlate with the XRD analysis results, which show the presence of the main characteristic elements of the mineral phases.



SEM imaging at different magnifications and EDX spectra for TheraCal LC surfaces at 3 days after the setting reaction

The EDX spectrum is in good agreement with the results of the XRD analysis. The presence of the P element was most likely due to a phosphate phase that was below the detection limit of the diffractometer or had a low degree of crystallinity. The presence of $Bi_2 O_3$, in the form of rods, and of the main hydration phases - calcium silicate hydrate (CSH) in the form of needles/folded sheets and calcium hydroxide (CH) in the form of hexagonal plates - was also visible.



SEM images for BIO MTA+ hardened at 3 days, 37ºC, R.H.=100%, with detail on particles Bi_2O_3 and characteristic EDX spectrum

4.3.4. Atomic force microscopy

The surface topography of both samples was determined by AFM measurements. For the TheraCal LC sample, AFM measurements showed a relatively smooth surface with an average roughness (Ra) of 29.9 nm and a root mean square roughness (Rms) of 38.3 nm. As for BIO MTA+, the sample had a rough surface with an average roughness (Ra) of 0.173 µm and a root mean square roughness (Rms) of 0.215 µm .

Average surface roughness of TheraCal LC and MTA, obtained from AFM measurements

Biomaterial	**Medium roughness**	**Roughness root mean square**
TheraCal LC	0.029 µm	0.038 µm
BIO MTA+	0.173 µm	0.215 µm

4.3.5. Determination of contact angle

For the hardened TheraCal LC and BIO MTA+ samples the contact angle was determined. The surfaces of the two materials show a hydrophilic character, with similar values for the two samples. The TheraCal LC sample showed a contact angle value of 62.83°, indicating a slightly more hydrophilic character than the BIO MTA+ sample, which recorded a contact angle value of 66.64° .

Contact angle measurements for TheraCal LC and BIO MTA+ biomaterials

Biomaterial	Contact angle	Standard deviation
TheraCal LC	62,83°	0,81
BIO MTA+	66,64°	4,15

4.3.6. Determination of Vickers microhardness

The surface microhardness of the two materials was determined by Vickers microhardness measurements. A microhardness of 16 HV was recorded for TheraCal LC and 64 HV for BIO MTA+.

4.4 Discussion

Surface characterisation is an important step in materials science because of the changes that can occur during processing and testing.

X-ray diffraction is an important technique for characterising crystalline materials. X-ray diffraction, used for composition and crystalline phase characterisation, performed on TheraCal LC samples, showed the presence of the following mineral phases: Ca_3SiO_5 , $BaZrO_3$ and $SrZrO_5$. In addition, SEM images showed a uniform distribution of these phases in the polymer matrix and EDX spectra demonstrated the presence of barium and strontium as radio-opacifying elements.

The diffractogram performed on the anhydrous powder of BIO MTA+ showed the presence of calcium silicates with the following main phases - $Ca_3 SiO_5$, $Ca_2 SiO_4$ and $Bi_2 O_3$, the results being in accordance with other studies. These aspects are in accordance with the main solid phases,

identified by XRD analysis of Portland cement (cement with similar composition of MTA: tricalcium silicate (alite / $Ca_3\ SiO_5$) and dicalcium silicate (belite / $Ca_2\ SiO_4$), together with the main oxide components: CaO, SiO_2 , $Al_2\ O_3$ and $Fe_2\ O_3$, in a study carried out by Scrivener, K.L. et al.

The addition of the radiopaque element Bi_2O_3 is necessary, radiopacity being one of the basic requirements of a dental material, and the resulting hydrocompounds from the setting reaction have been deposited on the surface of the material.

AFM measurement results showed that the TheraCal LC sample had a smoother surface than the BIO MTA+. The smaller mineral crystals were slightly dispersed in the resin matrix of TheraCal LC, making the surface more homogeneous.

The contact angle or surface energy is an important property of materials that determines whether a surface is hydrophilic or hydrophobic. Both BIO MTA+ and TheraCal LC materials exhibited hydrophilicity without significant difference.

In terms of the microhardness of the two surfaces, it was observed that the microhardness of the TheraCal LC material is lower than that of the MTA cement. This can be explained by the presence of the resin component in the composition of TheraCal LC material, which influences its elasric modulus.

4.5. Conclusions

1. Both materials have calcium silicate as the main mineralogical phase, except that MTA cement contains more mineral phases and TheraCal LC contains light-cured resin in its composition.
2. Bismuth oxide, identified in the BIO MTA+ composition, can be a crystallisation substrate for the newly formed mineral phases, confirmed by the presence of CSH compounds in it.
3. Both materials exhibit rough surfaces, with MTA reaching a higher value than TheraCal LC, and both biomaterials exhibit hydrophilicity of the surfaces.
4. In terms of surface microhardness, BIO MTA+ is superior to TheraCal LC, recording a value similar to the microhardness of dentin, which indicates it in sandwich crown restoration techniques.

CHAPTER 5. *IN VITRO* BIOMINERALIZATION OF TWO CALCIUM SILICATE-BASED PULP CAPPING MATERIALS

5.1. Introduction

Hydroxyapatite is the main calcium phosphate mineral in the hard tissues of the body, such as bones or teeth. Hydroxyapatite is a compound of inorganic nature that is found in the highest amount in the composition of tooth enamel and is mainly composed of calcium and phosphate ions. For example, in the case of enamel, demineralisation involves the loss of calcium and phosphate ions from the enamel structure, while remineralisation involves the accumulation of calcium and phosphate, from over-saturated oral fluids, in the enamel.

Calcium silicate-based materials and their derivatives have the potential to form crystalline structures in the dentin layer. *In vitro* and clinical studies have shown an increase in mineral deposition in dentin when calcium silicate cements were applied to artificially created carious lesions *in vitro* and carious lesions *in vivo*, being associated with intratubular and superficial mineral deposition.

MTA consists mainly of calcium oxide in the form of tricalcium silicate, dicalcium silicate and tricalcium aluminate with bismuth oxide added for radiopacity and is considered a silicate cement, rather than a mixture of oxides, capable of inducing crystallization of the dentin substrate. TheraCal LC, a tricalcium silicate-based material containing a light-cured resin matrix, is indicated for pulp capping and in turn has a stimulatory potential for hydroxyapatite formation and induction of tertiary dentin deposition.

The aim of the study was to compare, in terms of bioactivity, surface and microstructure characteristics, two biomaterials that perform the same clinical function (dentin barrier formation) but are binder systems with different setting reaction mechanisms: TheraCal LC - a light-cured tricalcium silicate and BIO MTA+, an mineral trioxide aggregate cement. Both materials exhibit bioactive properties that interact with the aqueous environment and form apatite phases, which contribute to surface mineralisation.

5.2. Materials and methods

5.2.1. Materials

The in vitro bioactivity of both materials was evaluated on cured samples of TheraCal LC, by photo-polymerization, and samples of BIO MTA+, cured for 3 days at 37ºC, R.H. For this, the hardened samples were immersed in a simulated body fluid solution (SBF).

Composition of SBF solution

Reactant	Quantity
NaCl	7.996 g/L
$NaHCO_3$	0.350 g/L
KCl	0.224 g/L
K_2 HPO $_4$· $3H_2$ O	0.228 g/L
$MgCl_2 \cdot 6H_2$ O	0.305 g/L
1 Kmol / m^3 HCl (87.28 ml of 35.4% HCl)	40 cm^3 / 1000 mL
$CaCl_2$	0.278 g/L
At_2 SO_4	0.071 g/L
$(CH_2$ OH_3)CNH_2 (tris)	0.057 g/L
1 Kmol / m^3 HCl for pH adjustment to 7.25	

5.2.2. Methods

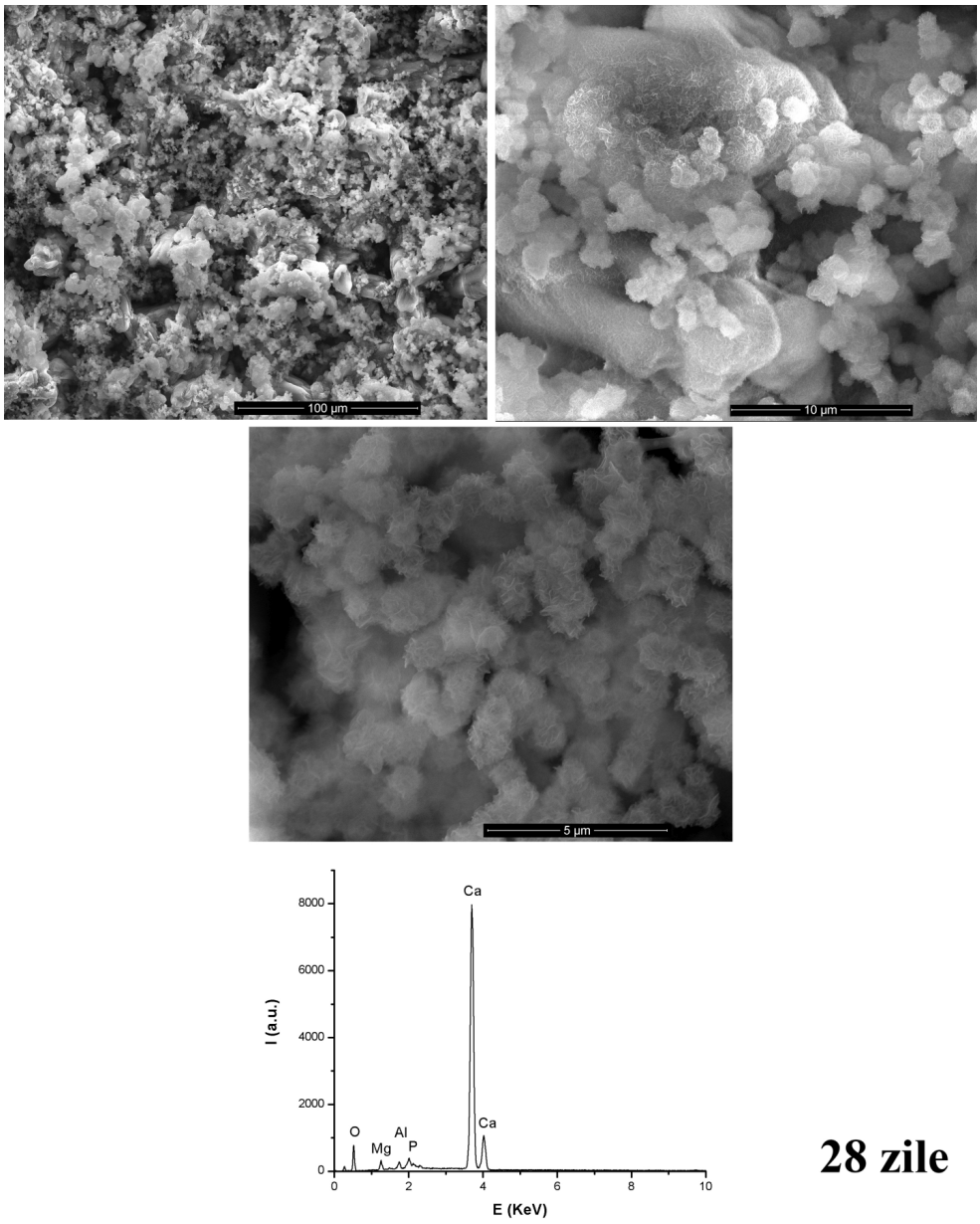
In vitro bioactivity was qualitatively assessed by SEM coupled with EDX and FT-IR, showing surface mineralization.

5.3. Results

5.3.1. SEM microscopy coupled with EDX microprobe

It is observed that by immersion in SBF solution at 7, 14 and 28 days, the surface of the materials was mineralized due to the formation of apatitic phases in the form of plates that formed spheres. The formation of these apatitic phases was supported by EDX spectra, where a significant intensity of the element P was observed.

For the TheraCal LC sample, Si was observed together with Ca, suggesting the formation of hydrosilicates in the presence of SBF. For the BIO MTA+ sample immersed in SBF solution, the Si intensity decreased with the time of immersion in SBF, suggesting the deposition of apatite phases on the CSH surfaces.

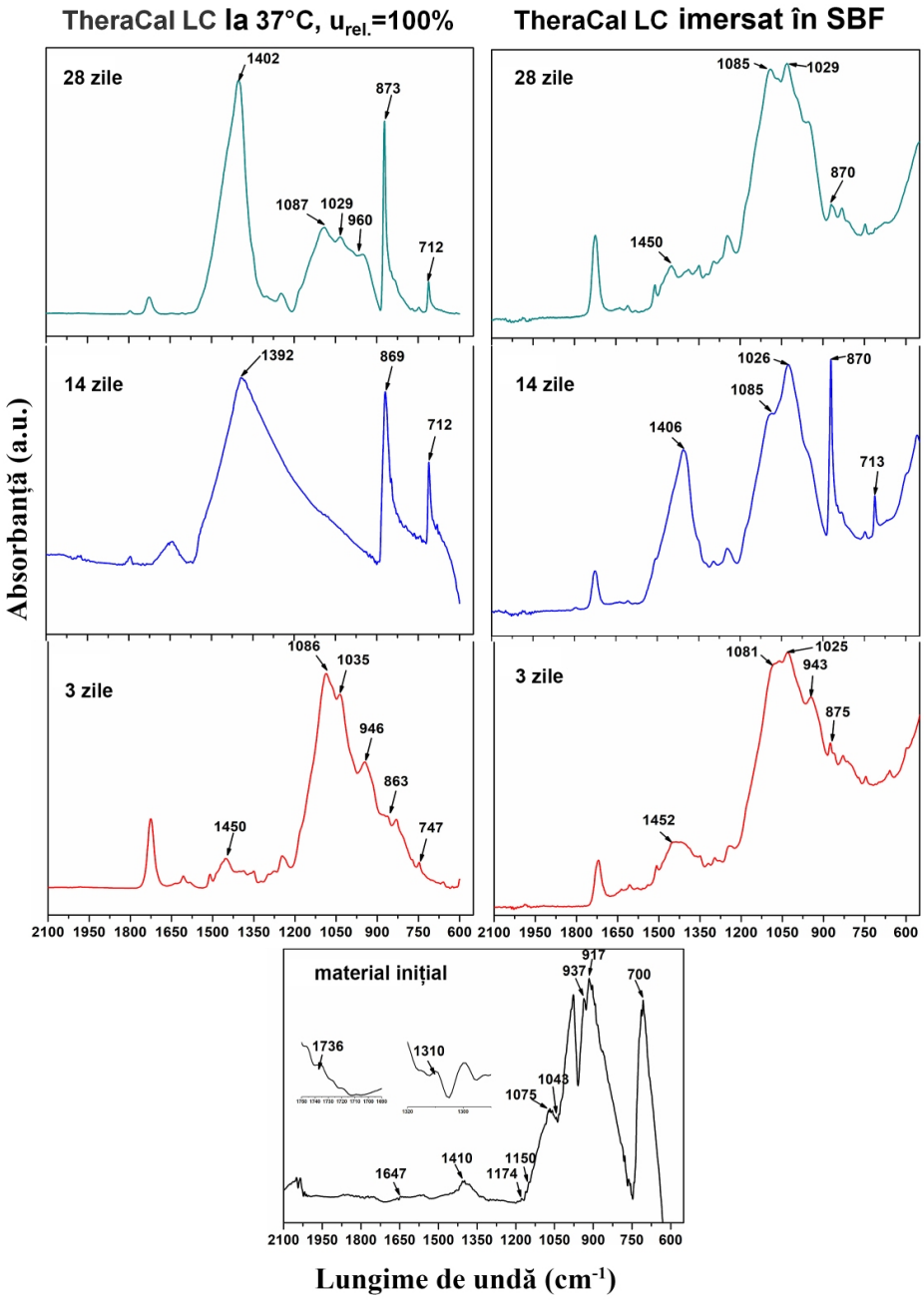


SEM micrographs at different magnifications and elemental surface analysis of BIO MTA+ at 28 days showing mineralization

5.3.2. Fourier-transform infrared spectroscopy

The initial FT-IR spectrum for TheraCal LC showed the presence of CO_3^{2-} bands at 700 cm^{-1} and a band at 917 cm^{-1} , attributed to SiO_4^{4-} , indicating the alite phase. A characteristic bending band for alite (Ca_3SiO_5) was visible at 937 cm^{-1} . Bands for PO_4^{3-} clusters were observed at 1043 and 1075 cm^{-1} and a band for asymmetric CO_3^{2-} bending at 1410 cm^{-1} . These bands were attributed to apatite and calcite, which could not be identified by XRD analysis.

The FT-IR spectra for BIO MTA+ powder showed differences when compared to those originally obtained for TheraCal LC. A band for SO_4^{2-} from anhydrite (anhydrous calcium sulphate) or gypsum (calcium sulphate) was identified at 620 cm^{-1} . The band at 917 cm^{-1} was assigned to SiO_4^{4-} from alite (Ca_3SiO_5); bands at 878 - 873 cm^{-1} indicated the presence of SiO_4^{4-} from alite. A band at 960 cm^{-1} was assigned to CSH following hydration.



FT-IR spectra for TheraCal LC during the uptake reaction and after immersion in SBF

5.4. Discussion

The ability to form apatite-like structures is due to the potential to release hydroxyl ions (OH^-) and calcium ions (Ca^{2+}) into the ambient environment when the surface of the material comes into contact with physiological fluids containing phosphate. Precipitates are formed by the dissolution of calcium hydroxide that is formed during the initial hydration reaction, causing the pH and Ca^{2+} ion concentration to increase and resulting in the over-saturation of the phosphate-containing fluid relative to the calcium phosphate and therefore favouring precipitation.

Bioactive and biocompatible materials such as MTA can modulate tissue repair and facilitate the deposition of dental hard tissue by promoting the deposition of carbonated apatite. This is important because pulp capping materials can induce remineralisation of dentin affected by carious processes and activate the process of neodentinogenesis with the formation of a new tertiary dentin bridge.

The in vitro behaviour of the two investigated materials was studied by FT-IR spectroscopy and SEM imaging at 3, 14 and 28 days after immersion of the samples in the SBF solution. In the case of both materials, the formation of apatitic phases was identified by the formation of spheres composed of aggregate plates. These aspects were confirmed by EDX spectra associated with SEM measurements. SEM imaging typical of mineralization was given by the presence of spherical agglomerations formed by apatite plates. The CSH formations were observed in the form of needles.

Similar aspects have been described by Abu Zeid et al. in the SEM characterization of two commercial MTA cements. In the case of TheraCal LC, the presence of calcium phosphate at the surface was evidenced by the presence of the element P in the EDX spectrum and PO_4^{3-} in the FT-IR spectrum. Hydroxyapatite formation on the surfaces of White MTA and TheraCal LC 7 days after immersion in phosphate buffered saline (PBS) was observed by Yamamoto, S. et al. SEM images from the same study showed the presence of crystalline precipitates of different sizes on the surface, with a spherical appearance with acicular (needle-like) micro-projections. In another study conducted by Guimarães, B.M. et al. the presence of calcium phosphate was observed on the surfaces of MTA and fluid MTA cements, respectively, after their immersion in an SBF solution for 28 days. The same study identified bismuth oxide as a radiopacifying agent in the composition of MTA cement, and SEM imaging showed the presence of bismuth oxide particles of variable size in the cement matrix on the irregular surface of the material.

FT-IR analysis results for TheraCal LC showed similar bands for alite (Ca_3SiO_5) and apatite (HA-$C_{10}(PO_4)_6(OH)_2$), CO_3^{2-}, SiO_4^{4-}, CSH and calcium carbonate, similar to literature data, and after completion of the setting reaction, bands could be identified for CSH. By immersion in SBF it was observed that the characteristic bands of the phosphate groups increased, due to mineralization of the surface of the hardened material. FT-IR analysis performed on the anhydrous BIO MTA+ powder showed bands for CO_3^{2-} and alit, similar to those obtained by Abu Zeid et al. and Gandolfi et al. By hydration at 37°C, R.H. = 100%, calcium silicates were transformed into calcium hydrosilicates, their presence being demonstrated by SEM images, needle/sheet shapes and FT-IR spectra. The presence of amorphous resin matrix and a dense microstructure with rhomboidal calcium phosphate crystals at the surface of TheraCal LC was observed. EDX analysis identified higher intensities for calcium. These aspects are similar to those identified in the personal study.

Biomineralization of MTA cement was experimentally observed by Lim M. et al. by the addition of calcium chloride ($CaCl_2$) 5%, a mixture that resulted in increased proliferation and gene expression of human pulpal stem cells without affecting solubility and surface roughness. The increased surface roughness of BIO MTA+ and the lack of resin matrix may lead to better hydroxyapatite deposition after 28 days of immersion in SBF solution. Factors such as calcium ion concentration, as well as pH of the environment, may influence the formation of apatite phases at the surface of a biomaterial by accelerating this process.

5.5. Conclusions

1. Both tested materials have bioactive properties, being able to develop apatite on surfaces after immersion in a solution similar to the human internal physiological environment.
2. The two biomaterials exhibit the ability to form hydrosilicates in the presence of SBF solution.
3. Although both materials contain silicates, BIO MTA+ contains more mineral phases.
4. Mineralization was better in the BIO MTA+ material, with both the anhydrous and hydrated phases forming apatite deposits at the surface.
5. BIO MTA+ appears to be a better choice than TheraCal LC in terms of its ability to stimulate dentin remineralisation.

CHAPTER 6. *IN VIVO* EVALUATION OF TWO PULP CAPPING BIOMATERIALS

6.1. Introduction

Pulp capping biomaterials must have the ability to stimulate the regenerative potential of the dental pulp by activating neodentinogenesis and tertiary dentin formation in the form of a dentinal bridge by activating odontoblasts at the pulp level. These issues arise when the dental pulp is directly exposed to the oral environment, necessitating the clinical manoeuvre of direct pulp capping. The biomaterial used in pulp capping may dictate the prognosis and success of the clinical procedure.

Numerous biomaterials have been proposed over the years for clinical pulp capping. MTA, a calcium silicate cement, has become the ideal pulp capping material in recent years, becoming the "gold standard" in this category of biomaterials. MTA has been successfully used in in *vivo* tests in both human subjects and animal models, with results indicating good biocompatibility, effective marginal closure, antimicrobial activity, low pulpal inflammatory response and stimulation of pulp cell proliferation with odontoblast differentiation. TheraCal LC, a tricalcium silicate modified with light-cured resin, has been successfully used in *vivo* in direct pulp capping. However, there have been reports of an unfavourable response of the dental pulp in certain situations, which may be a consequence of the toxic effects of unpolymerised resin monomers.

The aim of the *in vivo* animal model experiment was to compare the effect of the two biomaterials on the dental pulp following direct pulp capping. The objectives were to evaluate the pulp inflammatory grade and the activation of the neodentinogenesis process in the form of tertiary dentin deposition.

6.2. Materials and methods

6.2.1. Materials

6.2.1.1. Experimental animals

The in vivo experiment consisted of creating buccal cervical cavities in the maxillary and mandibular rabbit incisors, opening the pulp chamber and placing biomaterials (BIO MTA+ and TheraCal LC) which, in contact with the dental pulp, stimulate odontoblasts in the periphery of

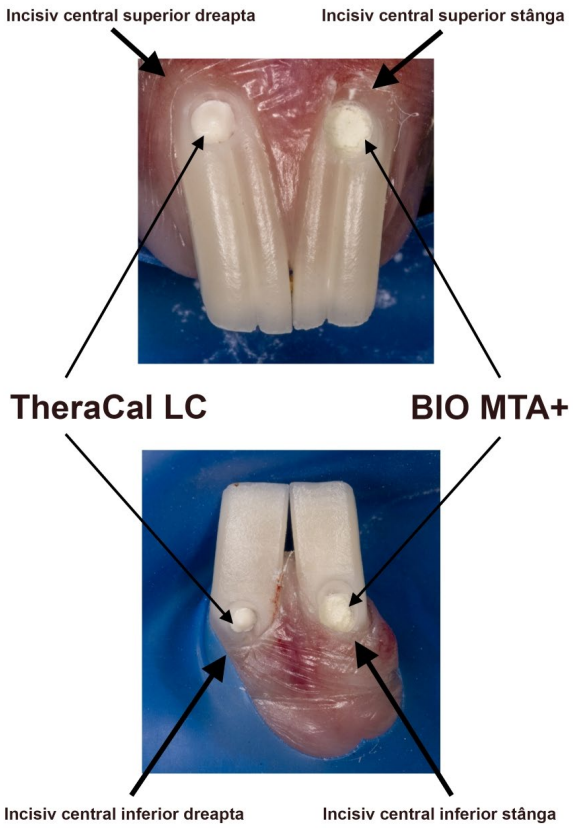
the dental pulp to secrete tertiary dentin (neodentinogenesis process), forming a barrier of dentin tissue at the site of the pulp chamber exposure.

The animals used for the *in vivo* neodentinogenesis experiment were rabbits of the *New Zealand White* strain. The site of the *in vivo* study was the Laboratory Animal Breeding Facility - INCDMI "Cantacuzino".

6.2.1.2. Armamentarium

6.2.1.3. Biomaterials

The materials used were pulp capping agents - TheraCal LC and BIO MTA+. The materials were prepared according to the manufacturers' instructions.



Distribution of TheraCal LC and BIO MTA+ experimental materials in rabbit maxillary and mandibular incisors

6.2.2. Methods

6.2.2.1. *In vivo* direct pulp capping

6.2.2.2. Obtaining dental hard sections

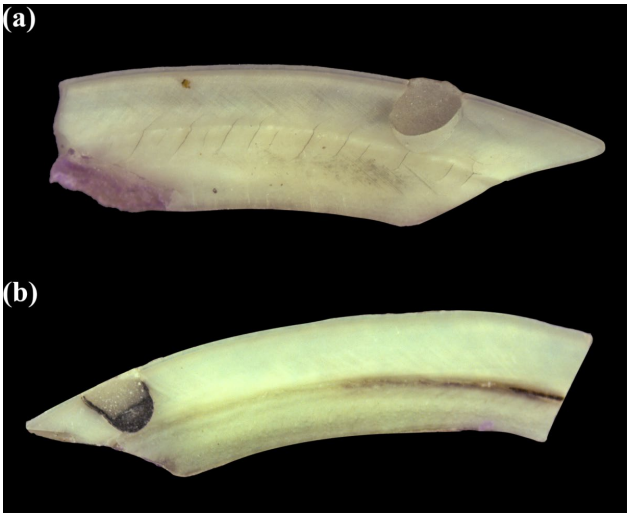
6.2.2.3. Histological processing of rabbit teeth

6.2.2.4 Examination of histological slides and dental hard sections

6.2.2.5. Immunohistochemistry

6.3. Results

6.3.1. Dental hard sections



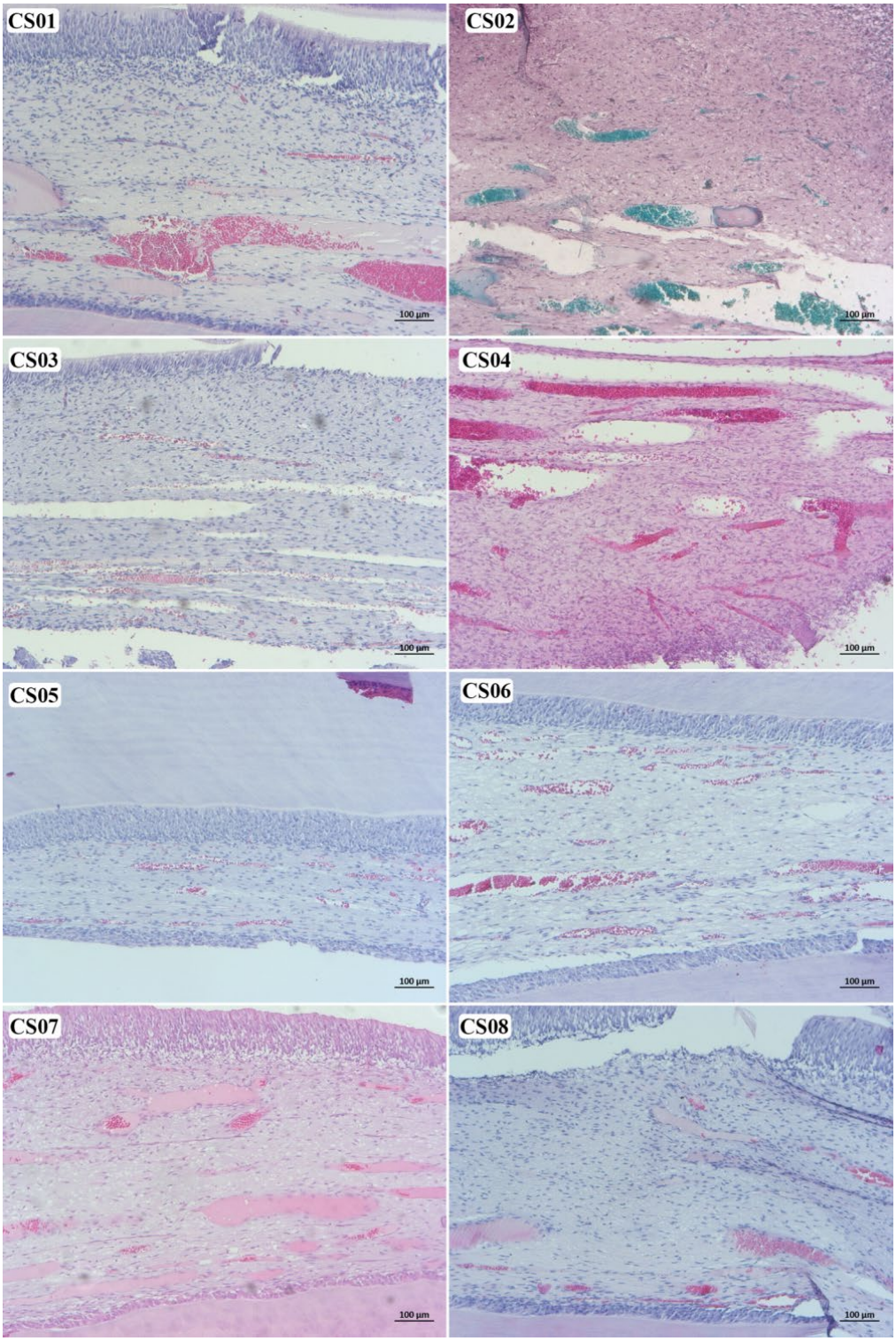
Hard sections through pulp-capped upper incisors with TheraCal LC (a) and BIO MTA+ (b)

6.3.2. Histological study

6.3.2.1. Inflammatory pulp status

Pulp status of the teeth involved in the study in terms of necrosis

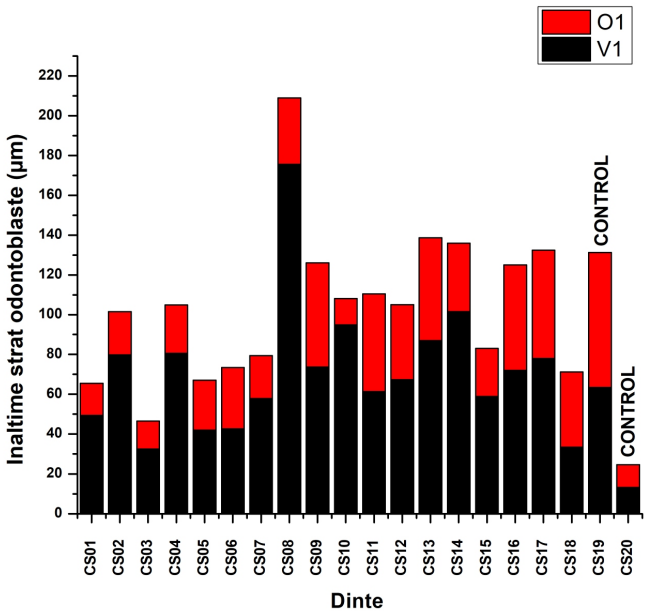
Pulp stasis in terms of presence (Score=1) or absence (Score =0) of necrosis			
TOOTH	**SCORE**	**TOOTH**	**SCORE**
CS01	0	**CS11**	0
CS02	0	**CS12**	0
CS03	0	**CS13**	0
CS04	0	**CS14**	0
CS05	0	**CS15**	0
CS06	0	**CS16**	0
CS07	0	***CS17***	0
CS08	0	***CS18***	0
CS09	0	***CS19***	0
CS10	0	***CS20***	0



Histological images of CS01-CS08 tooth pulps

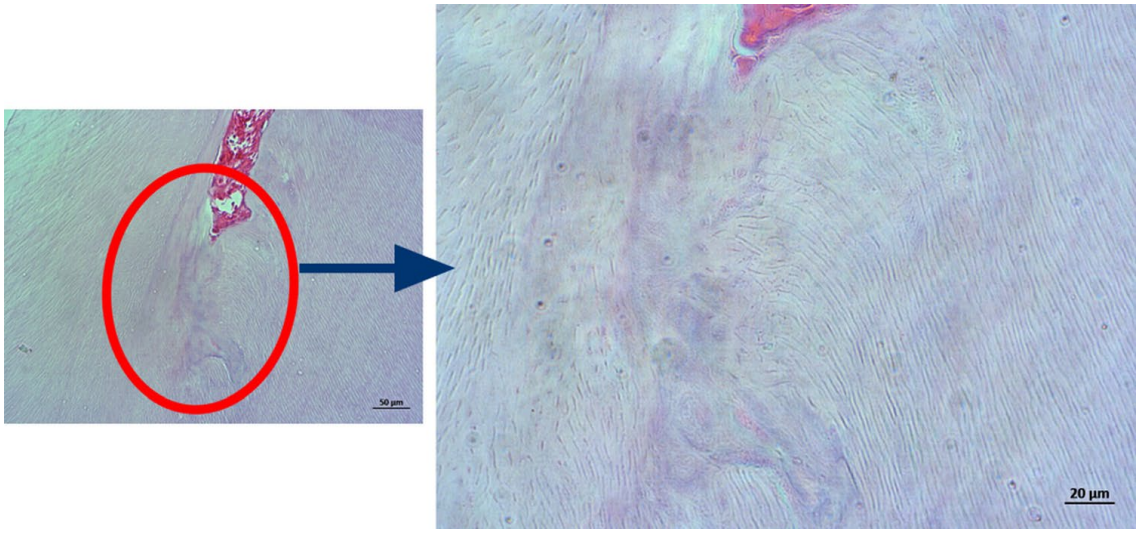
6.3.2.2. Measurements of the odontoblastic layers

Measurements of the height of the buccal and oral odontoblastic layers were performed. For each tooth involved in the study, two measurements were taken for the buccal odontoblastic layer (V_1 and V_2) and two for the oral layer (O_1 and O_2), respectively.



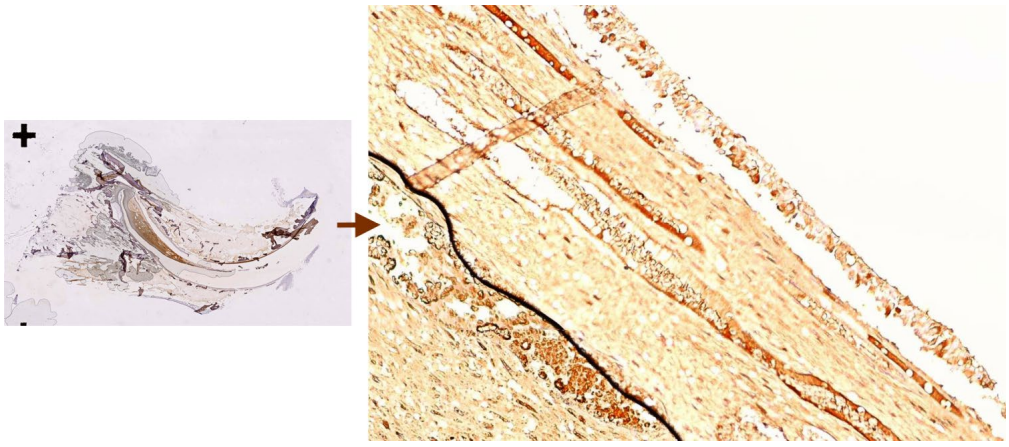
Height measurements of odontoblast layers at points V_1 and O_1

6.3.2.3. Dentinal bridge formation



Dentin bridge formed in tooth CS04. Magnification 20x and 40x. HE staining.

6.3.2.4. Immunohistochemistry



IHC staining for tooth CS02

6.4. Discussions

The clinical success of direct pulp capping can be influenced by a number of factors such as the size of the pulp exposure surface, the presence of microorganisms, the age of the patient or the type of capping biomaterial used and the pulp response generated. Numerous pulp capping materials have been proposed and used over the years, and MTA can now be seen as the standard for the calcium silicate-based biomaterial class. The novelty of MTA lies in the ability of this cement to perform its setting reaction in high moisture environments.

In addition to the formation of new dentinal tissue, pulp inflammation that may occur following direct pulp capping was also monitored. An ideal pulp capping material should not induce pulp inflammation, which may be followed by pulp necrosis. Both materials tested experimentally resulted in minimal, possibly localised or minimally diffuse pulpal inflammation, and pulpal necrosis was absent in all pulp capped teeth. These aspects certify an increased biocompatibility for both TheraCal LC and BIO MTA+. *In vivo* animal model studies confirm the optimal biocompatibility of the two materials with a low presence of pulpal inflammation.

In vivo studies by Cannon, M. et al. and Li, X. et al. showed that TheraCal LC, a hybrid pulp capping agent, induced dentinal hard tissue deposition at the exposure site with low inflammatory cell response. This is a consequence of CH formation following the hydration reaction, which created the premise of high pH with antibacterial activity and calcium ion formation that induced morphodifferentiation and proliferation of odontoblast cells at the pulp exposure site [203]. Another study reported the absence of dentinal bridge formation, most likely

due to the presence of pulp necrosis, as well as the presence of necrotic pulp tissue and blood clot under the pulp exposure site, at 70 days following direct pulp capping.

Tertiary dentin has a dystrophic appearance, with a more sinuous and disorganised course of the dentinal tubules or an atubular structure may present. These features are similar to the newly formed, complete dentinal bridge seen in the CS04 tooth, which shows an irregular tubular structure in the periphery and a central homogeneous area of hard, atubular tissue. Newly formed, complete or incomplete dentinal bridges were also observed in other teeth (CS01, CS02 and CS05).

Immunohistochemistry results showed the presence of tertiary dentin formation in two teeth, one pulp capped with TheraCal LC (CS10) and the other with BIO MTA+ (CS02).

Pulp biomaterials are successfully used in minimally invasive procedures in vital pulp therapies. The response that the material generates in the pulp-dentin complex should be optimal and generate maximum benefit by activating the neodentinogenesis process. A considerable number of studies support biocompatibility, stimulation of dentin barrier formation and a low pulpal inflammatory response for most MTA and calcium silicate cements.

6.5. Conclusions

1. Both materials induced minimal pulpal inflammation following direct pulp capping.
2. No teeth showed pulp necrosis following direct pulp capping.
3. The presence of tertiary dentin in the form of complete or incomplete dentinal bridges was observed.
4. IHC evaluation of dentin and odontoblasts was observed in both materials, indicating tertiary dentin formation.
5. The New Zealand rabbit is not an ideal model for *in vivo* direct pulp capping because of the constant growth of the incisors.

CHAPTER 7. GENERAL CONCLUSIONS AND PERSONAL CONTRIBUTIONS

7.1. General conclusions

Maintaining the vital status of the dental pulp is a necessity for keeping teeth in the dental arches for as long as possible. Direct pulp capping biomaterials have been used for decades for the treatment of clinical situations where the dental pulp is directly exposed to the oral environment following various pathologies. Technological progress in recent years has led to the development of increasingly elaborate and complex pulp capping materials with superior physicochemical, mechanical and biological properties compared to older generations of materials.

The three research strands have a multi- and multidisciplinary approach. Two biomaterials based on calcium silicates have been tested, which clinically fulfil the same role, i.e. stimulating the formation of tertiary dentin by activating the process of neodentinogenesis following direct pulp capping, but with different mechanisms of the setting reaction. Correlation of *in vitro* data on the composition of the two biomaterials, by complex thermal analysis and X-ray diffraction determinations, with those obtained from SEM imaging and elemental analysis by EDX, with determination of surface topography by AFM and hydrophobic-hydrophilicity by CA, Vickers microhardness mechanical strength testing with *in vitro* biomineralisation of the tested surfaces immersed for 28 days in SBF solution, with data obtained from in *vivo* pulp capping in an animal model, provides originality to the study.

Two pulp capping biomaterials were characterized physicochemically, mechanically and by SEM imaging and EDX analysis.

- Complex thermal analysis and XRD analysis, performed on both materials, revealed the nature of the main crystalline phases, with the exception that MTA cement contains several mineral phases and TheraCal LC material contains light-cured resin in its composition.
- XRD analysis of the calcined and uncalcined TheraCal LC material revealed the main mineralogical phase of tricalcium silicate as well as two Ba and Sr-rich phases attributed to barium zirconate and strontium zirconate, while the uncalcined sample of TheraCal LC showed the presence of the organic phase.

- The surfaces of the biomaterials were characterized by SEM imaging, indicating a dense microstructure for BIO MTA+ cement and a dispersed particle appearance in the organic matrix for TheraCal LC.
- Bismuth oxide has been identified by SEM imaging in BIO MTA+ in the form of rods, which may be the crystallization substrate for the newly formed mineral phases.
- AFM measurements showed a much rougher surface for the BIO MTA+ cement, due to the lack of organic component in its composition, and the hydrophilicity of both materials was determined by contact angle measurements. Given the placement of the placement of the materials directly in close proximity to a cell layer (odontoblastic), a surface with roughness and hydrophilic character becomes indicated.
- The Vickers microhardness showed a value four times higher for BIO MTA+ cement, similar to that of dentin, which favours it in sandwich coronal filling techniques.

Biomineralization of the surfaces of the two biomaterials was evaluated over a period of 28 days, following immersion of the samples in a solution resembling the internal physiological medium.

- Both materials have bioactive properties and can develop apatite on surfaces after immersion in SBF solution.
- The two cements have the ability to form hydrosilicate compounds on surfaces after immersion in SBF.
- Even though both materials contain calcium silicates, especially tricalcium silicate, BIO MTA+ contains more mineral phases, as shown by SEM images and FT-IR analysis.
- Mineralization was better in the BIO MTA+ material, with both the anhydrous and hydrated phases forming apatite deposits at the surface, as shown by SEM imaging.
- Given the higher number of mineral phases of BIO MTA+ and the lack of resin component, it seems a much better choice than TheraCal LC in terms of dentin regeneration.

In vivo testing of the two biomaterials in animal models was performed after clinical direct pulp capping.

- Both TheraCal LC and BIO MTA+ resulted in minimal pulpal inflammation following pulp capping. No rabbit teeth showed pulpal necrosis.

- Measurements of the height of the buccal odontoblastic layer in the coronal third of the pulp showed a greater thickness than the lingual layer in all teeth with direct pulp capping, compared to teeth included in the negative control group, where the two layers showed similar heights or a greater thickness than the lingual layer.
- Complete or incomplete dentinal bridges have been generated as a result of activation of the neodentinogenesis process.
- IHC evaluation, with Anti-osteocalcin antibody, indicated by specific labelling the presence of tertiary dentin for two pulp capped teeth with the two biomaterials included in the study.

7.2. Personal contributions

The present research addresses a topic of interest and topicality in dentistry, aiming to investigate *in vitro* and *in vivo* two direct pulp cappind materials with similar composition but different mechanisms of the setting reaction, which are used in direct pulp capping procedures, in regenerative pulp therapies, in minimally invasive dentistry. The state of knowledge is presented in the first two chapters, the general part of the paper and represents a review of the literature.

The personal research part starts with **Chapter 4**, in which the two biomaterials were evaluated *in vitro* in terms of chemical composition, surface and microstructural characteristics and mechanical strength. Considering the similar composition of the two cements, based on calcium silicates, in particular tricalcium silicate, with mention of the existence of light-curing resin in one of the materials, the premise of testing the two cements was created. Evaluations by complex thermal analysis, XRD, SEM, EDX, AFM, CA and Vickers microhardness indicated both the advantages and disadvantages of each material, providing a comparative analysis of the two.

In **Chapter 5** the two pulp capping materials were compared *in vitro* in terms of bioactivity, surface characteristics and microstructure. Placing both materials in a simulated environment, similar to the internal environment of the human body, resulted in the formation and deposition of hydroxyapatite on their surfaces. SEM characterization of the samples showed biomineralization at different time intervals through the presence of apatitic phases at the surfaces, supported by EDX analysis through the presence of the element P in apatite. Given the nature of the composition of MTA cement, it developed several inorganic phases at its surface. FT-IR analysis showed the presence of bands characteristic of apatitic phases, which in turn confirms the bioactivity of the two materials.

In **Chapter 6** the biological effect of materials on dental pulp was evaluated in *vivo* in an animal model. *In vivo* testing of the two biomaterials provides the closest and most appropriate response to their activity in the dental pulp. Both materials exhibited good biocompatibility following direct pulp capping, inducing minimal pulpal inflammation and no pulpal necrosis in the teeth tested. Both materials showed biological activity at the pulp level, activating the process of neodentinogenesis and tertiary dentin formation. These aspects were confirmed by dentinal bridge formation and IHC staining.

The topic addressed in this paper makes important contributions by providing an in-depth understanding of the behaviour of some pulp capping biomaterials directly *in vitro* and in vivo and may represent a starting point for future new research with applicability in dental practice. Despite the diversity of animal or tooth models, pulp exposure type and direct pulp capping conditions, the success rate from translational research for some of the calcium silicate cements could cover a wide range of human permanent or temporary teeth.

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