



**Medical University "Prof. Dr. Paraskev Stoyanov" –  
Varna  
Faculty of Dental medicine  
Department of Periodontology and Dental Implantology**

**Dr. Sabina Plamenova Keremedchieva**

**Investigating the role of marginal adaptation of indirect  
restorations as a plaqueretentive factor and its  
influence on the attachment level**

**ABSTRACT**

of the dissertation for the award of educational and scientific degree "DOCTOR"

**SCIENTIFIC SPECIALTY:**

Therapeutic Dentistry

**ACADEMIC ADVISORS:**

Prof. Dr. Stefan Vassilev Peev, **PhD, DSc**

Assoc. Prof. Dr. Angela Zdravkova Gusiyska, **Phd**

**Varna, 2024**

This dissertation contains 209 standard pages and is illustrated with 99 tables, 118 figures and 9 appendices. 283 literary sources are cited. The numbering of tables and figures in the abstract does not correspond to those in the dissertation.

The dissertation was accepted for public defense by the Faculty Council of the Department of Periodontology and Dental Implantology on January 18th, 2024.

The official defense of the dissertation will be held on April 18th, 2024 at 12 o'clock in Auditorium 103 "Assoc. Dr. Dimitar Klisarov" of the Faculty of Dental medicine at the Medical university "Prof. Dr. Paraskev Stoyanov" - Varna, in a meeting of the Scientific Jury.

The defense materials are available in the Scientific Department at MU-Varna and are published on the website of MU-Varna.

# CONTENTS

ABBREVIATIONS	4
1. INTRODUCTION	5
2. AIM AND TASKS	7
3. RESEARCH	8
3.1. Materials and methods Task №1	8
3.2. Results, analysis, discussion Task №1	18
3.3. Materials and methods Task №2	20
3.4. Results, analysis, discussion Task №2	27
3.5. Materials and methods Task №3	27
3.6. Results, analysis, discussion Task №3	41
4. SUMMARY	46
5. CONCLUSIONS	48
6. CONTRIBUTIONS	49
PHD ARTICLES	50

## **ABBREVIATIONS**

CAL – Clinical attachment level  
PD – Probing depth  
MG – Margo gingivalis, gingival margin level  
CMR – Cervical margin relocation  
CM – Cervical margin  
CEJ – Cemento-enamel junction  
HC – Hybrid ceramic  
LD – Lithium disilicate  
GIC – Glass-ionomer cement  
MO – Medio-occlusal  
DO – Disto-occlusal  
MOD – Медно-disto-occlusal  
IDS – Immediate dentin sealing  
IOS – Intraoral scanner  
bis-GMA – bisphenol A-glycidil methacrylate  
UDMA – Urethan methacrylate  
TEGDMA – Triethylene glycol dimethacrylate  
HEMA – 2-hydroxyethyl methacrylate

# 1. INTRODUCTION

Defects of the crown of the tooth can be caused by numerous reasons, the most common in clinical practice being caries, fracture, occlusal trauma and abrasion. Caries is the most widespread dental problem in the world. In addition to defects of the crown, caries lesions may also lead to mastication dysfunction and poor aesthetics. Large carious lesions involving both the occlusal and proximal aspects of the tooth present a challenge to clinicians, especially in cases involving distal teeth with the cervical margin of the defect located subgingivally, below the cemento-enamel junction (CEJ).

The dentist should take into consideration a number of aspects related to the selection of the correct technique and material for restoration. These clinical decisions are of utmost importance for the success of the applied treatment. Such type of defects can be treated directly with a composite obturation or using an indirect restoration. Indirect restorations can be made of a variety of materials: composite, various types of ceramic, etc. In case of choosing to restore the defect with an indirect restoration, additional questions regarding the execution technique and the right dental cement for cementation also follow. The technique for execution is divided into classic and hybrid. According to the classic technique, an impression is taken from the cavity preparation in order to fabricate the indirect restoration in a dental laboratory conditions. According to the hybrid technique, before the impression, a layer of composite is placed in the area of the cervical margin of the cavity to elevate it supragingivally. The hybrid technique makes it easier to perform the adhesive protocol and impression taking. The impression can be taken conventionally, with an impression tray and a suitable impression material, or digitally, using an intraoral scanner. The choice of a cementing agent is also an important step in the restoration of teeth with indirect restorations. There are different types of dental cements with their specific composition and properties.

Each step of the treatment has multiple aspects that must be taken into account and well thought through by the dentist in order to obtain a long-lasting, functional and aesthetic result. The ultimate goal is to achieve a restoration with an excellent marginal adaptation, which doesn't cause plaque retention and has a natural tooth-like appearance.

Plaque-retentive factors can be natural and iatrogenic. Iatrogenic plaque-retentive factors include poorly adapted fillings, inlays, onlays, overlays, crowns, bridges, etc. They represent a predilection site for the accumulation of bacterial biofilm,

which has a negative impact on the structures of the periodontium. One of the leading local risk factors for the occurrence of periodontitis is the presence of plaque-retentive factors in the oral cavity. Periodontitis is a destructive disease resulting from an inflammatory process affecting the tissues of the periodontium - gingiva, periodontium, root cementum and the so-called "bundle" bone. The clinical attachment level (CAL) represents the distance from the CEJ to the bottom of the periodontal pocket. Changes in the clinical attachment level can be gain or loss of attachment and reflect the degree of periodontal tissue regeneration or destruction, respectively. Any dental material placed subgingivally for an extended period of time affects the level of attachment in that area.

The wide variety of materials and techniques raises the question which is the best approach for the restoration of class II crown defects with subgingivally located cervical margins.

## **2. AIM AND TASKS**

### **AIM:**

To determine the influence of different methods on the precision of indirect restorations and the clinical attachment level in the respective area.

### **TASKS:**

#### **Task #1:**

In vitro inspection of the marginal adaptation of indirect restorations (ceramic inlays) class II (MO/DO) on specimens.

#### **Task #2:**

In vitro inspection of microleakage in indirect restorations (ceramic inlays) class II (MO/DO) on specimens.

#### **Task #3:**

A 6-month follow-up of the clinical attachment level of patients with direct (composite obturations) or indirect (ceramic inlays) Class II restorations (MO/DO/MOD) and cervical margins located subgingivally, below the CEJ at the primary visit.

### **3. RESEARCH**

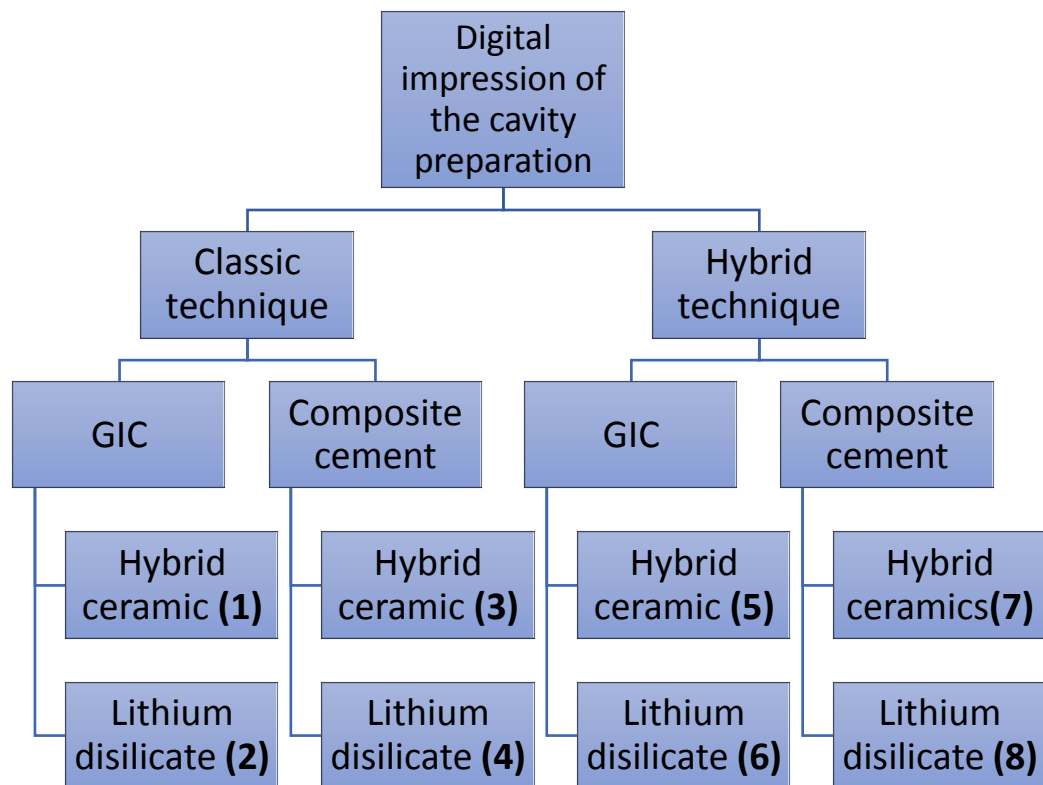
#### **3.1. MATERIALS AND METHODS TASK №1**

According to Task №1, the subject of experimental research is 40 sections of extracted permanent premolars and molars of patients over 18 years of age. The teeth have an intact crown, complete root development and no endodontic treatment. Immediately after extraction, the teeth are thoroughly cleaned and examined under a microscope for cracks. The teeth were stored in 4% buffered aqueous formaldehyde solution for a short period of time. On each tooth, a class II cavity preparation (MO or DO) was made for the fabrication of a ceramic inlay according to standardized criteria. The cervical margin was located 1mm below the CEJ. In certain groups, hybrid technique with cervical margin relocation (CMR) using bulk fill composite was performed. Digital impression method was used. Ceramic inlays were manufactured and cemented. The sample teeth were then thermocycled, embedded in epoxy resin and sectioned in a medio-distal direction through the middle of the restoration.

The unit of observation is the thickness of the dental cement layer in the area of the cervical margin. Measurements were made using Leica DM1000 LED light microscope at x40 magnification.

8 groups were formed according to the use of:

- different techniques: classic; hybrid (CMR)
- various dental cements: Fuji Plus (GC); composite cement EsteCem II (Tokuyama Dental)
- different materials for fabrication of ceramic inlays: hybrid ceramic (Vita Enamic); lithium disilicate (IPS e.max CAD, Ivoclar Vivadent)



Task №1 includes the following 8 groups:

- Group 1: teeth with classic technique indirect restorations, cemented with GIC, fabricated out of hybrid ceramic
- Group 2: teeth with classic technique indirect restorations, cemented with GIC, fabricated out of lithium disilicate
- Group 3: teeth with classic technique indirect restorations, cemented with composite cement, fabricated out of hybrid ceramic
- Group 4: teeth with classic technique indirect restorations, cemented with composite cement, fabricated out of lithium disilicate
- Group 5: teeth with hybrid technique indirect restorations, cemented with GIC, fabricated out of hybrid ceramic
- Group 6: teeth with hybrid technique indirect restorations, cemented with GIC, fabricated out of lithium disilicate
- Group 7: teeth with hybrid technique indirect restorations, cemented with composite cement, fabricated out of hybrid ceramic
- Group 8: teeth with hybrid technique indirect restorations, cemented with composite cement, fabricated out of lithium disilicate

#### Description of work stages:

1. Fixation
2. Preparation of a standardized cavity for ceramic inlays
3. Digital impression
4. Fabrication of ceramic inlays
5. Cementation of ceramic inlays with dental cement
6. Thermocycling
7. Epoxy resin embedding
8. Obtaining slices
9. Polishing the slices
10. Microscopic examination

#### 1. Fixation

Immediately after extraction, the teeth were thoroughly cleaned of calculus and granulations. They were examined under a microscope for the presence of defects and cracks. They were then stored in 4% buffered aqueous formaldehyde solution for a short period of time. Afterwards, the teeth were well dried. The tips of the apices of each tooth were fixed using cast wax in the center of a plastic tube. The tube was then filled with epoxy resin (EpoThin, Buehler) to cover the root portion of the tooth up to 3 mm apical from the CEJ (Figure 1, Figure 2). In the following way, stability of the specimens was ensured for easier processing.



*Figure 1*



*Figure 2*

## 2. Preparation of a standardized cavity for ceramic inlays

The following parameters were taken into consideration when preparing a class II cavity (MO or DO) for ceramic inlays:

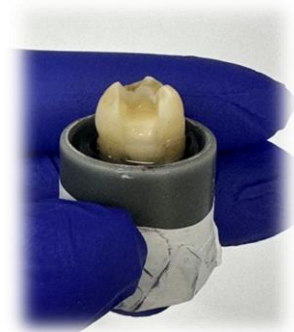
- Cavity preparation was performed using Expert Set 4562 for ceramic inlays and partial crowns (Komet Dental) (Figure 3).
- The prepared cavities had an inclination of the walls of 6-10°; minimum depth 1.5 mm, counted from the deepest part of the fissure; minimum width at the isthmus 2.5 mm; cervical margin located 1 mm below the CEJ; horizontal pulp base; rounded axiopulpal margin (Figure 4, Figure 5). Compliance with the cavity preparation criteria was checked with a UNC 15 periodontal probe (Figure 6).
- In Group 5,6,7,8, for the implementation of the hybrid technique, cervical margin relocation (CMR) was performed with the application of Estelite bulk fill flow composite (Tokuyama Dental) (Figure 7). The procedure was completed with thorough polishing using 4652-204 Polishing kit for composite (Komet Dental).



*Figure 3*



*Figure 4*



*Figure 5*



*Figure 6*



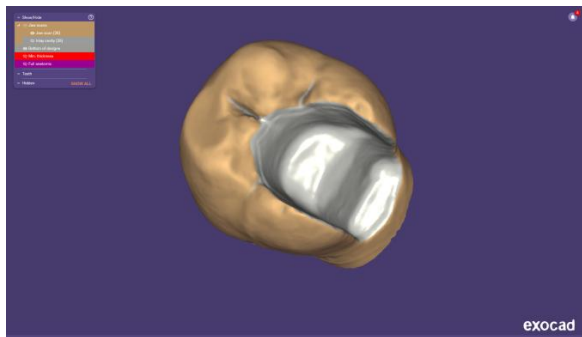
*Figure 7*

### 3. Digital impression

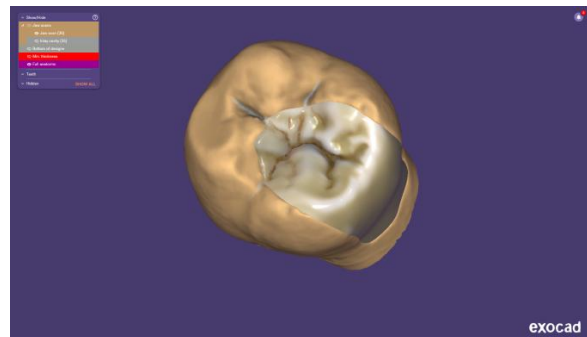
A digital impression was taken of the cavity preparations using True Color Texture Scan UP3D UP560 laboratory scanner.

### 4. Fabrication of ceramic inlays

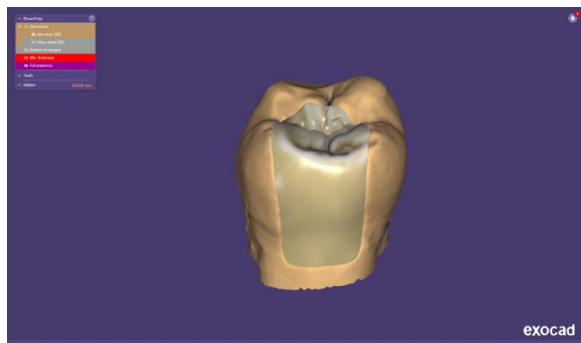
In Group 2, 4, 6, 8, the ceramic inlays were manufactured out of milled lithium disilicate (IPS e.max CAD, Ivoclar Vivadent). In Group 1,3,5,7, the inlays were fabricated out of hybrid ceramic (Vita Enamic). After taking a digital impression, the design of the restorations was modeled on Exocad (Figure 8, 9, 10, 11). The restorations were fabricated in a dental laboratory using CAD/CAM (Figure 12) according to the manufacturer's instructions (Figure 13).



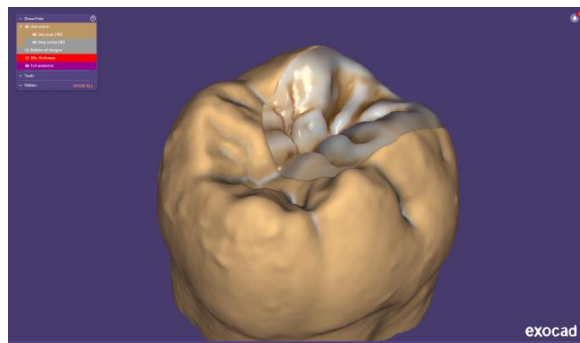
*Figure 8*



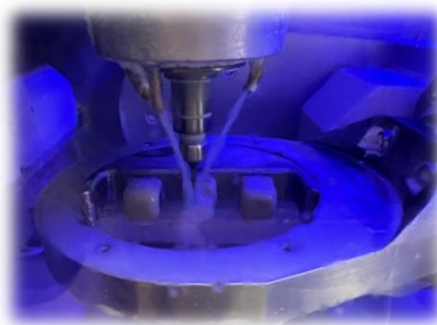
*Figure 9*



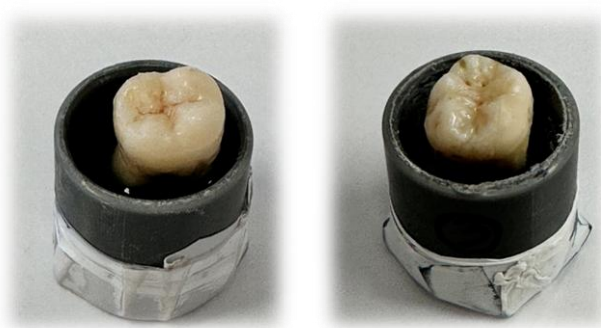
*Figure 10*



*Figure 11*



*Figure 12*



*Figure 13*

#### 5. Cementation of ceramic inlays with dental cement

In Group 1, 2, 5, 6, the cementing agent used was GIC reinforced with resin Fuji Plus (GC) (Figure 14). The following protocol was used: Fuji Plus Conditioner is applied to the cavity preparation for 20 seconds, then rinsed with water and air-dried without overdrying the surface. After mixing the Fuji Plus powder and liquid components in a 1:1 ratio, a layer of cement is applied to the inner surface of the indirect restoration and placed in the cavity with pressure applied for a few seconds (Figure 15). Excess cement was removed approximately 1 minute after placement. The working time from the start of cement mixing at room temperature is 2 minutes and 30 seconds. Polishing can begin 4 minutes and 30 seconds after placement of the restoration.

In Group 3,4,7,8, the cementing agent used was EsteCem II composite cement (Tokuyama Dental) (Figure 16). The following protocol was used: an etching gel containing 9.5% hydrofluoric acid (Yellow Porcelain Etch, Cerkamed) is applied to the inner surface of the ceramic inlay for 60 seconds. It is then washed thoroughly with water and air-dried. A layer of silane (Silan, Cerkamed) is applied on the inner surface of the inlay. Etching gel containing 39% phosphoric acid (etching gel HV, Tokuyama Dental) is placed on the walls of the cavity preparation for 10-15 sec, then rinsed for at least 15 sec and air-dried. The two components of Universal Bond (Tokuyama Dental) are mixed and placed on the inner surface of the ceramic inlay and on the walls of the cavity preparation. The air nozzle is used to spread the bond layer evenly. The inner surface of the inlay is evenly coated with EsteCem II composite cement (Tokuyama Dental) and placed in the prepared cavity with moderate pressure. This is followed by photopolymerization for 2-4 sec in the beginning in order to remove excess cement, then final photopolymerization for 20 sec (Figure 15).



*Figure 14*



*Figure 15*



*Figure 16*

## 6. Thermocycling

The thermocycling method chosen was that of Aguir Mabrouk Najet et al. The specimens were subjected to thermal cycles with a difference in temperature from 6°C to 60°C. A daily cycle of 45 min at 6°C in a refrigerator (Figure 17) followed by a 45 min cycle at 60°C in an incubator (Figure 18) was performed 4 times in a row for 5 consecutive days. In this way, the thermal stress to which restorations in the oral cavity are subjected was experimentally simulated.



*Figure 17*



*Figure 18*

## 7. Epoxy resin embedding

After the completion of thermal cycles, the specimens were thoroughly dried. As a continuation of the tube in which they were initially fixed, another plastic tube was placed coronally, which completely covered the crown of the teeth with cemented indirect restorations. The transition between the two tubes was insulated with Teflon tape. The newly placed plastic tube was then filled with epoxy resin (EpoThin, Buehler), thereby fully incorporating the preparations into the resin (Figure 19, Figure 20).



Figure 19



Figure 20

## 8. Obtaining slices

The specimens were cut in a medio-distal direction through the middle of the ceramic inlay (Figure 21) using IsoMet1000 precision cutting machine (Buehler) (Figure 22).



Figure 21

## 9. Polishing the slices

The samples were polished with EcoMet30 (Buehler) grinding and polishing machine (Figure 23) after changing several polishing discs of decreasing abrasiveness.



*Figure 22*



*Figure 23*

## 10. Microscopic examination

Marginal adaptation of hybrid ceramic and lithium disilicate inlays was investigated by recording the thickness of dental cement between the restoration and the cavity wall of the tooth, in the area of the cervical margin. Each preparation was placed under Leica DM 1000 LED stereomicroscope at x40 magnification (Figure 24). The resulting images were transferred to the computer connected to the microscope. Measurements were made with the ruler tool in the Leica Application Suite program. The thickness of the cement layer in the area of the cervical margin was recorded in mm at the following 3 points (Figure 25, 26):

- T.1 represents the thickness of the dental cement at the outer edge of the cervical margin;
- T.2 represents the thickness of the cement in the middle of the cervical margin;
- T.3 corresponds to the thickness of the cement in the area of the rounded inner corner of the cervical margin;
- To locate point T.2, additional measurements were made:
- D.1 distance – represents the distance between the outer edge of the cervical margin and the rounded inner corner of the cervical margin at the dental cement-tooth interface;
- D.2 distance – equals half of D.1 distance;

The mean value of the 3 measurements (at points T.1, T.2 and T.3) of the thickness of dental cement in the area of the cervical margin was calculated for each sample.



Figure 24

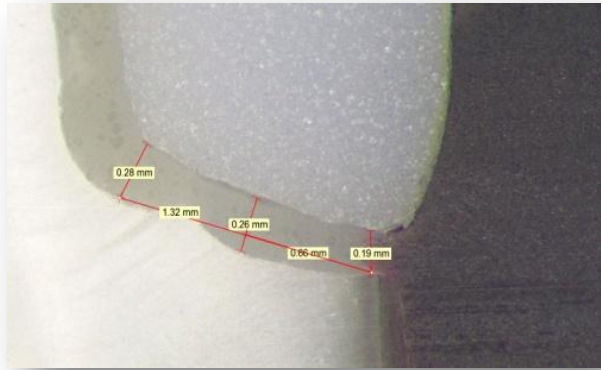


Figure 25



Figure 26

### 3.2. RESULTS, ANALYSIS, DISCUSSION TASK #1

Task #1: Inspection of the marginal adaptation of indirect restorations (ceramic inlays) class II (MO/DO) on specimens. For this purpose, the average thickness of the dental cement layer in the area of the cervical margin was measured and compared. All tooth specimens were divided into 8 groups.

The difference in average values was studied according to: the different groups, the type of technique, the type of dental cement and the type of material.

The following statistical tests were used: Shapiro-Wilk test, One-way ANOVA, Independent t-test, Student's t-distribution. Significance level of  $\alpha = 0.05$  was chosen

There were no statistically significant differences in the thickness of the dental cement layer in the area of the cervical margin between the 8 studied groups, representing combinations of the type of technique, cementing agent and fabrication material.

When comparing the "type of technique" parameter - classic (direct cavity scan, without cervical margin relocation) or hybrid (cervical margin relocation (CMR) with composite before direct cavity scanning), no statistically significant differences were reported. In their research, Zaruba M. et al. studied the influence of CMR on the marginal adaptation of ceramic inlays. The authors also concluded that no statistically significant differences were observed between the marginal adaptation of ceramic inlays with and without CMR.

Statistical analysis of the data according to the "type of cement" parameter - resin-reinforced glass ionomer cement (Fuji Plus, GC) and composite cement (EsteCem II, Tokuyama Dental) did not demonstrate statistically significant differences regarding the thickness of the cement layer in ceramic inlays. However, in Task #2, when comparing microleakage by the "type of cement" parameter, statistically significant differences were reported between the two types of cements, in favor of the composite cement. The composite cement demonstrated significantly better sealing and a lower volume of microleakage. With these results, the conclusion made by Romao W. Jr. et al. can be confirmed. There is no correlation between cement layer thickness and microleakage. The research team compared cement layer thickness and microleakage in Class II ceramic inlays made with different ceramic systems. The same conclusion was reached by Homayoun A. et al. in their experimental study on ceramic inlays. A number of studies documented in the

literature also found no correlation between microleakage and dental cement thickness.

When comparison was made according to the "type of material" parameter - hybrid ceramic (Vita Enamic) and milled lithium disilicate (IPS e.max CAD), no statistically significant differences were reported regarding the marginal adaptation of ceramic inlays. However, it can be noted that hybrid ceramic demonstrates slightly better marginal adaptation compared to lithium disilicate. This may be due to the lower surface hardness of the hybrid ceramic compared to milled lithium disilicate, which allows for easier and more accurate fabrication of the restoration and thinner margins. Materials with less surface hardness demonstrate a lower risk of chipping and better adaptation. A large part of the conducted experimental studies report a better marginal adaptation of hybrid ceramic than lithium disilicate. For example, Cabral, A. C. R. et al. explain the better adaptation of hybrid ceramic with its modulus of elasticity which is closer to dentin and makes the material extremely suitable for inlays. On the other hand, Sener-Yamaner I. D. et al. reported better marginal adaptation of lithium disilicate restorations. In their in vitro study, Uzgur R. et al. concluded that the marginal adaptation of hybrid ceramic and lithium disilicate partial restorations was very similar and without statistical difference. The authors used the exact same materials: hybrid ceramic - Vita Enamic and milled lithium disilicate - IPS e.max CAD, Ivoclar Vivadent for inlay manufacturing. Both materials demonstrated satisfactory marginal adaptation.

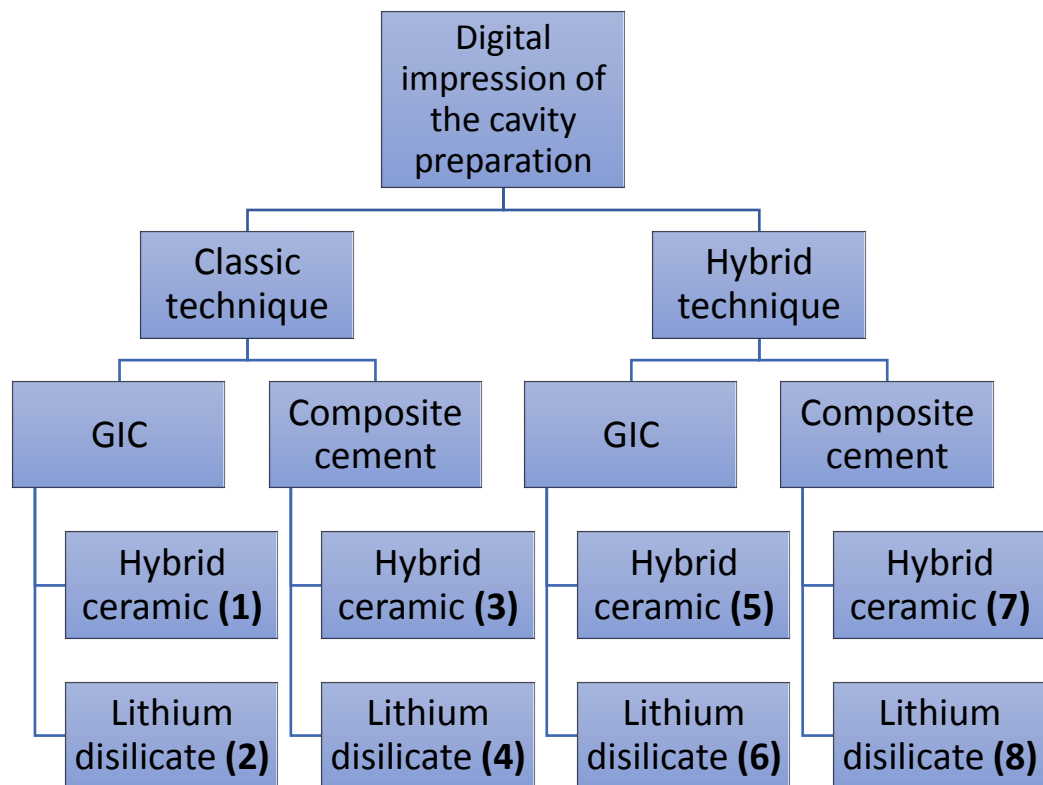
### 3.3. MATERIALS AND METHODS TASK №2

According to Task №2, the subject of experimental research is 40 sections of extracted permanent premolars and molars of patients over 18 years of age. The teeth have an intact crown, complete root development and no endodontic treatment. Immediately after extraction, the teeth are thoroughly cleaned and examined under a microscope for cracks. The teeth were stored in 4% buffered aqueous formaldehyde solution for a short period of time. On each tooth, a class II cavity preparation (MO or DO) was made for the fabrication of a ceramic inlay according to standardized criteria. The cervical margin was located 1mm below the CEJ. In certain groups, hybrid technique with cervical margin relocation (CMR) using bulk fill composite was performed. Digital impression method was used. Ceramic inlays were manufactured and cemented. The samples were then subjected to thermocycling, followed by fuchsin staining, embedding in epoxy resin and sectioning in a medio-distal direction through the middle of the restoration.

The unit of observation is the microleakage of the staining agent – fuchsin in the area of the cervical margin. Measurements were made using Leica DM1000 LED stereomicroscope at x40 magnification.

8 groups were formed according to the use of:

- different techniques: classic; hybrid (CMR)
- various dental cements: Fuji Plus (GC); composite cement EsteCem II (Tokuyama Dental)
- different materials for fabrication of ceramic inlays: hybrid ceramic (Vita Enamic); lithium disilicate (IPS e.max CAD, Ivoclar Vivadent)



Task №1 includes the following 8 groups:

- Group 1: teeth with classic technique indirect restorations, cemented with GIC, fabricated out of hybrid ceramic
- Group 2: teeth with classic technique indirect restorations, cemented with GIC, fabricated out of lithium disilicate
- Group 3: teeth with classic technique indirect restorations, cemented with composite cement, fabricated out of hybrid ceramic
- Group 4: teeth with classic technique indirect restorations, cemented with composite cement, fabricated out of lithium disilicate
- Group 5: teeth with hybrid technique indirect restorations, cemented with GIC, fabricated out of hybrid ceramic
- Group 6: teeth with hybrid technique indirect restorations, cemented with GIC, fabricated out of lithium disilicate
- Group 7: teeth with hybrid technique indirect restorations, cemented with composite cement, fabricated out of hybrid ceramic
- Group 8: teeth with hybrid technique indirect restorations, cemented with composite cement, fabricated out of lithium disilicate

#### Description of work stages\*:

1. Fixation
2. Preparation of a standardized cavity for ceramic inlays
3. Digital impression
4. Fabrication of ceramic inlays
5. Cementation of ceramic inlays with dental cement
6. Thermocycling
7. Fuch sine staining
8. Epoxy resin embedding
9. Obtaining slices
10. Polishing the slices
11. Microscopic examination

\*Figures are attached only to the stages that differ from the stages in Task №1

#### 1. Fixation

Immediately after extraction, the teeth were thoroughly cleaned of calculus and granulations. They were examined under a microscope for the presence of defects and cracks. They were then stored in 4% buffered aqueous formaldehyde solution for a short period of time. Afterwards, the teeth were well dried. The tips of the apexes of each tooth were fixed using cast wax in the center of a plastic tube. The tube was then filled with epoxy resin (EpoThin, Buehler) to cover the root portion of the tooth up to 3 mm apical from the CEJ (Figure 1, Figure 2). In the following way, stability of the specimens was ensured for easier processing.

#### 2. Preparation of a standardized cavity for ceramic inlays

The following parameters were taken into consideration when preparing a class II cavity (MO or DO) for ceramic inlays:

- Cavity preparation was performed using Expert Set 4562 for ceramic inlays and partial crowns (Komet Dental) (Figure 3).
- The prepared cavities had an inclination of the walls of 6-10°; minimum depth 1.5 mm, counted from the deepest part of the fissure; minimum width at the

isthmus 2.5 mm; cervical margin located 1 mm below the CEJ; horizontal pulp base; rounded axiopulpal margin (Figure 4, Figure 5). Compliance with the cavity preparation criteria was checked with a UNC 15 periodontal probe (Figure 6).

- In Group 5,6,7,8, for the implementation of the hybrid technique, cervical margin relocation (CMR) was performed with the application of Estelite bulk fill flow composite (Tokuyama Dental) (Figure 7). The procedure was completed with thorough polishing using 4652-204 Polishing kit for composite (Komet Dental).

### 3. Digital impression

A digital impression was taken of the cavity preparations using True Color Texture Scan UP3D UP560 laboratory scanner.

### 4. Fabrication of ceramic inlays

In Group 2, 4, 6, 8, the ceramic inlays were manufactured out of milled lithium disilicate (IPS e.max CAD, Ivoclar Vivadent). In Group 1,3,5,7, the inlays were fabricated out of hybrid ceramic (Vita Enamic). After taking a digital impression, the design of the restorations was modeled on Exocad (Figure 8, 9, 10, 11). The restorations were fabricated in a dental laboratory using CAD/CAM (Figure 12) according to the manufacturer's instructions (Figure 13).

### 5. Cementation of ceramic inlays with dental cement

In Group 1, 2, 5, 6, the cementing agent used was GIC reinforced with resin Fuji Plus (GC) (Figure 14). The following protocol was used: Fuji Plus Conditioner is applied to the cavity preparation for 20 seconds, then rinsed with water and air-dried without overdrying the surface. After mixing the Fuji Plus powder and liquid components in a 1:1 ratio, a layer of cement is applied to the inner surface of the indirect restoration and placed in the cavity with pressure applied for a few seconds (Figure 15). Excess cement was removed approximately 1 minute after placement. The working time from the start of cement mixing at room temperature is 2 minutes and 30 seconds. Polishing can begin 4 minutes and 30 seconds after placement of the restoration.

In Group 3,4,7,8, the cementing agent used was EsteCem II composite cement (Tokuyama Dental) (Figure 16). The following protocol was used: an etching gel containing 9.5% hydrofluoric acid (Yellow Porcelain Etch, CerKamed) is applied to the inner surface of the ceramic inlay for 60 seconds. It is then

washed thoroughly with water and air-dried. A layer of silane (Silan, Cerkamed) is applied on the inner surface of the inlay. Etching gel containing 39% phosphoric acid (etching gel HV, Tokuyama Dental) is placed on the walls of the cavity preparation for 10-15 sec, then rinsed for at least 15 sec and air-dried. The two components of Universal Bond (Tokuyama Dental) are mixed and placed on the inner surface of the ceramic inlay and on the walls of the cavity preparation. The air nozzle is used to spread the bond layer evenly. The inner surface of the inlay is evenly coated with EsteCem II composite cement (Tokuyama Dental) and placed in the prepared cavity with moderate pressure. This is followed by photopolymerization for 2-4 sec in the beginning in order to remove excess cement, then final photopolymerization for 20 sec (Figure 15).

## 6. Thermocycling

The thermocycling method chosen was that of Aguir Mabrouk Najet et al. The specimens were subjected to thermal cycles with a difference in temperature from 6°C to 60°C. A daily cycle of 45 min at 6°C in a refrigerator (Figure 17) followed by a 45 min cycle at 60°C in an incubator (Figure 18) was performed 4 times in a row for 5 consecutive days. In this way, the thermal stress to which restorations in the oral cavity are subjected was experimentally simulated.

## 7. Fuchsin staining

After completion of the thermal cycles, the preparations were thoroughly dried. The entire surface of the teeth was covered with 2 layers of varnish (Figure 27), except for the restoration and 2 mm beyond its borders. Filtered 0.5% fuchsin staining solution was freshly prepared (Figure 28). The samples were placed in a container and completely covered by fuchsin for a period of 24 h (Figure 29). Then they were thoroughly washed under running water for 20 min and dried.



*Figure 27*



*Figure 28*



*Figure 29*

## 8. Epoxy resin embedding

After the completion of thermal cycles, the specimens were thoroughly dried. As a continuation of the tube in which they were initially fixed, another plastic tube was placed coronally, which completely covered the crown of the teeth with cemented indirect restorations. The transition between the two tubes was insulated with Teflon tape. The newly placed plastic tube was then filled with epoxy resin (EpoThin, Buehler), thereby fully incorporating the preparations into the resin (Figure 19, Figure 20).

## 9. Obtaining slices

The specimens were cut in a medio-distal direction through the middle of the ceramic inlay (Figure 21) using IsoMet1000 precision cutting machine (Buehler) (Figure 22).

## 10. Polishing the slices

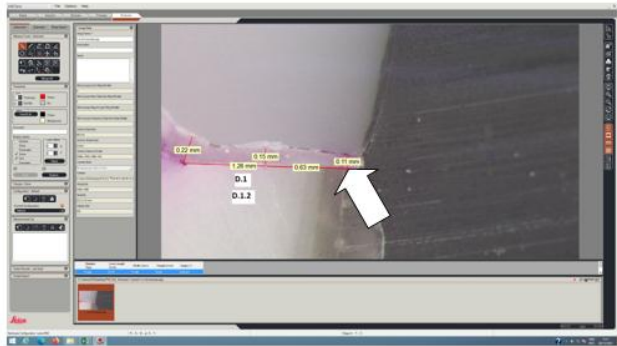
The samples were polished with EcoMet30 (Buehler) grinding and polishing machine (Figure 23) after changing several polishing discs of decreasing abrasiveness.

## 11. Microscopic examination

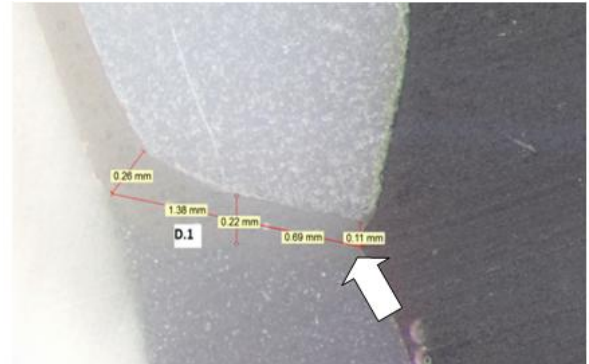
The microleakage of specimens with hybrid ceramic and lithium disilicate inlays was investigated by recording the penetration of the staining agent fuchsin in the cervical margin region, at the dental cement-tooth interface. Each preparation was placed under Leica DM 1000 LED stereomicroscope at x40 magnification. The resulting images were transferred to the computer connected to the microscope. Measurements were made with the ruler tool in the Leica Application Suite program. Microleakage in the area of the cervical margin was registered in %, and for this purpose the following calculations were made:

- D.1 distance – represents the distance between the outer edge of the cervical margin and the rounded inner corner of the cervical margin at the dental cement–tooth interface;
- D.1.2 distance – equals the distance up until the staining agent fuchsin penetrated at the dental cement-tooth interface.

The percentage of microleakage was determined by calculating how many % distance D.1.2 appears when compared to distance D.1 (Figure 30, Figure 31, the arrow indicates the position of the studied interface).



*Figure 30*  
(D.1=D.1.2)



*Figure 31*  
(D.1.2=0)

### **3.4. RESULTS, ANALYSIS, DISCUSSION TASK №2**

Task №2: Inspection of microleakage in indirect restorations (ceramic inlays) class II (MO/DO) on specimens. Penetration of 0,5% fuchsin solution at the cervical margin of the restoration is compared. Fuchsin infiltration at the dental cement–tooth interface was recorded in percentage. When hybrid technique (CMR) was used (group 5, 6, 7, 8), the microleakage of the dental cement–composite interface was taken into consideration. All prepared specimens were divided into 8 groups. The difference in average values was investigated: by group, by type of technique, by type of cement and by type of material.

In order to do the statistical analysis the following tests were used: Shapiro-Wilk test, Kruskal Wallis test, Mann Whitney test. Significance level  $\alpha = 0.05$  was selected.

When microleakage was compared between groups, statistically significant differences and a large effect size were found. Group 7 and Group 8 were not included in the analysis because their microleakage values were equal to 0, which means that no microleakage was observed in the studied interface. It should be noted, however, that in Group 7 and Group 8, hybrid technique (CMR) with composite was used and composite cement to cement the ceramic inlays. Although no microleakage was reported at the composite cement–composite interface, fuchsin penetration was reported at the composite–tooth interface, where the original position of the cervical margin was prior to elevation. These findings question the overall positive effect of the hybrid technique. The main purpose of cervical margin relocation is to facilitate isolation, impression technique, cementation and polishing. In their in vitro study, Koken S. et al. investigated the effect of cervical margin relocation prior to cementation of CAD/CAM onlays on marginal sealing. The team used two types of composite with different densities in order to execute the hybrid technique. They concluded that microleakage at the dentin–composite interface was significantly lower in cases where the hybrid technique was not used. A greater volume of microleakage was reported at dentin level than at enamel level. A number of authors conclude that cervical margin relocation should be subjected to further experimental and clinical studies, with long-term follow-up. The lowest percentage of microleakage of the remaining 6 groups participating in the statistical analysis was demonstrated by group #3 – classic technique for inlays, cemented with composite cement, fabricated out of hybrid ceramic. Statistically significant differences in microleakage with a large effect size were reported between the following groups: group #3 and group #2 –

classic technique for inlays, cemented with GIC, fabricated out of lithium disilicate (in favor of group #3); group #4 – classic technique for inlays, cemented with composite cement, fabricated out of lithium disilicate and group #2 (in favor of group #4) and between group #3 and group #6 – hybrid technique for inlays, cemented with GIC, fabricated out of lithium disilicate (in favor of group #3).

Regarding the type of technique and type of material, no statistically significant differences in microleakage were noted in the studied groups.

Regarding the type of cement, a statistically significant difference with a large effect size was found in favor of composite cement over GIC, for cementation of ceramic inlays. The composite cement demonstrated a significantly lower volume of microleakage at the cervical margin. The obtained result confirms the conclusion of an experimental study conducted by Rosentritt M. et al. that composite cements can be successfully used in the cementation of ceramic inlays, while resin-reinforced GICs have limited application. In a study conducted by Yüksel E. et al., the research team concluded that composite cement demonstrated a lower level of microleakage than GIC. The better sealing of the composite cement can be explained by its lower solubility. GIC is water-soluble and after being subjected to thermocycling to mimic thermal stress and conditions in the oral cavity, a more pronounced deterioration of its configuration was observed. It is possible that microfractures may also appear in the GIC layer as a result of the difference in temperatures during the thermal cycles. Another reason for the greater micropermeability of GIC compared to composite cement may be porous defects that form during the mixing of GIC components. These problems are a prerequisite for poor integrity of the GIC layer and an occurrence of more pronounced microleakage. The conclusion that can be drawn is that composite cement is the preferred material for ceramic inlays cementation.

### **3.5. MATERIALS AND METHODS TASK №3**

According to Task №3, the subject of the clinical examination are 50 teeth, premolars and molars, of male and female patients, over 18 years of age, examined at the University medical and dental Center - Varna or during classes at the Department of "Periodontology and dental implantology" at the Faculty of Dental medicine at MU-Varna. All study participants had vital premolars or molars with class II defects (MO, DO, or MOD) and a cervical margin positioned subgingivally, below the CEJ at the primary visit. Completed cases were followed up for 6 months, taking into account probing depth (PD), margo gingivalis level (MG) and clinical attachment level (CAL) before and 6 months after restoration placement.

Male and female patients who meet the following criteria were included in the clinical study of Task №3:

- Patients over 18 years of age
- Patients in good general health
- Satisfactory personal oral hygiene
- Presence of vital premolars or molars with subgingival class II defects
- Signed informed consent paper
- Completed health questionnaire
- Signed consent to X-ray examination

All patients included in the study were informed about the expected benefits and risks. Participation was voluntary and took place after the patient was given the information materials regarding the study, signed an informed consent paper, filled out a health questionnaire and signed a consent to perform an X-ray examination.

The criteria for excluding patients from the study was the following:

- Patients under 18 years of age
- Patients with severe systemic diseases
- Patients currently undergoing radiation or chemotherapy
- Patients whose teeth do not meet the criteria for inclusion in the study
- Refusal to complete the necessary documentation

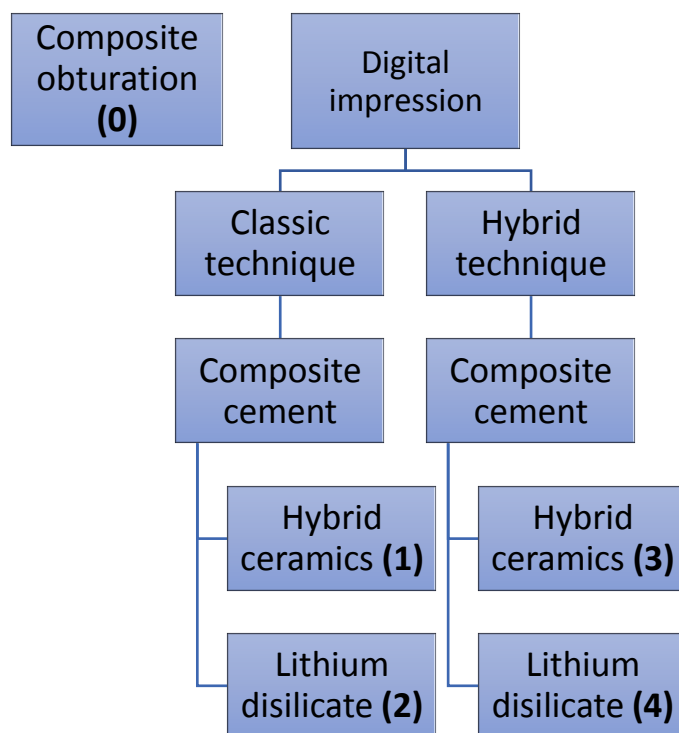
For each participant in the clinical study of Task №3, bitewing radiography was performed 3 times with a dose of 30 mGy\*cm<sup>2</sup> and an exposure time of 9.9 seconds after signing an informed consent for X-ray examination. The first X-ray examination was performed before the start of treatment, the second immediately after the completion of the restorative treatment, the third - 6 months after the completion of the treatment at the control visit. X-rays were performed for documentary and diagnostic purposes.

Bitewing radiographs are effective in early detection of interproximal caries lesions. The methods and criteria for detecting a poorly adapted restoration or interproximal caries vary in the studies conducted and documented in the literature. In their studies, Lervik T. et al., as well as Pack A.R. et al., applied bitewing radiographs and a thorough clinical examination to determine the presence of poor marginal adaptation of the restorations. The combination of thorough clinical and radiographic examinations increases the chance of early detection of plaque-retentive factors, which is of great importance in terms of periodontal health. In their study, Millar B. and Blake K. used bitewing radiographs to examine the impact of ill-fitting restorations on interproximal levels of the alveolar bone. The authors conclude that a correlation can be established between poor marginal adaptation and increased alveolar bone resorption.

10 of the included cases in the clinical study, were restored with direct composite obturations as a control group. The composite material used was Estelite Asteria (Tokuyama Dental). In the remaining 40 cases, cavity preparations for ceramic inlays were performed according to standardized criteria. The impression method used was intraoral scanning with a 3Shape Trios intraoral scanner, the dental cement used for cementation was EsteCem II composite cement (Tokuyama Dental).

The cases featuring indirect ceramic restorations are divided into 4 groups, according to the use of:

- different techniques: classic; hybrid
- different materials for fabrication of indirect restorations: hybrid ceramic (Vita Enamic); lithium disilicate (IPS e.max CAD, Ivoclar Vivadent).



All groups featured in Task №3 are formed as follows:

- Group 0 (control group): teeth with direct composite restorations
- Group 1: teeth with classic technique indirect restorations, fabricated out of hybrid ceramic
- Group 2: teeth with classic technique indirect restorations, fabricated out of lithium disilicate
- Group 3: teeth with hybrid technique indirect restorations, fabricated out of hybrid ceramic
- Group 4: teeth with hybrid technique indirect restorations, fabricated out of lithium disilicate

Description of work stages for patients with direct composite restorations:

1. Anamnesis and status
2. Photodocumentation
3. X-ray before the beginning of treatment
4. Preparation of MO, DO or MOD cavity
5. Gingival margin level measurement

6. Probing depth measurement
7. Clinical attachment level calculations
8. Fabrication of direct composite restorations using Estelite Asteria (Tokuyama Dental)
9. X-ray after the end of treatment
10. Follow-up visit and X-ray after 6 months

1. Anamnesis and status

Detailed anamnesis and status were registered using the forms designated for the purpose.

2. Photodocumentation

Photodocumentation was done using Canon EOS 700D camera, a retractor and a set of mirrors for intraoral pictures.

3. X-ray before the beginning of treatment

Study participants underwent a bitewing radiographic examination with a dose of 30 mGy\*cm<sup>2</sup> and an exposure time of 9.9 sec after signing an informed consent for radiographic examination.

4. Preparation of MO, DO or MOD cavity

The following parameters were taken into consideration when preparing a class II cavity (MO, DO or MOD) for ceramic inlays:

- Cavity preparation was performed using Expert Set 4562 for ceramic inlays and partial crowns (Komet Dental).
- The prepared cavities had an inclination of the walls of 6-10°; minimum depth 1.5 mm, counted from the deepest part of the fissure; minimum width at the isthmus 2.5 mm; cervical margin located 1 mm below the CEJ; horizontal pulp base; rounded axiopulpal margin. Compliance with the cavity preparation criteria was checked with a UNC 15 periodontal probe.
- In Group 5,6,7,8, for the implementation of the hybrid technique, cervical margin relocation (CMR) was performed with the application of Estelite bulk fill

flow composite (Tokuyama Dental). The procedure was completed with thorough polishing using 4652-204 Polishing kit for composite (Komet Dental).

#### 5. Gingival margin level measurement

#### 6. Probing depth measurement

#### 7. Clinical attachment level calculations

For the purposes of these stage 5, 6 and 7, a periodontal probe UNC15 and a periodontal chart, introduced in the Department of Periodontology and dental implantology at the Faculty of dental medicine - Varna by Prof. Dr. Stefan Peev, MD, were used. All the measurements are in mm. In the periodontal chart, 6 values are filled in for each tooth, subject to clinical examination - 3 from the vestibular (MV, V, DV) and 3 from the palatal/lingual (ML, L, DL) side registering the level of margo gingivalis (MG) and probing depth (PD), clinical attachment level was also calculated. The measurements were registered during the patient's visit for cavity preparation from the side of the subgingival cervical margin.

Margo gingivalis (MG) level is the distance between the gingival margin and the CEJ, measured in mm using a periodontal probe. Depending on whether the gingival margin is located coronally, apically or at the level of the CEJ, a value is marked with "+", "-" or "0", respectively.

Probing depth (PD) is defined as the distance from margo gingivalis to the bottom of the sulcus or periodontal pocket, measured in mm using a periodontal probe. The probe is inserted parallel to the long axis of the tooth until resistance is felt by the soft tissues. After insertion of the periodontal probe to the bottom of the sulcus or periodontal pocket, we do not remove it until we move it with a step forward of 1 mm and an up-down movement of 1-2 mm. For each of the 6 parts of the tooth, we register the highest recorded value.

Clinical attachment level (CAL) represents the distance from the CEJ to the bottom of the periodontal pocket. It is usually calculated taking into account the other two parameters – probing depth (PD) and level of margo gingivalis (MG). A gain or loss of CAL can be observed as the distance between the CEJ and the bottom of the pocket decreases or increases, respectively. More than 1 periodontal status needs to be accounted for to track CAL dynamics. Changes in CAL provide information on the degree of destruction or acquisition of periodontal tissues.

## 8. Fabrication of direct composite restorations using Estelite Asteria (Tokuyama Dental)

The working filed is isolated with a rubber dam. An etching gel containing 39% phosphoric acid - Etching gel HV (Tokuyama Dental) was placed on the walls of the cavity preparation for 10-15 seconds. It was followed by thorough rinsing with water for a minimum of 15 seconds and drying using the air nozzle. After mixing the two components of Universal Bond (Tokuyama Dental), the bond was placed on the walls of the cavity preparation and air-dried. Estelite Asteria photopolymer composite (Tokuyama Dental) was then layered (Figure 32) with LM-Arte modeling tools. Each portion was polymerized with a photopolymerization lamp for 20 seconds. This was followed by inspection of the articulation contacts and thorough polishing using 4652-204 Polishing kit for composite (Komet Dental) (Figure 33).



*Figure 32*



*Figure 33*

## 9. X-ray after the end of treatment

Study participants underwent a bitewing radiographic examination immediately after completion of the composite obturation.

## 10. Follow-up visit and X-ray after 6 months

6 months after placement of the direct composite restoration, the patient was invited for a follow-up visit, during which photodocumentation was done again. Margo gingivalis level, probing depth and clinical attachment level were recorded for a second time. The study participant was sent for his 3rd bitewing X-ray.

## **Description of working stages for patients with indirect ceramic restorations:**

1. Anamnesis and status
2. Photodocumentation
3. X-ray before the beginning of treatment
4. Preparation of MO, DO or MOD cavity for ceramic inlays
5. Gingival margin level measurement
6. Probing depth measurement
7. Clinical attachment level calculations
8. Digital impression
9. Temporary obturation
10. Fabrication of ceramic inlays
11. Cementation of ceramic inlays with composite cement
12. X-ray after the end of treatment
13. Follow-up visit and X-ray after 6 months

### **1. Anamnesis and status**

Detailed anamnesis and status were registered using the forms designated for the purpose.

### **2. Photodocumentation**

Photodocumentation was done using Canon EOS 700D camera, a retractor and a set of mirrors for intraoral pictures.

### **3. X-ray before the beginning of treatment**

Study participants underwent a bitewing radiographic examination with a dose of 30 mGy\*cm<sup>2</sup> and an exposure time of 9.9 sec after signing an informed consent for radiographic examination.

### **4. Preparation of MO, DO or MOD cavity**

The following parameters were taken into consideration when preparing a class II cavity (MO, DO or MOD) for ceramic inlays:

- Cavity preparation was performed using Expert Set 4562 for ceramic inlays and partial crowns (Komet Dental).

- The prepared cavities had an inclination of the walls of 6-10°; minimum depth 1.5 mm, counted from the deepest part of the fissure; minimum width at the isthmus 2.5 mm; cervical margin located 1 mm below the CEJ; horizontal pulp base; rounded axiopulpal margin. Compliance with the cavity preparation criteria was checked with a UNC 15 periodontal probe.

- In Group 5,6,7,8, for the implementation of the hybrid technique, cervical margin relocation (CMR) was performed with the application of Estelite bulk fill flow composite (Tokuyama Dental). The procedure was completed with thorough polishing using 4652-204 Polishing kit for composite (Komet Dental).

## 5. Gingival margin level measurement

## 6. Probing depth measurement

## 7. Clinical attachment level calculations

For the purposes of these stage 5, 6 and 7, a periodontal probe UNC15 and a periodontal chart, introduced in the Department of Periodontology and dental implantology at the Faculty of dental medicine - Varna by Prof. Dr. Stefan Peev, MD, were used. All the measurements are in mm. In the periodontal chart, 6 values are filled in for each tooth, subject to clinical examination - 3 from the vestibular (MV, V, DV) and 3 from the palatal/lingual (ML, L, DL) side registering the level of margo gingivalis (MG) and probing depth (PD), clinical attachment level was also calculated. The measurements were registered during the patient's visit for cavity preparation from the side of the subgingival cervical margin.

Margo gingivalis (MG) level is the distance between the gingival margin and the CEJ, measured in mm using a periodontal probe. Depending on whether the gingival margin is located coronally, apically or at the level of the CEJ, a value is marked with "+", "-" or "0", respectively.

Probing depth (PD) is defined as the distance from margo gingivalis to the bottom of the sulcus or periodontal pocket, measured in mm using a periodontal probe. The probe is inserted parallel to the long axis of the tooth until resistance is felt by the soft tissues. After insertion of the periodontal probe to the bottom of the sulcus or periodontal pocket, we do not remove it until we move it with a step

forward of 1 mm and an up-down movement of 1-2 mm. For each of the 6 parts of the tooth, we register the highest recorded value.

Clinical attachment level (CAL) represents the distance from the CEJ to the bottom of the periodontal pocket. It is usually calculated taking into account the other two parameters – probing depth (PD) and level of margo gingivalis (MG). A gain or loss of CAL can be observed as the distance between the CEJ and the bottom of the pocket decreases or increases, respectively. More than 1 periodontal status needs to be accounted for to track CAL dynamics. Changes in CAL provide information on the degree of destruction or acquisition of periodontal tissues.

## 8. Digital impression

Before taking a digital impression with 3Shape Trios 3 Pod intraoral scanner (IOS) (Figure 34), calibration of the IOS was performed (Figure 35). A retraction cord soaked in an astringent solution was placed in order to visualize better the cervical margin on the intraoral scan. A digital impression was taken of the finished cavity preparation by scanning the patient's upper jaw, lower jaw and bite (Figure 36, Figure 38). A suitable color was chosen for the future restoration.

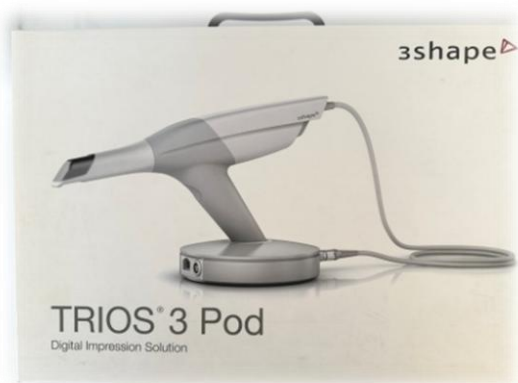


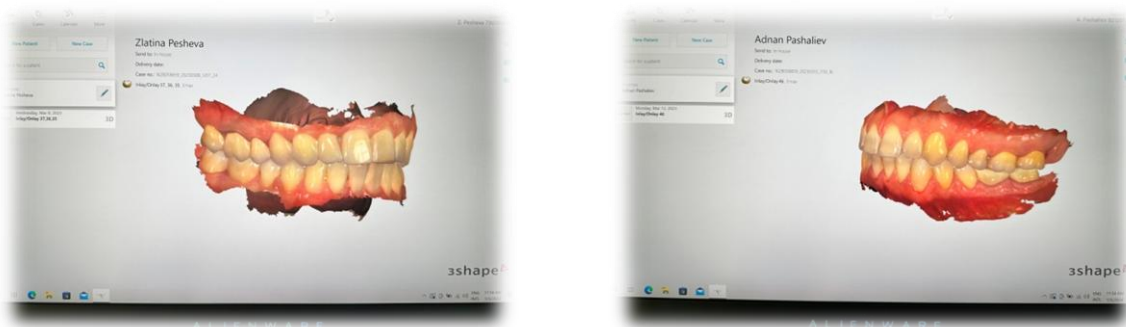
Figure 34



Figure 35



Figure 36



*Figure 37*

## 9. Temporary obturation

Telio Inlay/Onlay (Ivoclar Vivadent) temporary obturation material (Figure 38) in combination with LM-Arte modeling tools were used to place a temporary restoration. The placement was followed by photopolymerization for 20 sec. Articulation contacts were checked and adjusted.



*Figure 38*

## 10. Fabrication of ceramic inlays

In Group 2, 4, the indirect ceramic restorations were made out of milled lithium disilicate (IPS e.max CAD, Ivoclar Vivadent) (Figure 39), and in Group 1, 3 – out of hybrid ceramic (Vita Enamic) (Figure 40). After taking a digital impression, the design of the restorations was modeled on Exocad. The inlays were fabricated in a dental laboratory using CAD/CAM according to the manufacturer's instructions (Figure 41, 42, 43, 44).



*Figure 39*



*Figure 40*



*Figure 41*



*Figure 42*



*Figure 43*



*Figure 44*

#### 11. Cementation of ceramic inlays with composite cement

In all the groups with indirect ceramic restorations, the cementing agent used was EsteCem II composite cement (Tokuyama Dental). The working protocol is as follows: isolation of the working field with a rubber dam, after which an etching gel containing 9.5% hydrofluoric acid (Yellow Porcelain Etch, Cerkamed) is applied to the inner surface of the ceramic inlay for 60 seconds. It is then washed thoroughly with water and air-dried. A layer of silane (Silan, Cerkamed) is applied onto the inner surface of the inlay. An etching gel with 39% phosphoric acid content (etching gel HV, Tokuyama Dental) is placed on the walls of the cavity preparation for 10-15 sec, then rinsed for at least 15 sec and air-dried. The two components of Universal Bond (Tokuyama Dental) are mixed and placed on the inner surface of the ceramic inlay and on the walls of the tooth cavity preparation. The air nozzle is used to spread the bond layer evenly. The inner surface of the inlay is evenly coated with EsteCem II composite cement (Tokuyama Dental) and placed in the prepared cavity with moderate pressure. This is followed by photopolymerization for 2-4 sec in the beginning to remove excess dental cement, then a final photopolymerization for 20 sec is executed.

## 12. X-ray after the end of treatment

Every study participant underwent a bitewing radiographic examination immediately after the cementation of the ceramic inlay.

## 13. Follow-up visit and X-ray after 6 months

6 months after placement of the indirect ceramic restoration, the patient was invited for a follow-up visit, during which photodocumentation was done again. Margo gingivalis level, probing depth and clinical attachment level were recorded for a second time. The study participant was sent for his 3rd bitewing X-ray.

### 3.6. RESULTS, ANALYSIS, DISCUSSION TASK №3

Task №3: Follow-up for a period of 6 months of the clinical attachment level in patients with direct (composite obturations) and indirect (ceramic inlays) class II restorations (MO/DO/MOD) with a subgingival cervical margin (below the CEJ) at the primary visit.

The difference in average values is studied for all groups, for each group separately, by type of technique and by type of material.

In order to do the statistical analysis the following were used: descriptive statistics, Paired t-test and Student's t-distribution. Level of significance  $\alpha = 0.05$  was selected.

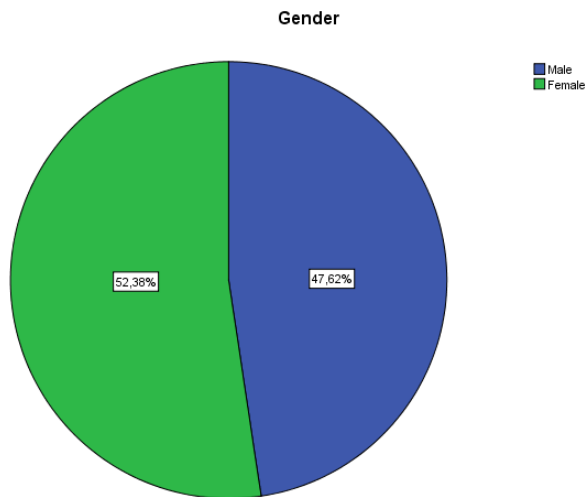
#### Descriptive statistics

Descriptive statistics – graphs (histograms, box and whisker charts and pie charts), frequency tables and numerical characteristics (mean, median, minimum, maximum value and standard deviation).

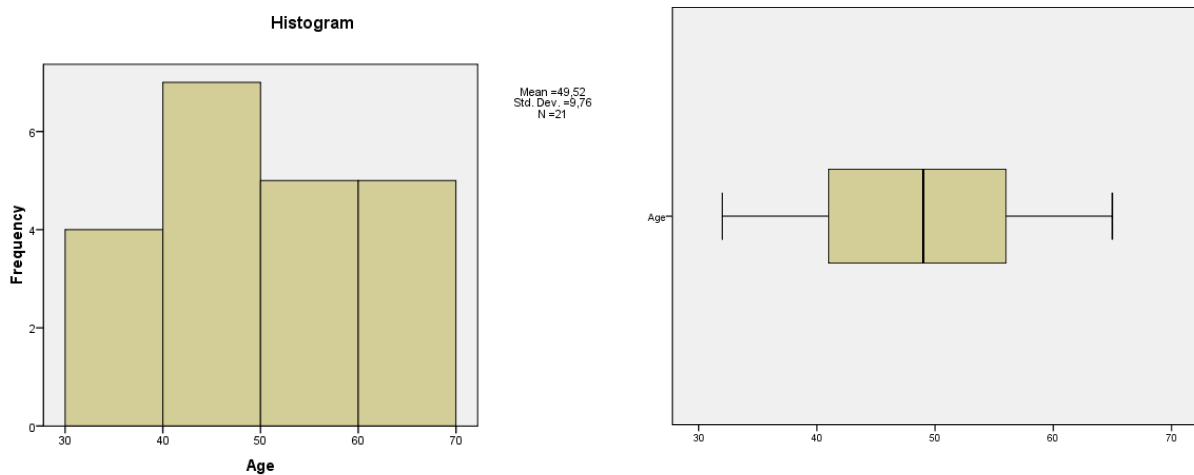
The sample includes 50 teeth, vital premolars or molars. In some cases, more than 1 tooth was treated on the same patient.

The patients were evenly distributed according to their gender – 47.6% men and 52.4% women.

Gender					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	10	47,6	47,6	47,6
	Female	11	52,4	52,4	100,0
	Total	21	100,0	100,0	



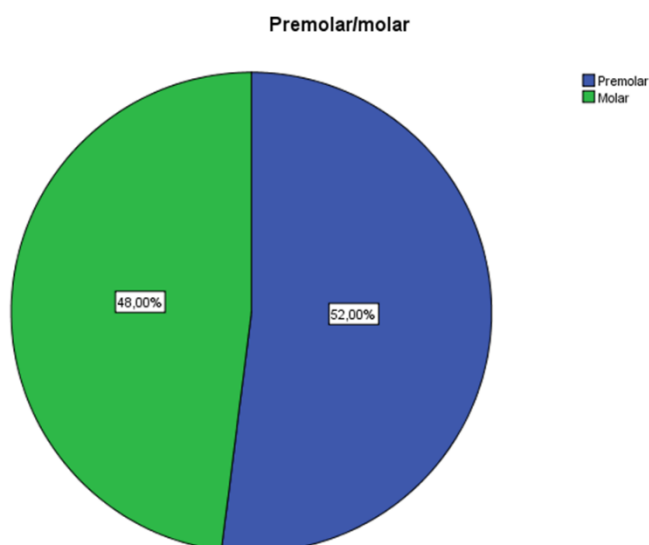
The study included patients in the age range of 32–65 years, who were almost symmetrically distributed. The mean age of the patients was 49.5 years. Half of the patients were 49 years of age or younger, and the other half were 49 years of age or older. Most of the patients included in the study were in the 40-50 age range.



The sample teeth were almost evenly distributed by type (molars and premolars), with 52% being premolars and 48% being molars.

**Premolar/molar**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Premolar	26	52,0	52,0	52,0
	Molar	24	48,0	48,0	100,0
	Total	50	100,0	100,0	



The sample teeth, according to the position of their cervical margin (medial and distal), were also almost evenly distributed, with 44% positioned on the medial side and 56% positioned on the distal side of the tooth.

**C.M. M/D**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Mesial	22	44,0	44,0	44,0
	Distal	28	56,0	56,0	100,0
	Total	50	100,0	100,0	

In the statistical analysis of the data from all groups (group 0, 1, 2, 3, 4) it is found that we have a statistically significant difference in probing depth before and 6 months after placement of the direct or indirect restoration, with a large effect size . The average probing depth decreased from 4.12 mm before to 3.53 mm after. The

clinical attachment level value also decreased from 3.89 mm before to 3.59 mm after, which means that we have clinical attachment level gain in the area of the subgingivally located cervical margin. A statistically significant difference was observed, with a small effect size. At the margo gingivalis level, a statistically significant difference was observed, again with a small effect size. The tendency is towards the formation of a slight recession, from 0.23 mm before to -0.06 after.

Although no statistically significant differences were reported in the clinical attachment level when comparing the groups separately, it can be noted that the greatest clinical attachment level gain was demonstrated by group 1 – classic technique for inlays, fabricated out of hybrid ceramic. Followed immediately by group 2 – classic technique for inlays, fabricated out of lithium disilicate. Control group 0 – direct obturation, ranks 3rd. In group 3 and group 4 - hybrid technique for inlays, fabricated out of hybrid ceramic and lithium disilicate, the clinical attachment level gain was of very small effect size.

In a clinical study, Ferrari M. et al. investigated the impact of the hybrid technique on periodontal health. The authors concluded that increased bleeding on probing was observed in the groups of hybrid technique compared to the group involving classic technique, which questions the overall positive effect of the hybrid technique. Bleeding on probing is an indicator of inflammation in the area, which under certain conditions can lead to deterioration of periodontal health over time. Cervical margin relocation aims to facilitate impression-taking, isolation and cementation of indirect restorations, but cannot improve the quality of the cement/dentin adhesive bond, nor avoid progressive degradation of the hybrid layer. Defects in the composite material due to inclusion of air during application, fractures and microleakage can be observed. Moreover, the porosity of the composite can lead to plaque retention with all the subsequent negative effects on the periodontal tissues.

A number of studies documented in the literature conclude that the evidence for the overall beneficial effect of CMR prior to the fabrication of partial indirect restorations is insufficient. This conclusion is also confirmed by the results obtained in task #3, since the groups with hybrid technique (group 3 and group 4) perform more unsatisfactory in clinical conditions compared to the other groups included in the study. Additional studies with long-term follow-up are needed in order to draw a more definitive conclusion about the effect of the hybrid technique on periodontal tissues and the survival rate of the restorations.

When comparing groups by the type of technique, a statistically significant difference in terms of clinical attachment level gain was reported for the classic

technique. It demonstrates slightly better clinical results compared to the direct composite obturation. One of the possible reasons is the polymerization shrinkage of composite materials, which is reduced to the thin layer of composite cement in ceramic inlays. Polymerization shrinkage can lead to microleakage, poor marginal adaptation and secondary caries. In a review article addressing the topic of direct vs. indirect restorations, Opdam, N. concluded that although traditionally indirect restorations are preferred because they are more stable, longer lasting and have a higher success rate in teeth with cervical margins of the defect located below the CEJ, direct restorations gradually start to demonstrate satisfactory results as well. The reason for this may be with the innovations in modern dentistry in terms of composite materials and adhesive systems. However, there are cases in which choosing an indirect restoration is preferred: when optimal contour and aesthetics of the restoration are needed, in case of more extensive defects, in case of difficulty in performing the direct technique. The choice of a restoration technique must be made according to a number of factors specific for each clinical case.

Regarding the choice of material, no statistically significant differences were reported with respect to the clinical attachment level. Statistically significant differences with a greater effect of decreasing probing depth were reported for ceramic materials compared to composite materials for direct obturation, with medium effect size. A number of studies prove the satisfactory qualities of indirect ceramic restorations.

## 4. SUMMARY

The answer to the question which is the most appropriate way to restore a class II defect of the dental crown with cervical margins located subgingivally is not definitive. A number of parameters must be taken into account, such as execution technique, restoration material, cementing agent, impression technique, aesthetic and functional qualities, time and resources that the patient is willing to invest in the treatment. It is of utmost importance for periodontal health to place a restoration that is non-plaqueretentive, although we are obstructed by the subgingivally located restoration margins.

In terms of marginal adaptation, no statistically significant differences were reported between the hybrid ceramic and milled lithium disilicate inlays. However, the results show a slightly better marginal adaptation of hybrid ceramic restorations. According to some authors, this may be due to the lower surface hardness of hybrid ceramic, which makes it easier to be manufactured and precisely cut using CAD/CAM technologies.

In terms of microleakage, composite cement demonstrated significantly better results than GIC when cementing ceramic inlays, and a recommendation can be made for its use in clinical practice.

In terms of clinical attachment level, the best result was demonstrated by indirect restorations made using the classic technique, followed by direct composite obturations. This finding may confirm the advantages of indirect restorations over direct restorations in the presence of Class II defects with a subgingivally located cervical margins. It cannot be definitively concluded that hybrid technique has an entirely positive effect.

In a number of studies, the research teams concluded that further investigation with longer follow-up is needed to conclude on the beneficial effect of hybrid technique with cervical margin relocation. The conclusion of this PhD supports this opinion. Numerous in vivo and in vitro studies documented in the literature demonstrate the positive qualities of indirect ceramic restorations. The best clinical performance in the limitations of the conducted in vivo study was demonstrated by inlays made according to the classic technique, fabricated out of hybrid ceramic.

We consider it necessary to continue the follow-up of clinical cases over time in order to gain even greater clarity about the effect on periodontal tissues and survival rate of the investigated techniques and materials for restoration of class II defects with subgingival cervical margins.

## **5. CONCLUSIONS**

1. Most patients who took part in the clinical study are in the age range of 40-50 years.
2. The teeth sampled for the clinical study, according to their type, molars and premolars, were almost evenly distributed, with 52% being premolars and 48% being molars.
3. Overall, all cases in the clinical study have a statistically significant decrease in probing depth and some degree of clinical attachment level gain.
4. Inlays made using the classic technique, fabricated out of hybrid ceramic demonstrate the most satisfactory clinical results.
5. Statistical significance in terms of the most favorable effect on clinical attachment level was demonstrated by the classic technique for indirect restorations.
6. Clinical cases with cervical margin relocation demonstrate the least positive effect on clinical attachment level.
7. In the experimental study investigating marginal adaptation, no statistically significant differences between the individual groups were reported, but it can be noted that hybrid ceramic demonstrated the best results.
8. In the experimental study investigating microleakage, statistically significant differences were registered in terms of the dental cements used for ceramic inlays cementation, in favor of composite cement over GIC.
9. For cementation of ceramic restorations, it is preferable to use composite cement.

## **6. CONTRIBUTIONS**

### **Original contributions**

1. For the first time, an in vitro comparison is made between the combinations of restorative techniques, dental cements and restorative materials studied in the PhD.

2. For the first time, an in vivo comparison is made between the combinations of restoration techniques and restoration materials investigated in the PhD.

### **Original contributions to the country**

1. For the first time, experimental studies are carried out on sample teeth, fully embedded in epoxy resin.

### **Affirmative contributions**

1. Composite cement is the preferred material for cementation of indirect ceramic restorations.

2. Hybrid ceramic inlays demonstrate the best results both experimentally and clinically, out of all the studied materials.

3. Ceramic CAD/CAM restorations have satisfactory clinical performance.

## **PHD ARTICLES**

1. Keremedchieva, S., Peev, S., Gusiyska, A., & Sabeva, E. (2020). Cervical margin relocation - basic principles and influence on the periodontal tissues. Scripta Scientifica Medicinae Dentalis, 6(2), 12-16. doi:<http://dx.doi.org/10.14748/ssmd.v6i2.7377>

2. Keremedchieva, S., Peev, S., & Parushev, I. (2023). Marginal adaptation of ceramic inlays—an in vitro study. *Scripta Scientifica Medicinae Dentalis*, 9(2), 61-67. doi:<http://dx.doi.org/10.14748/ssmd.v9i2.9365>
3. Keremedchieva, S., & Peev, S. (2023). Microleakage in composite and ceramic restorations—a review of staining protocols. *Scripta Scientifica Medicinae Dentalis*, 9(2), 27-34. doi:<http://dx.doi.org/10.14748/ssmd.v9i2.9364>