MEDICAL UNIVERSITY
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Study of the properties of temporary filling materials

ABSTRACT

of a dissertation for acquiring of an

educational and scientific degree

"doctor"

SCIENTIFIC SPECIALTY:

Therapeutic dentistry

SCIENTIFIC SUPERVISOR:
Assoc. Prof. Miglena Balcheva – Eneva, MD, PhD

Varna 2024

The dissertation has been approved and sent for defense at a meeting of the Department of "Conservative Dentistry and Oral Pathology" at the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

The dissertation contains 165 standard pages and is illustrated with 34 tables and 55 figures. The bibliography consists of 211 sources, of which 9 are in Cyrillic and 202 are in Latin.

The public defense of the dissertation work will take place on 15.07.2024 at 15:00, before a scientific jury composed of:

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

Note: The numbers of the figures and tables in the abstract do not correspond to the numbers in the dissertation.

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Abbreviations

AMA	Antimicrobial activity
AME	Antimicrobial effect
BHI	Brain Heart Infusion Broth
BisGMA	Bisphenol A-glycidyl methacrylate
BMS	Burning Mouth Syndrome
CA	Contact allergy to dental materials
CHX	Chlorhexidine
DA	Drug allergy
EBA	Ethoxybenzoic acid
EGDMA	Ethylene glycol dimethacrylate
FA	Food allergy
GIC	Glass ionomer cement
HDT	Hard dental tissues
2-HEMA	2-Hydroxyethyl Methacrylate
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MMA	Methyl methacrylate
PA	Pollen allergy
TEGDMA	Triethylene glycol dimethacrylate
UDMA	Urethane Dimethacrylate
Zn	Zinc
ZOE	Zinc oxide-eugenol cement

I. INTRODUCTION

Dental caries is a pathological process characterized by demineralization and destruction of enamel, dentine and cementum. It affects not only the physical but also the mental health of the individual. It develops in conditions of a changed oral environment and its final phase is a pronounced carious lesion. Oral microflora, saliva and acidity in the oral cavity play a role in its etiology.

Untreated, it can quickly lead to the involvement of the pulp and periodontium, which requires a different treatment time, the application of complex manipulations and the use of a large set of different instruments, medical products and dental materials.

Treatment of dental caries and its complications sometimes requires a multi-stage approach, which can be the result of a number of reasons such as persistent infection or lack of time on the part of the patient or the dental practitioner. For optimal results, the filled cavity should be sealed between visits. Its sealing is achieved with temporary filling materials. The aim is to protect the pulpo-dentinal complex in the case of caries or to prevent the entry of microorganisms that could compromise an ongoing root canal treatment. In addition, these materials must meet a number of other requirements such as restoration of occlusal relationships and lost tooth function.

A number of temporary obturation materials are available on the market, which differ in composition and properties. The choice of a specific temporary obturation material depends on various factors.

II. AIM AND TASKS

Aim:

Study the properties of temporary filling materials and their capabilities for HDT protection and tooth/cavity sealing in multi-stage treatment.

Task:

1. Study the antibacterial qualities of materials for temporary obturation.
    1. Determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)
    2. Use cup plate technique
    3. Track the dynamics of the vitality of the microbial culture in a closed system
2. Study the microleakage in different types of temporary filling materials.
    1. Study the microleakage in teeth with temporary obturation for a 2-day period
    2. Study the microleakage in teeth with temporary obturation for a 14-day period
3. Establish the sensitizing potential of temporary filling materials.

III. MATERIALS AND METHODS

Materials and methods for task 1.

Materials and methods for subtask 1.1. Determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

The study investigates the antimicrobial effect (AME) of eugenol and zinc oxide as part of the composition of a material for temporary obturation of the endodontic cavity. As a positive control, we used chlorhexidine gluconate 2%, whose antimicrobial activity (AMA) was also determined.

The Eugenol and ZnO (brand: i-ZOE) were provided by i-dental, and the chlorhexidine gluconate (brand: Gluco-CHeXIN 2%) was provided by PPH CERKAMED. AME of the described compounds was studied against five reference bacterial strains:

- *Esherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 29213
- *Klebsiella pneumoniae* ATCC 13883
- *Pseudomonas aeruginosa* ATCC 27853
- *Streptococcus pyogenis* ATCC 12384

and one strain of Candida fungi:

- *Candida albicans* ATCC 10231

All microbial strains are from the brand Micro Swap and provided by Ridacom, Bulgaria.

The microbiological studies were carried out in MC-Varna, in the period May-June 2023.

We used brain-heart broth (HiMedia, Ridacom, Bulgaria) to make the **serial dilutions** of eugenol, ZnO and chlorhexidine gluconate. The eugenol was tested at five different concentrations in the range of 50% (v/v) – 3%(v/v), using only brain-heart infusion broth (BHI broth) as a diluent. ZnO was pre-diluted with sterile distilled water (5% w/v) and serial dilutions in the range 2.5% (w/v) – 0.15% (w/v) were subsequently made. A combination of eugenol and ZnO was also proposed, where the eugenol was of varying concentrations 50% – 3% (v/v), and the concentration of ZnO was constant in each test tube at 5% (w/v).

We examined the positive control of chlorhexidine gluconate in concentrations of 1-0.06% suspension. All concentrations of antimicrobial agents were tested in 1 ml of BHI broth for each strain in four rows of five test tubes.

After making the serial dilutions, 0.1 ml of a densitometer-standardized microbial suspension with a density corresponding to 0.5 MF was added to each compound. All samples were triplicated. Six positive controls with 1 ml of BHI broth and 0.1 ml of each microbial strain standardized to 0.5 MF were made, as well as negative controls in four tubes with 1 ml of BHI broth and the highest test concentrations of eugenol (50% v/v), ZnO (5% w/v), chlorhexidine (XX) – 1% (v/v) and pure BHI broth.

All samples were incubated aerobically for 24 hours at 37°C and for 48 hours at 45°C for Candida albicans. After the incubation period, the turbidity of each suspension was visually monitored for the presence of microbial growth and absence of an inhibitory effect on the part of the test agent. For MIC, we assumed

the highest concentration of the respective compound at which no visual turbidity was observed.

When determining MBC, we used the test samples to determine MIC. From all suspensions in which no microbial growth was observed visually, we transferred a suspension to a volume taken once with an inoculating loop in blood agar. We cultivated the agar media for one day in a thermostat at a temperature of 37°C for all bacterial species and for 48 hours at 45°C for Candida albicans.

For MBC, we adopted the lowest concentration of the antimicrobial agent at which microbial growth was kept to 99.9%.

Materials and methods for subtask 1.2. Cup plate technique

The study investigates the AME of eugenol and zinc oxide as part of the composition of a material for temporary obturation of the endodontic cavity. As a positive control, we used chlorhexidine gluconate 2%, whose AMA was also determined.

Eugenol and ZnO are identical to those in subtask 1.1. AME of the described compounds was studied against five reference bacterial strains and one Candida fungal strain – the same as in subtask 1.1. The microbiological studies were carried out in MC – Varna, in the period May-June 2023. We tested the AME of pure eugenol, 5% ZnO solution, 2% chlorhexidine digluconate and eugenol with ZnO in a ratio necessary to obtain ZOE, described by the manufacturer in the accompanying instructions for use.

We determined the antimicrobial effect of the investigated compounds in Müller-Hinton agar (HiMedia, Ridacom, Bulgaria). We initially created solid inoculations on the surface of agar media with standardized 0.5 MF microbial

strains. After drying the agar surfaces for 15 minutes at room temperature, we made fossa with a diameter of 6 mm with a sterile Corkborer (HiMedia, Ridacom, Bulgaria). We placed 100 μl of each of the investigated antimicrobial agents in the resulting fossa. All samples were made in triplicate.

All bacterial strains were cultivated for 24 hours in aerobic conditions at a temperature of 37°C, and C. albicans – for 48 hours at a temperature of 45°C. After the incubation period, the absence/presence of AME was determined in the zones of inhibition around the agar fossa and their diameter in mm.

Materials and methods for subtask 1.3. Tracking the dynamics of the vitality of the microbial culture in a closed system

The study investigates the AME of eugenol and zinc oxide as part of the composition of a material for temporary obturation of the endodontic cavity. As a positive control, we used chlorhexidine gluconate 2%, whose AMA was also determined.

The eugenol and ZnO are identical to those of subtask 1.1. AMD of the described compounds was studied against two reference bacterial strains:

- *Esherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 29213

and one strain of Candida fungi:

- *Candida albicans* ATCC 10231

The microbiological studies were carried out in MC – Varna, in the period May-June 2023.

This research technique involves introducing microbes into a nutrient medium along with an inhibitory factor and monitoring the viability of the microbial population over various time intervals. Viability is tested by removing a test sample from the closed system and transferring it to a medium in order to equate the number of viable cells per milliliter of microbial suspension (the so-called microbial count). The purpose of this technique is not only to determine the AMA of the inhibitory agent, but also to monitor these effects at specific time intervals.

We prepared the following test solutions:

• BHI broth with 50% (v/v) eugenol solution (0.5 ml brain-heart broth with 0.5 ml 100% eugenol);

• BHI broth with 5% (v/v) zinc oxide solution (0.5 ml BHI broth with 0.5 ml 10% (w/v) zinc oxide aqueous solution);

• BHI broth with 1% (v/v) XX digluconate solution (0.5 ml BHI broth with 0.5 ml 2% chlorhexidine solution). Chlorhexidine was used as a positive control in the study;

• To monitor the optimal microbial growth of the test microorganisms, we also set a negative control containing only 0.5 ml of BHI broth along with 0.5 ml of sterile distilled water.

In all test solutions, we placed 0.1 ml of a microbial suspension of the respective microbial strain, standardized with a densitometer to 0.5 MF units. All samples were created three times.

We followed the dynamics of microbial growth through periodical inoculations of 0.1 ml on the surface of blood agar using a sterile Drigalski spatula.

The intervals and times of measurement/screening were as follows: 0 hour, 30 min, 1 hour, 3 hour and 6 hour.

To determine the microbial number (number of living cells per milliliter of suspension) in each interval we removed 0.1 ml of microbial suspension, with which we made a serial dilution. This technique is necessary because it is possible that when directly transferring a volume of the sample onto an agar medium, the number of microbial colonies is too high and impossible to enumerate (over 400 colonies on the surface of the agar) and equate to a milliliter of sample. The additional tenfold dilution of the sample from 10^{-1} to 10^{-5} allows the enumeration of an acceptable number of colonies on blood agar (up to 300-400 colonies) and their equating to 1 ml of sample. The technique for making a serial dilution involves withdrawing 0.1 ml of sample from the test tube and mixing it with 0.9 ml of sterile saline (dilution 10^{-1}). We mixed the suspension for a minimum of 5 seconds using a vortex (CappRondo, Ridacom, Bulgaria) and transferred 0.1 ml to another tube with 0.9 ml of saline solution (10^{-2}). We repeated the procedure to a dilution of 10^{-5}, taking out 0.1 ml of suspension in blood agar from each solution using an automatic pipette and distributing the liquid evenly with a sterile Drigalski spatula. Blood agar samples were incubated for 24 hours at 37°C for both bacterial strains and for 48 hours at 45°C for Candida albicans. Cultivation took place under aerobic conditions. All triplicated samples were averaged; the values in the time intervals were entered into Excel, Microsoft Office Professional Plus 2013 and visualized with appropriate charts.

Materials and methods for task 2

Materials and methods for subtask 2.1. Study of microleakage in teeth with temporary obturation for a 2-day period

30 freshly extracted human teeth without visible defects on the smooth surfaces and without cavitating carious lesions occlusally, stored in distilled water, were used. To rule out fractures, cracks and other defects, the teeth were previously examined under a microscope. Photographs were taken using an Olympos SZ51 optical microscope with x10 magnification and digital microscope camera TP6080000B.

Study algorithm: The prepared 30 teeth were divided into 3 groups of 10 teeth each, which were filled with three different materials for a period of 2 days (Table 1). Cavities were made on the lingual or occlusal surface of the teeth, using conventional tools – diamond drills with a green marking for a high-speed tip under water-air cooling. After preparation, the teeth are again inspected for defects, cracks and fractures.

Table 1: Distribution of teeth by groups

Obturation material	Brand	Total number	Incisors	Canines	Premolars
Citodur	DoriDent	10	4	4	3
IRM	Dentsply	10	3	4	3
i-Pro	i-dental	10	4	3	3

The first group was filled with Citodur (DoriDent) (Fig. 1), the second group – with zinc oxide-eugenol cement IRM (Dentsply) (Fig. 2) and the third group – with photopolymerizing material for temporary filling i-PRO LC (i-dental) (Fig. 3).

All cavities are made before placement of the packing material, depending on the manufacturer's recommendations.

In the first group, after the preparation, the cavity is wiped with a cotton pellet soaked in chlorhexidine gluconate and dried. Citodur is applied in one portion using a reverse spatula and adjusted to the cavity walls.

Figure 1: Citodur

Figure 2: IRM

Figure 3: i-PRO

In the second group, after the preparation, the cavity is wiped with a cotton pellet soaked in chlorhexidine gluconate and dried. ZOE is applied in one portion using a reverse spatula and adjusted to the walls of the cavity.

In the third group, the same protocol as the previous one was used for the light-cured temporary restorations. The material is applied using a reverse spatula and light-cured for 20 seconds, according to the manufacturer's instructions.

After obturation, the teeth are isolated with contrast varnish except for the cavities and 2 mm around (Fig. 4). All samples were thermally cycled under the following regime: 6 cycles, temperature 5-55°C, dwell time 20s. They were placed

in a dye – 1% solution of fuchsine for 8 hours, after which they were washed and cut vertically through the crown and the root with a diamond separator (Fig. 5).

Figure 4: Varnished teeth

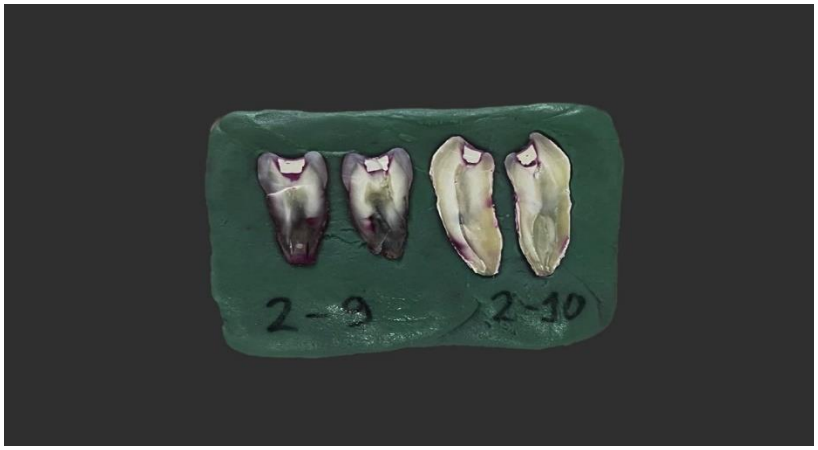

Figure 5: Ready samples

Obturated cavities were examined with an optical microscope Olympos SZ51 with x8, x15 and x25 magnifications. The degree of microleakage was reported by measuring the penetration depth dm of the dye between the obturation and the cavity wall, lingually and buccally. For this purpose, a Zuzi Ucmos digital microscope camera TP6080000B and TourView software were used.

Average microleakage values in the buccal (Mb) and lingual (Ml) regions of the cavity were calculated. Relative microleakage (%) was calculated in the buccal (Mb rel) and lingual (Ml rel) regions of the cavity for the individual groups. The

average relative microleakage M rel (%) for the individual groups was calculated according to the following formula:

$$\text{M rel} = \frac{\text{Mb rel} + \text{Ml rel}}{2}\% \quad (1)$$

Materials and methods for subtask 2.2. Study of microleakage in teeth with temporary obturation for a 14-day period

30 freshly extracted human teeth without visible defects on the smooth surfaces and without cavitating carious lesions occlusally, stored in distilled water, were used. They are selected in the same way as in subtask 2.1.

Study algorithm: The prepared 30 teeth are divided into 3 groups of 10 teeth each, identical to the groups in subtask 2.1., and obturated with three different materials for a period of 14 days. The cavities and materials are also identical to those in subtask 2.1. All samples were thermally cycled under the following regime: 42 cycles, temperature 5-55°C, dwell time 20s. They were placed in dye – 1% fuchsine solution for 8 hours, then washed and cut vertically through the crown and root with a diamond separator. The examination and determination of microleakage is identical to the way specified in subtask 2.1.

Materials and methods for task 3.

To confirm allergy to dental materials, we used standard allergens from dental materials from Chemotechnique Diagnostics (Vellinge, Sweden). Hypoallergenic patches with polyethylene chambers IQ Ultra Chambers® and IQ Ultimate Chambers®, as well as the skin marker were from the same company.

We compiled a panel of 9 allergens – ingredients of temporary filling materials with established strong sensitizing properties (Table 2). We applied them through patch test to confirm contact hypersensitivity. We placed the allergens according to the requirements of the test on the skin of the back (healthy, hairless and without tan) in the area between the first thoracic vertebra, crista iliaca and the spine. We removed them after the 48th or 72nd hour, and the results were reported according to the requirements of the International Contact Dermatitis Research Group (ICDRG) on the scale indicated in Table 3. The additional condition for the patients was to take no antihistamines during the testing and 7 days beforehand, and no corticosteroids for at least 14 days before.

Table 2: A panel of standard dental allergens for patch testing

№	Dental allergen	Concentration
1	Methyl methacrylate (MMA)	2,0% petrolatum
2	Triethylene glycol dimethacrylate (TEGDMA)	2,0% petrolatum
3	Ethylene glycol dimethacrylate (EGDMA)	2,0% petrolatum
4	Bisphenol A glycerolate dimethacrylate (BIS-GMA)	2,0% petrolatum
5	2-Hydroxyethyl methacrylate (2-HEMA)	2,0% petrolatum
6	Eugenol	2,0% petrolatum
7	Zinc (Zn)	2,5% petrolatum
8	Epoxy resin (Bisphenol A)	1,0% petrolatum
9	Urethane dimethacrylate (UDMA)	2,0% petrolatum

Table 3: A scale for reporting the results of patch testing

Code	Reaction
-	Negative reaction
?	Doubtful reaction
+	Weak positive reaction (non-vesicular)
++	Strong positive reaction (edematous or vesicular)
+++	Extreme positive reaction (ulcerative or bullous)
IR	Irritant reaction

Statistical methods

For statistical analysis of the data, a specialized statistical analysis package IBM SPSS Statistics 20 was used. A p-value that is less than the chosen significance level α was chosen as the significance level.

For the purposes of the study, the following statistical methods were applied – descriptive analysis, statistical inference, correlation analysis, non-parametric methods.

IV. RESULTS AND DISCUSSION

Results and discussion for task 1

Results and discussion for subtask 1.1. Determining the MIC and MBC of eugenol, zinc oxide and chlorhexidine

When determining the MIC of eugenol, ZnO, and the combination of eugenol and ZnO against 7 microbial strains, we observed some opalescence and yellowish color in the eugenol solutions and a white powdery precipitate in the zinc oxide solutions compared to the set negative controls of the suspensions (BHI with eugenol, BHI with ZnO and BHI with ZnO and eugenol) (Fig.6).

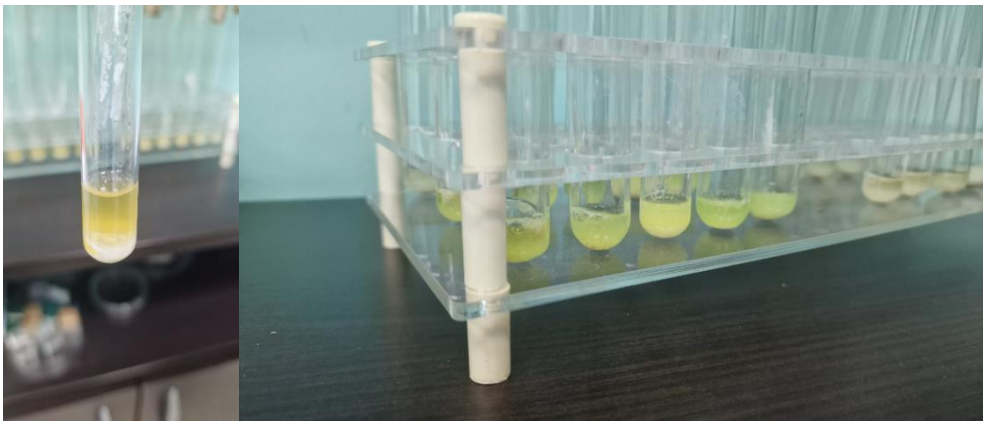

Figure 6: Determining the MIC of eugenol, ZnO and eugenol + ZnO combination against 7 microbial strains

We reported MIC for all solutions. We reported the strongest AME in eugenol solutions against gram-negative strains of E. coli, K. pneumoniae, P. aeruginosa with MPC lower than 3% (v/v), against S. aureus – 25% (v/v) and lack of AME of eugenol against C. albicans and S. pyogenes at concentrations 50% (v/v) and lower ($p<0.05$) (Fig.7).

A number of other authors presented results on the strong antimicrobial activity of eugenol, and some of them also mentioned reporting a more pronounced

AME of the compound against Gram-negative isolates using the method of serial dilutions for the determination of MIC.

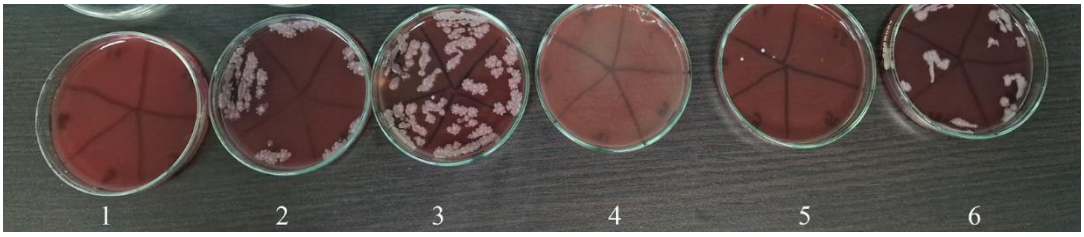

Figure 7: Determination of MBC of eugenol (starting concentration 50% (v/v)) against 1 – E. coli, 2 – S. aureus, 3 – C. albicans, 4 – K. pneumoniae, 5 – P. aeruginosa and 6 – S. pyogenes

Eugenol also demonstrated high AMA to multidrug-resistant clinical isolates of the types we studied, such as methicillin-resistant S. aureus – MRSA strains, fungal strains and others.

ZnO inhibits microbial growth both in liquid suspension and on agar medium at its highest concentration of E. coli, K. pneumoniae, C. albicans (MIC – 2.5% (w/v)), for the other strains – S. aureus, S. pyogenes, P. aeruginosa we did not detect AME at the set concentrations related to pronounced antimicrobial activity to any of the investigated strains ($p>0.05$) (Fig. 8).

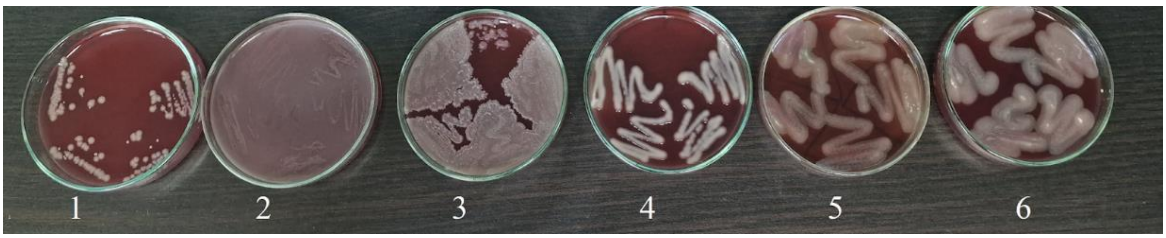

Figure 8: Determining the MBC of ZnO (starting concentration 5% (w/v)) against 1 – E. coli, 2 – S. aureus, 3 – C. albicans, 4 – K. pneumoniae, 5 – P. aeruginosa and 6 – S. pyogenes

When eugenol was combined with ZnO, the results overlapped to some extent with eugenol alone – *E. coli, K. pneumoniae* and *P. aeruginosa* with MIC and MBC < 3% (v/v) (Fig.9). With the combination, we also reported AME against *S. aureus* – MIC – 50% (v/v) eugenol + 5% (w/v) ZnO. On agar medium, the growth of *S. aureus* was not completely restricted at the target concentrations

including 50% (v/v), but the number of bacterial colonies was significantly lower. In relation to *C. albicans* and *S. pyogenes*, we did not report an inhibitory effect on microbial growth ($p>0.05$).

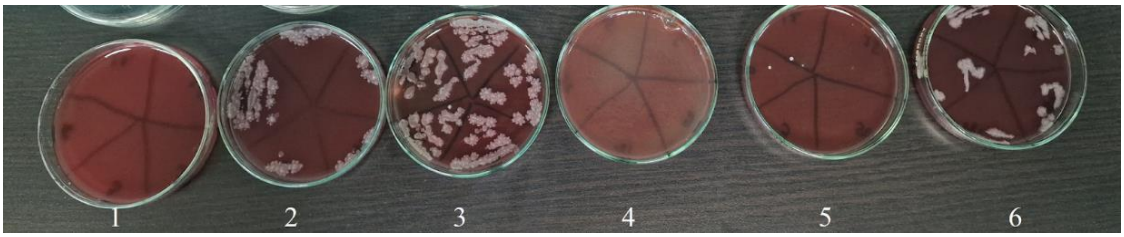

Figure 9: Determining the MBC of eugenol (starting concentration 50% (v/v)) with ZnO (5% (w/v)) against 1 – E. coli, 2 – S. aureus, 3 – C. albicans, 4 – K. pneumoniae, 5 – P. aeruginosa and 6 – S. pyogenes

The AME of chlorhexidine is well known and researched in a number of studies. Control samples with chlorhexidine showed AME against all tested bacteria – *E. coli, K. pneumoniae, P. aeruginosa, S. pyogenes* at all concentrations – MIC and MBC < 0.06%, compared to *C. albicans* MIC is higher – 0.12%, MBC – 0.25%, therefore chlorhexidine is a universal antimicrobial agent ($p>0.05$) (Fig. 10).

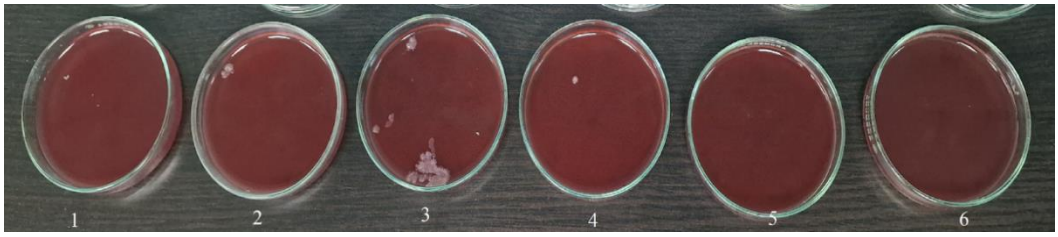

Figure 10: Determination of MBC of chlorhexidine (starting concentration 1%) against 1 – E. coli, 2 – S. aureus, 3 – C. albicans, 4 – K. pneumoniae, 5 – P. aeruginosa and 6 – S. pyogenes

Results and discussion for subtask 1.2. Cup plate technique

We created the cup plate technique with 100% eugenol solution, 5% (w/v) ZnO solution, 2% XX solution and prepared cement from eugenol and ZnO in the ratio set by the manufacturer. All compounds demonstrated diffusibility in agar medium to varying degrees. Eugenol demonstrated antimicrobial action against all

tested microbes with the largest inhibition zone for *K. Pneumoniae* – 25 mm and the smallest for *S. pyogenes* – 16 mm. The rest of the zones of inhibition of the eugenol are as follows: *E. coli* – 21 mm, *S. aureus* – 16 mm, *C. albicans* – 20 mm and *P. aeruginosa* – 18 mm ($p<0.05$) (Fig. 11).

Figure 11: Antimicrobial activity test of eugenol (E), ZnO, eugenol+ZnO and chlorhexidine (CHX) against E. coli (left) and S. aureus (right)

In contrast to the results in liquid medium, where the highest concentrations of eugenol applied were a 50% (v/v) solution tested with the cup plate technique and a 100% eugenol solution, the compound demonstrated AME against all microbial strains.

The bacterial species we tested and the action of eugenol as an inhibitor of their growth have been investigated in a number of studies. Of particular interest is *P. aeruginosa*, as it is the causative agent of a number of diseases with varying degrees of complexity and a frequent isolate in nosocomial infections. *Kotan et al.* reported a high AMA of eugenol against *Pseudomonas* as tested in agar medium. Their study also suggested that in a number of cases, testing the inhibitory activity of eugenol using different methodologies could lead to ambiguous results. The choice of medium – liquid or agar, the type of strain, and the antibacterial source of the sample are important. Our study also showed some discrepancy between the AMA of eugenol tested in liquid medium (activity against *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus*) and solid agar medium (activity against *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. pyogenes* and *C. albicans*). The authors

consider the disc-diffusion methods and generally the agar techniques (cup plate technique) to be more reliable. The obtained zones of inhibition when following the effect of eugenol unambiguously show increased activity towards Gram-negative microbes with zones of inhibition between 18-25 mm. These results have also been obtained by other studies. For Gram-positive strains, the zones of inhibition varied with an average value of 16 mm, a result confirmed by other authors.

Only ZnO showed AMD against three of the six analyzed strains – *E. coli, C. albicans* and *K. pneumoniae* with inhibition zones as follows: *E. coli* – 16 mm, *C. albicans* – 20 mm, *K. Pneumonia* – 19±1 mm and no AME antimicrobial activity was reported against *S. aureus, P. aeruginosa* and *S. pyogenes* ($p<0.05$). (Fig. 12). The results completely overlapped with those obtained when reporting the MIC and MBC of ZnO in relation to the investigated microbes.

Figure 12: Antimicrobial activity test of eugenol (E), ZnO, eugenol+ZnO and chlorhexidine (CHX) against C. albicans (left) and K. pneumoniae (right)

According to *Mendes et al.* (2022), the antimicrobial action of zinc oxide has been the subject of a number of studies and has been successfully demonstrated, although the mechanism of its inhibitory activity has not been fully established. According to their in vitro studies, the compound has AME against not only *E. coli* but also *S. aureus* and *P. aeruginosa* at higher zinc oxide concentration.

Eugenol + ZnO, prepared as cement, in all samples showed an enhanced AME compared to the effect of eugenol and ZnO alone, i.e. demonstrate a synergistic interaction. Studies by other researchers confirm our results.

The maximum antimicrobial activity of the combination Eugenol + ZnO was found for *K. pneumoniae* with an inhibition zone of 37 mm, and the smallest antimicrobial activity and, respectively, the smallest inhibition zone for *P. aeruginosa* – 20 mm. The remaining zones of inhibition are as follows: *E. coli* and *S. aureus* – 30 mm each, *C. albicans* and *S. pyogenes* – 26 mm ($p<0.05$). Chlorhexidine has a more powerful effect on *P. aeruginosa* (Fig. 13).

Figure 13: Antimicrobial activity test of eugenol (E), ZnO, eugenol+ZnO and chlorhexidine (CHX) against P. aeruginosa (left) and S. pyogenes (right)

The zones of inhibition reported for all isolates were comparable to those reported for a number of antibiotics. Analogous studies conducted by other researchers also reported similar inhibitory activity of the combination against Streptococcus. The used positive control also demonstrated weaker antimicrobial activity.

Chlorhexidine showed a weaker effect compared to the eugenol + ZnO combination: respectively *E. coli* – 25 mm, *S. aureus* – 20 mm, *C. albicans* – 26 mm, *K. Pneumonia* – 24±1 mm, *S. pyogenes* – 25 mm. Only against *P. aeruginosa*, the action of chlorhexidine was reported to be more powerful – 25 mm zone of inhibition, compared to 20 mm for the eugenol + ZnO combination ($p<0.05$).

In the AMA agar test technique, eugenol and the eugenol + ZnO combination showed antimicrobial activity against all microbes tested, and no AME was reported against *C. albicans* and *S. pyogenes* in the MIC/MBC assay techniques. The most likely reasons for this are the spectrum of tested concentration – from 50% to 3%, and the choice of medium. In the cup plate technique, we applied a 100% solution where the compound inhibited the growth of all strains of microbes.

All results of the studied AMA (zones of inhibition, mm) of eugenol, ZnO, eugenol + ZnO and chlorhexidine against *E. coli, K. pneumoniae, S. aureus, C. albicans, P. aeruginosa, S. pyogenes* tested by cup plate technique are presented in Table 4.

Table 4: Determining the antimicrobial activity of inhibitory agents against *E. coli, S. aureus, K. preumoniae, S. pyogenes, P. aeruginosa* and *C. albicans* through the cup plate technique (zones of inhibition in mm)

	Eugenol	**ZnO**	**Eugenol+ZnO**	**CHX (control)**	**p-value**
E. coli	21	16	30	25	p<0.05
S. aureus	16	-	30	20	p<0.05
C. albicans	20	18	30	26	p<0.05
K. pneumoniae	25	19(±1)	37	24(±1)	p<0.05
P. aeruginosa	18	-	20	28	p<0.05
S. pyogenes	16	-	26	25	p<0.05
p-value	p<0.05	p<0.05	p<0.05	p<0.05	

Results and discussion for subtask 1.3. Tracking the dynamics of the vitality of the microbial culture in a closed system.

The dynamics of microbial vitality we tracked under the effect of 50% (v/v) eugenol (Table 5, Fig. 14) shows its powerful inhibitory role against *E. coli* and *C.*

albicans after only 30 minutes and against *S. aureus* after 1 hour of action. For these time intervals, the microbes were completely destroyed (after 1 hour all samples were sterile), and for the 6-hour follow-up, the viability of the microorganisms was not restored. These results, as well as the results obtained with the cup plate technique, lead us to conclude that although eugenol does not demonstrate AME against *C. albicans* when measured by MIC, it does in fact have an inhibitory effect against the strain.

Table 5: Dynamics of the microbial density (number of viable cells/milliliter sample - MP) of *E. coli*, *S. aureus* and *C. albicans* in culture medium with 50% (v/v) eugenol

	0 hour	0.5 hour	1 hour	3 hours	6 hours
E. coli	7000000	-	-	-	-
S. aureus	6500000	6000000	-	-	-
C. albicans	6000000	-	-	-	-

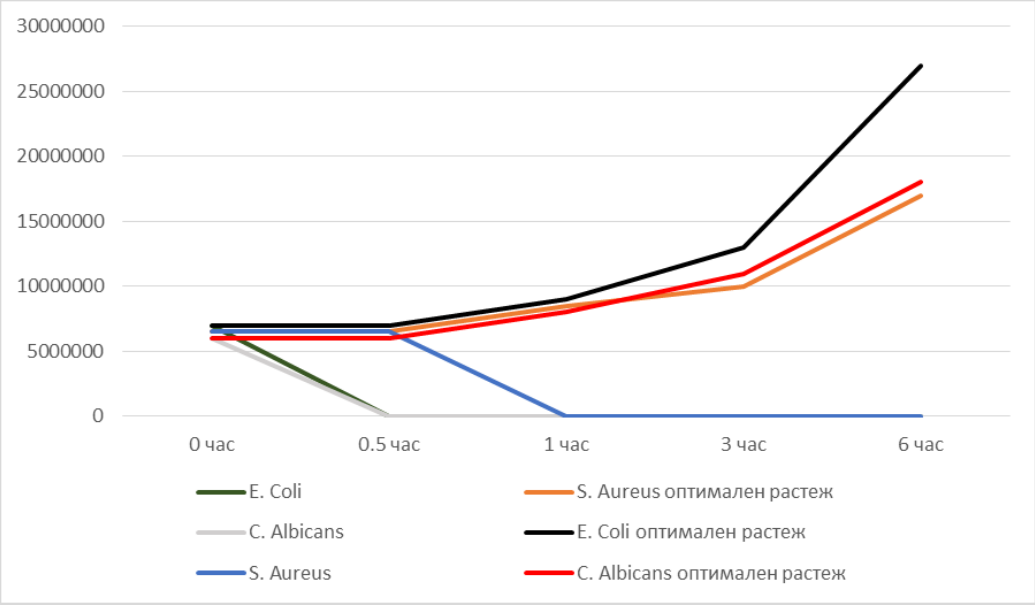

Figure 14: Microbial density dynamics (number of viable cells/milliliter sample - MIC) of *E. coli*, *S. aureus* and *C. albicans* in culture medium with 50% (v/v) eugenol compared with microbial density dynamics in culture medium with 50 % (v/v) distilled water (negative control)

The action of 1% (v/v) chlorhexidine digluconate, set as a positive control (Table 6, Fig. 15) is similar to that of eugenol – complete retention of microbial vitality after the 1st hour and until the end of the follow-up, no viable colonies are reported.

Table 6: Dynamics of microbial density (number of viable cells/milliliter sample - MIC) of *E. coli*, *S. aureus* and *C. albicans* in medium with 1% (v/v) chlorhexidine digluconate (positive control)

	0 hour	0.5 hour	1 hour	3 hours	6 hours
E. coli	7000000	30000	-	-	-
S. aureus	6500000	30000	-	-	-
C. albicans	6000000	600000	-	-	-

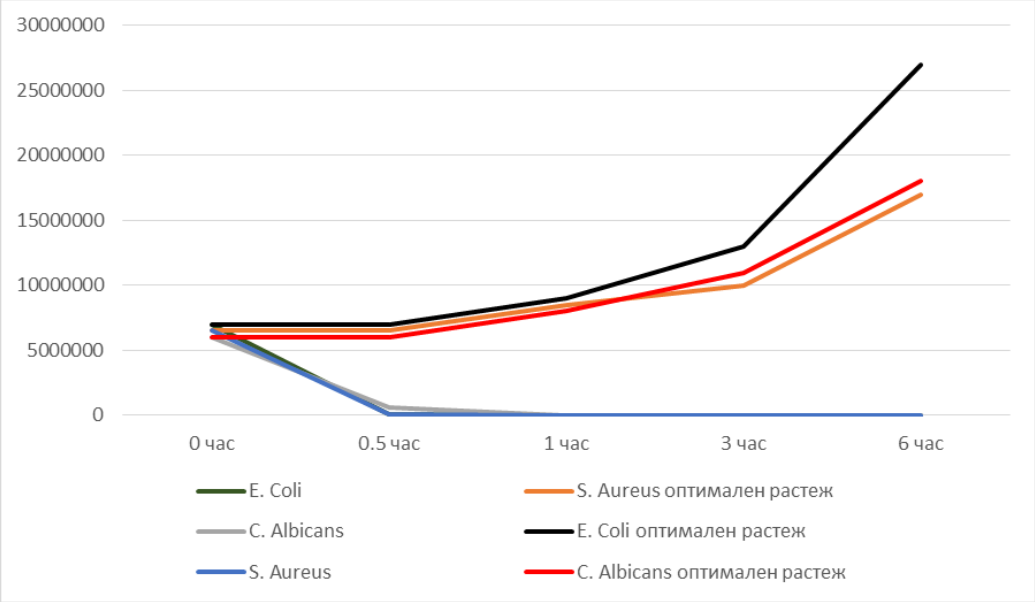

Figure 15: Microbial density dynamics (number of viable cells/milliliter sample - MIC) of *E. coli*, *S. aureus* and *C. albicans* in medium with 1% (v/v) chlorhexidine digluconate (positive control) compared with microbial density dynamics in culture medium with 50% (v/v) distilled water (negative control)

Microbial density dynamics of *E. coli* and *S. aureus* in medium with 5% (v/v) zinc oxide showed an increase in the number of viable bacterial cells until the

3rd hour and subsequently a significant decrease of two and more times per milliliter of sample (Table 7, Fig. 16).

Table 7: Dynamics of microbial density (number of viable cells/milliliter sample - MP) of *E. coli*, *S. aureus* and *C. albicans* in medium with 5% (w/v) zinc oxide

	0 hour	0.5 hour	1 hour	3 hours	6 hours
E. coli	7000000	7000000	9500000	18000000	8000000
S. aureus	6500000	6500000	8000000	30000000	8000000
C. albicans	6000000	6000000	7900000	12000000	18900000

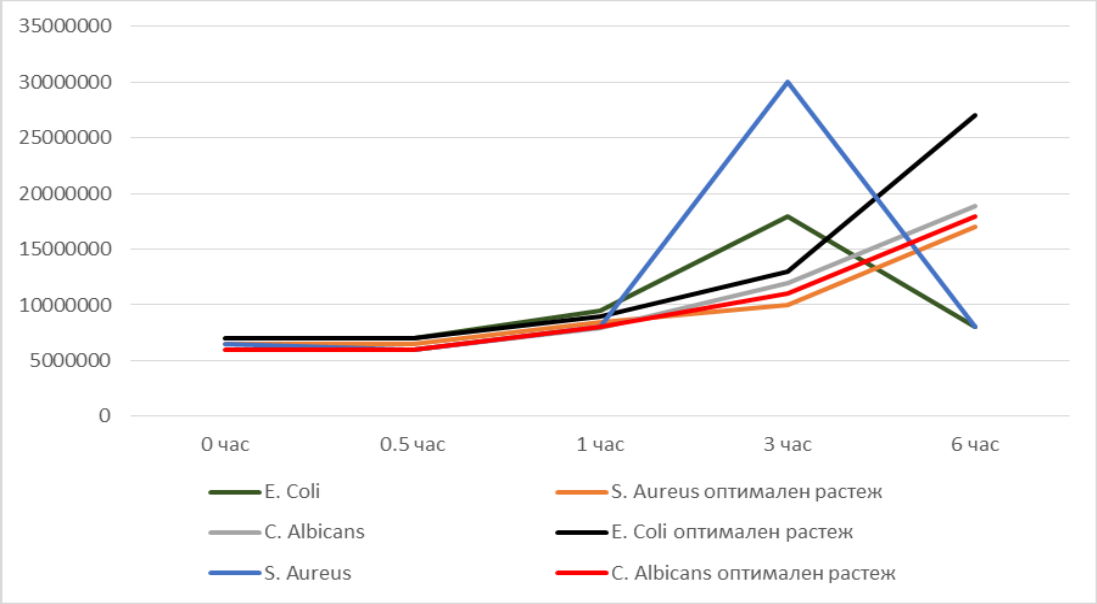

Figure 16: Microbial density dynamics (number of viable cells/milliliter sample - MIC) of E. coli, S. aureus and C. albicans in 5% (v/v) zinc oxide medium compared with microbial density dynamics in medium with 50% (v/v) distilled water (negative control)

For *E. coli*, the microbial count decreases from 18 million cells/ml. to 8 million cells/ml. In the medium, for *S. aureus* their number for the same time interval decreased from 30 million cells/ml. to 8 million cells/ml. When evaluating the effect of zinc oxide on *C. albicans*, the viable colony count was similar to that of the negative control, i.e. no fungicidal action is reported.

When monitoring the vitality and the number of microbes in the set negative control, these indicators grow in conformity with the measurements in the so-called batch cultivation. It is a closed system in which, after inoculation of a fresh medium, the number and mass of microorganisms in the culture change regularly over time, successively passing through several characteristic phases of development. The culture successively experiences a lag phase, an exponential growth phase, a stationary phase and a dying phase. The lag phase is the period between inoculation and the time when the culture reaches its maximum growth rate. The exponential phase is the period in which the culture maintains a constant maximum growth rate. Stationary phase is the period in which the rate of culture multiplication is approximately equal to the rate of cell death. The biomass reached by the culture in the stationary phase is maximum and is called yield. The dying phase is the last stage of culture development – in the medium, substrates are exhausted, toxic products are accumulated, growth and cell division practically stop, and the majority of old cells die and autolyze.

In Table 8 and Figure 17, the presented values reflect the first two phases of microbial growth in a closed system (the lag-phase and the exponential phase) in the control sample with *E. coli*, *S. aureus* and *C. albicans* in BHI broth.

Table 8: Dynamics of the microbial density (number of viable cells/milliliter sample – MIC) of *E. coli*, *S. aureus* and *C. albicans* in medium with 50% (v/v) distilled water (negative control)

	0 hour	0.5 hour	1 hour	3 hours	6 hours
E. coli	7000000	7000000	9000000	13000000	27000000
S. aureus	6500000	6500000	8500000	10000000	17000000
C. albicans	6000000	6000000	8000000	11000000	18000000

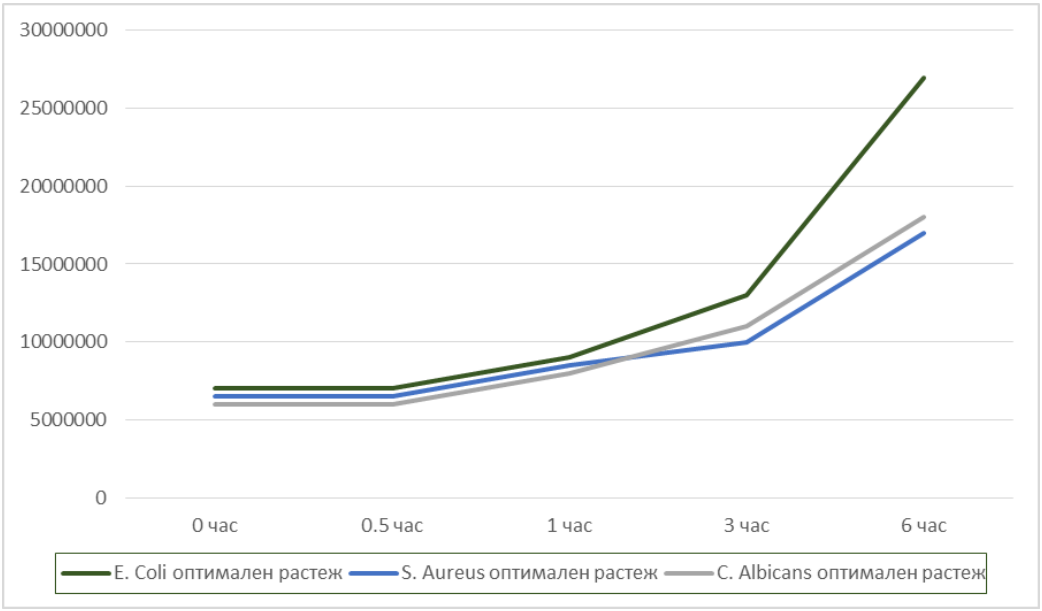

Figure 17: Dynamics of the microbial density (number of viable cells/milliliter sample - MIC) of *E. coli, S. aureus* and *C. albicans* in medium with 50% (v/v) distilled water (negative control)

When an inhibitory agent is introduced into the medium, the characteristic phases of the microbial growth kinetics can be significantly disturbed or some of them can be completely absent. This depends on the type and strength of the antimicrobial effect of the agents in the environment.

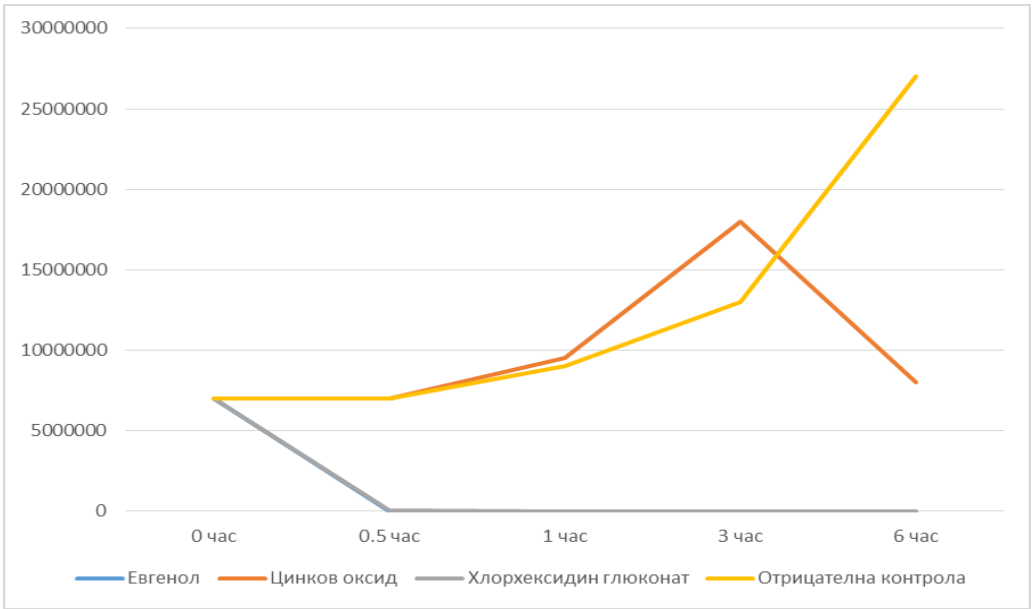

Figure 18: Dynamics of the microbial density (number of viable cells/milliliter sample – MP) of *E. coli* in medium with 50% (v/v) eugenol, in medium with 5% (v/v) zinc oxide, in medium with 1% (v/v) chlorhexidine digluconate and in medium with 50% (v/v) distilled water (negative control)

The presented results for the role of eugenol and a control sample with chlorhexidine show that the vitality of the microbial populations is compromised already in their first phase of development – the log phase, and zinc oxide reduces the number of viable cells in the exponential phase (Fig. 18, Fig. 19, Fig. 20).

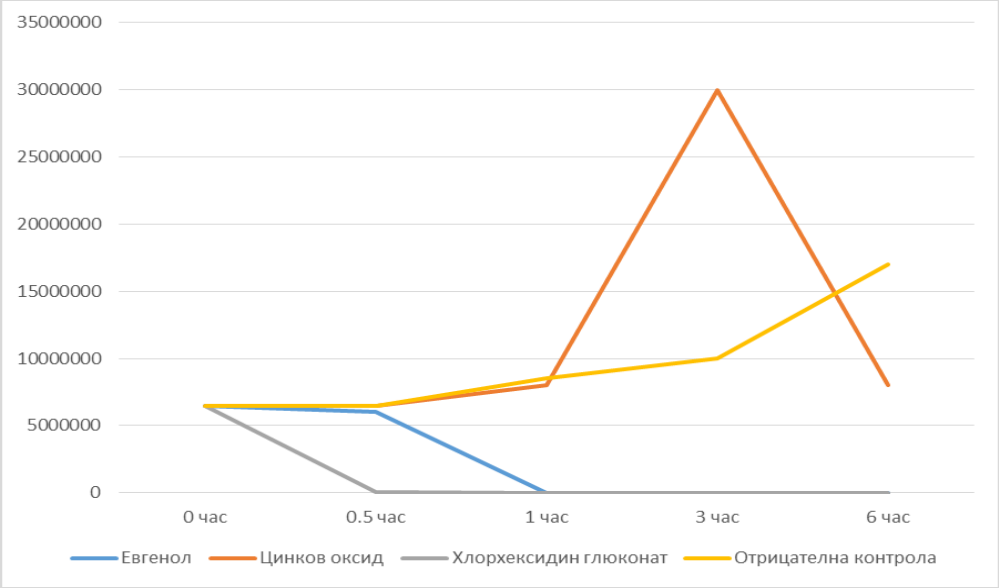

Figure 19: Dynamics of the microbial density (number of viable cells/milliliter sample – MP) of *S. aureus* in medium with 50% (v/v) eugenol, in medium with 5% (v/v) zinc oxide, in medium with 1% (v/v) chlorhexidine digluconate and in medium with 50% (v/v) distilled water (negative control)

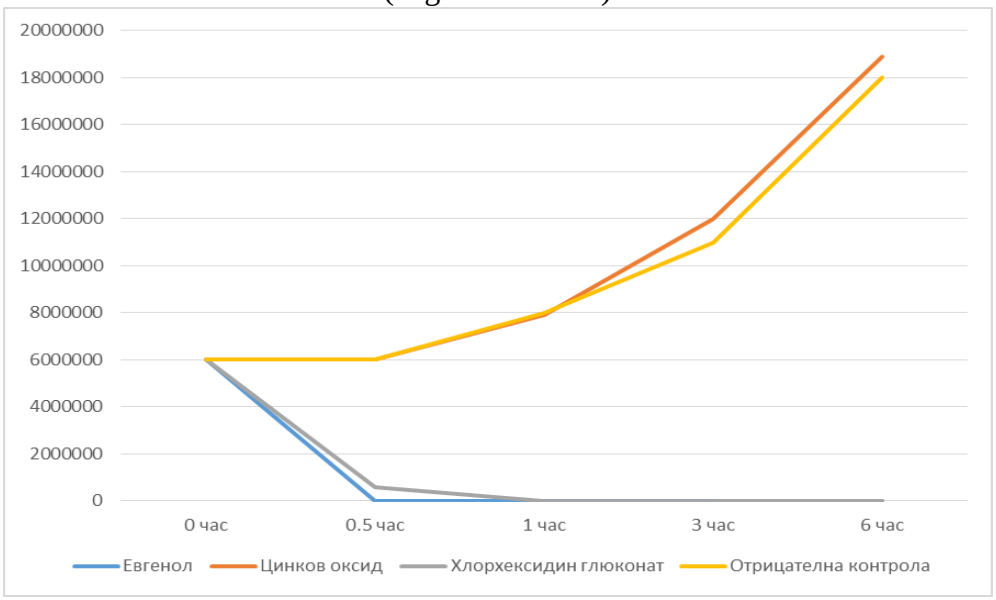

Figure 20: Dynamics of the microbial density (number of viable cells/milliliter sample – MF) of *C. albicans* in medium with 50% (v/v) eugenol, in medium with 5% (v/v) zinc oxide, in medium with 1% (v/v) chlorhexidine digluconate and in medium with 50% (v/v) distilled water (negative control)

Results and discussion for task 2

Results and discussion for subtask 2.1. A study of microleakage in teeth with temporary obturation for a 2-day period

- The cavities in the first group were obturated with temporary filling material Citodur, Dorident. The mean cavity depth ranged from 2.88 mm for incisors, 2.71 mm for canines and 2.75 mm for premolars, and the overall mean cavity depth for the given group was 2.78 mm.

After measuring the depth of the cavities, the depth of penetration of the dye in mm was investigated in two sections (Table 9) – it was discovered that the average vestibular microleakage was 1.67 mm and the average lingual microleakage was 1.33 mm. From these values, the overall average dye penetration was calculated, which was 1.5 mm. Microleakage was found in all cavities.

Table 9: Average microleakage values in mm for cavities obturated with Citodur

Tooth type	**Incisors**		**Canines**		**Premolars**	
Microleakage	Vestibular	Lingual	Vestibular	Lingual	Vestibular	Lingual
Average value (mm)	1.4450	1.2933	1.7737	1.2300	1.7817	1.4700
N	3	3	4	4	3	3
Standard deviation	.12124	.12513	.33597	.19566	.22827	.24622

The relative microleakage M rel (in %) for the entire group, calculated by formula (1), is 85.95%. In the photographs in Fig.21, it is clearly visible that the dye has penetrated more than 2/3 of the depth of the obturation.

- In the second group, the cavities were obturated with zinc oxide-eugenol cement IRM, Dentsply. The average cavity depth was measured and calculated – 2.45 mm for incisors, 2.06 mm for canines and 2.93 mm for premolars, and the overall average cavity depth was 2.24 mm.

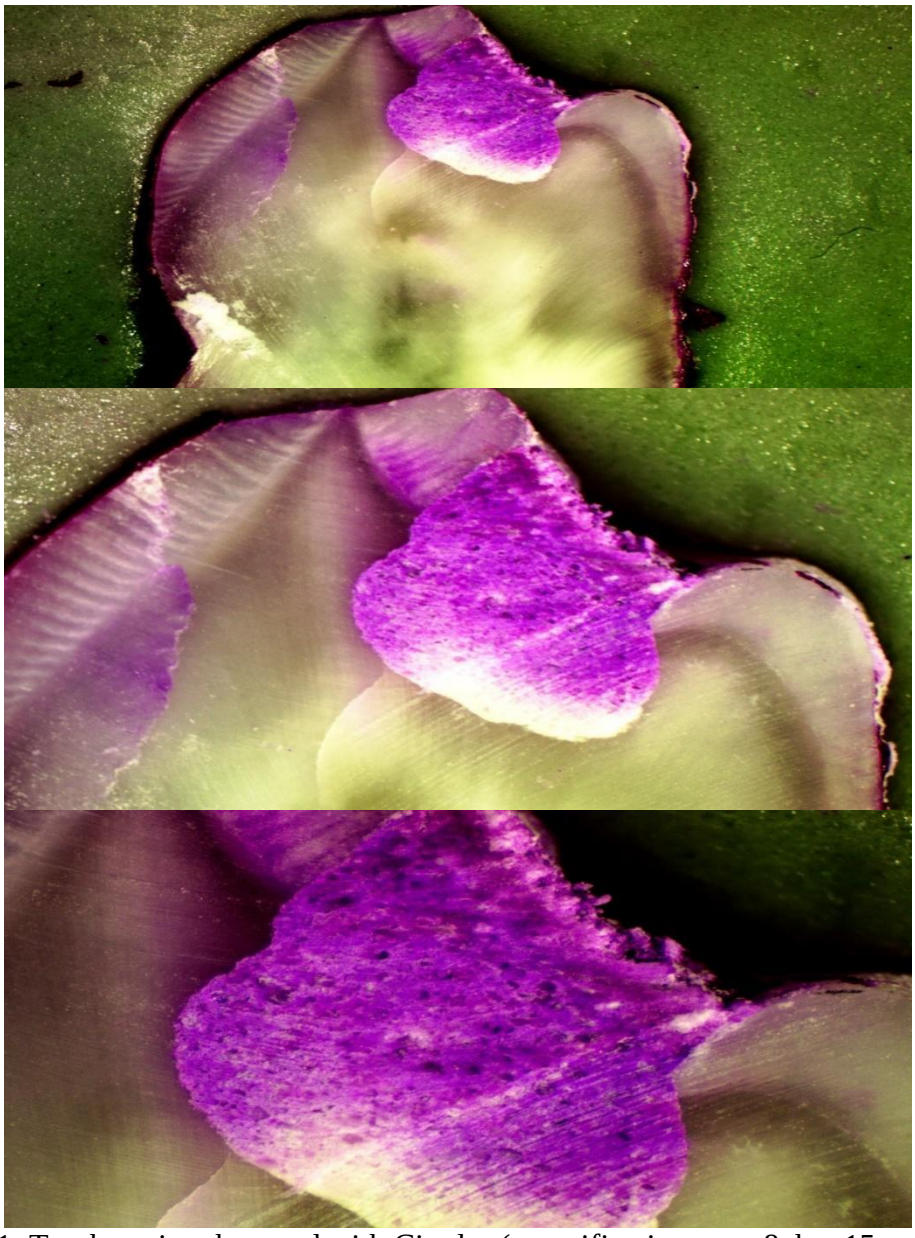

Figure 21: Tooth cavity obturated with Citodur (magnifications: a. x8, b. x15 and c. x25)

The depth of dye penetration in mm was investigated in two sections (Table 10) – the average vestibular microleakage was 1.70 mm and the average lingual microleakage was 1.28 mm. The overall average microleakage was 1.49 mm. All teeth are with microleakage. The relative microleakage M rel for the whole group, calculated by formula (1), is 87.92%. In the photographs in Fig.22, it is clearly visible that the dye has penetrated more than 2/3 of the depth of the obturation.

Table 10: Average microleakage values in mm for cavities obturated with IRM

Tooth type	Incisors		Canines		Premolars	
Microleakage	Vestibular	Lingual	Vestibular	Lingual	Vestibular	Lingual
Average value (mm)	1.4425	.9713	1.5817	1.1900	2.1400	1.6750
N	4	4	3	3	3	3
Standard deviation	.12652	.19843	.29472	.12971	.21290	.31548

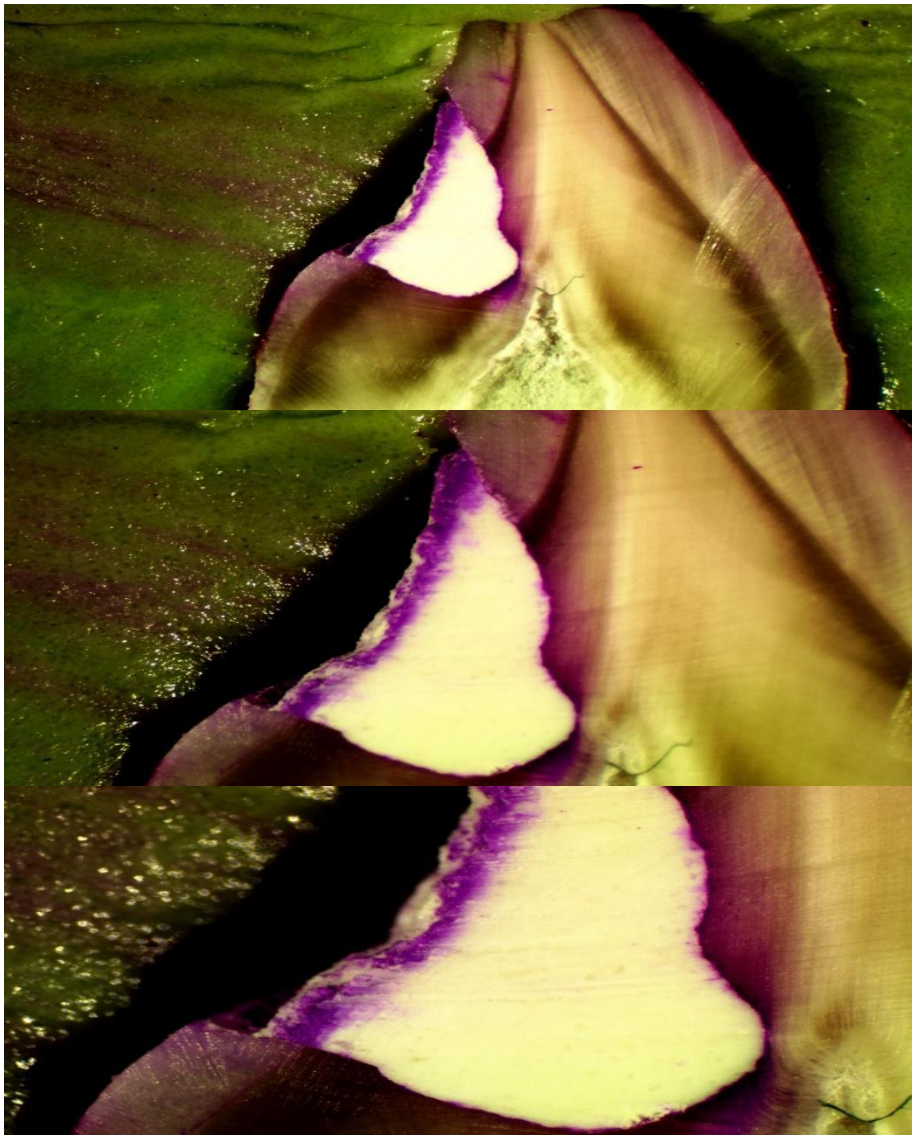

Figure 22: Tooth cavity obturated with IRM (magnifications: a. x8, b. x15 and c. x25)

- In the third group, the cavities were obturated with photopolymerizing material for temporary obturation i-PRO LC, i-dental. Average cavity depth

was calculated – 2.6 mm for incisors, 2.87 mm for canines and 2.87 mm for premolars. The overall mean cavity depth in the group was 2.78 mm.

The depth of penetration of the dye in mm was examined in two sections (Table 11) – an average vestibular microleakage of 1.56 mm and an average lingual microleakage of 1.13 mm were found. The overall mean microleakage was 1.35 mm. There weren't teeth without microleakage.

Table 11: Average microleakage values in mm for cavities obturated with i-PRO

Tooth type	**Incisors**		**Canines**		**Premolars**	
Microleakage	Vestibular	Lingual	Vestibular	Lingual	Vestibular	Lingual
Average value (mm)	1.5463	1.1487	1.3500	.9317	1.8033	1.3050
N	4	4	3	3	3	3
Standard deviation	.45357	.15596	.31177	.35105	.30856	.58602

The relative microleakage for the whole group, calculated by formula (1), is 72.83%. In the photographs in Fig.23, it is clearly visible that the dye has penetrated 1/3 of the depth of the obturation.

The relative microleakage for the entire group is the lowest compared to the two previous groups – 72.83%.

The comparative analysis of the depth of the cavities in the three groups of teeth shows that they are very close, and the microleakage values do not differ significantly in the cavities obturated with the different types of materials. The average microleakage is the highest for the cavities obturated with Citodur – 1.50 mm, followed by the cavities obturated with IRM – 1.49 mm, and the lowest microleakage is observed by the cavities of the third group obturated with i-PRO – 1.35 mm (Fig.24).

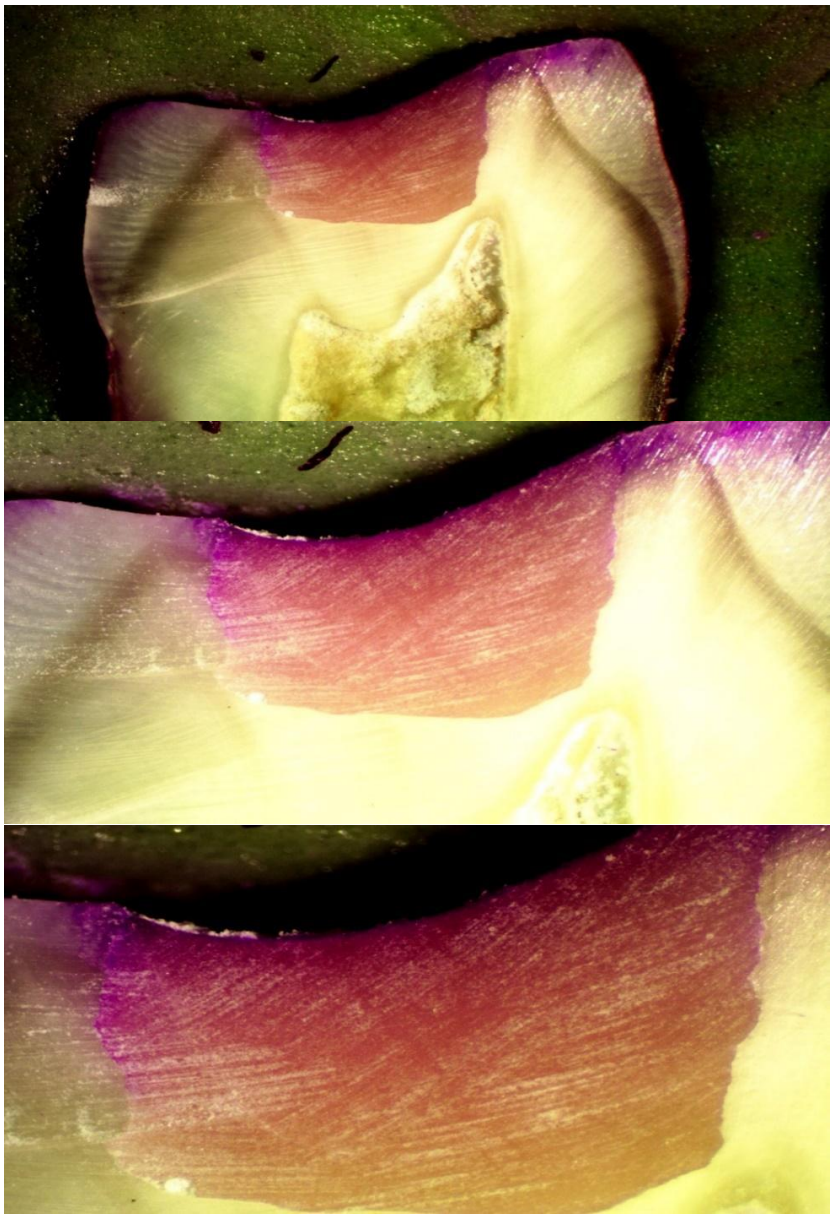

Figure 23: Tooth cavity obturated with i-PRO (magnifications: a. x8, b. x15 and c. x25)

Comparing the values obtained for all three groups of cavities, the lowest percentage of dye penetration was found in the cavities obturated with i-PRO. The percentage of microleakage was higher in cavities obturated with Citodur and IRM. Therefore, it can be concluded that the cavities obturated with photopolymerizable composite temporary obturation material are characterized by lower microleakage

compared to those obturated with the other two investigated materials, which show similar microleakage values.

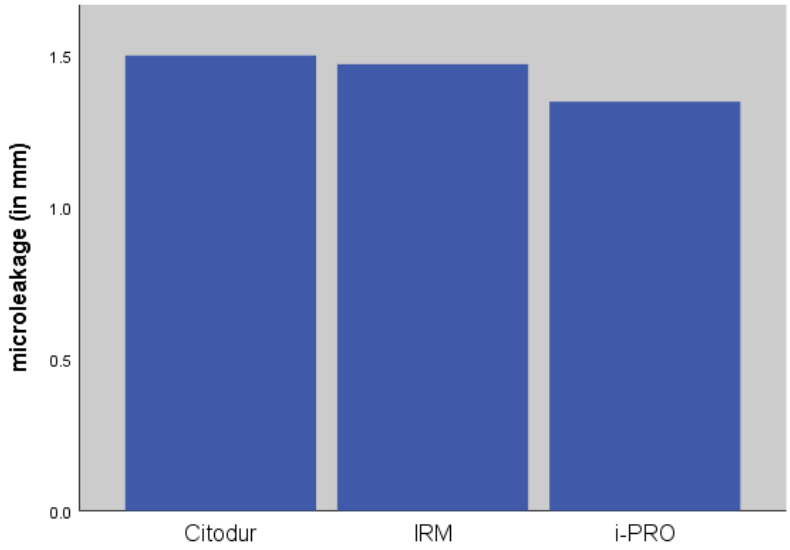

Figure 24: Average microleakage in individual groups

Results and discussion for subtask 2.2. A study of microleakage in teeth with temporary obturation for a 14-day period

- Cavities in the first group were obturated with temporary obturation material Citodur, Dorident. The mean cavity depth ranged from 2.3 mm for incisors, 2.7 mm for canines and 2.9 mm for premolars, with an overall mean cavity depth for the given group of 2.63 mm.

After measuring the depth of the cavities, the depth of penetration of the dye in two sections was also examined (Table 12) – it was found that the average vestibular microleakage was 1.73 mm and the average lingual leakage was 1.37 mm. The overall average dye penetration, which is 1.55 mm. Microleakage was found in all cavities. The relative microleakage M rel (in %) for the whole group,

calculated by formula (1), is 91.01%. In the photos of Fig. 25 it is clear that the dye has entered almost the entire depth of the obturation.

Table 12: Average microleakage values in mm for cavities obturated with Citodur

Tooth type	**Incisors**		**Canines**		**Premolars**	
Microleakage	Vestibular	Lingual	Vestibular	Lingual	Vestibular	Lingual
Average values (mm)	1.5138	1.1725	1.7750	1.3617	1.9117	1.5750
N	4	4	3	3	3	3
Standard deviation	.15819	.18118	.42729	.46261	.21502	.40234

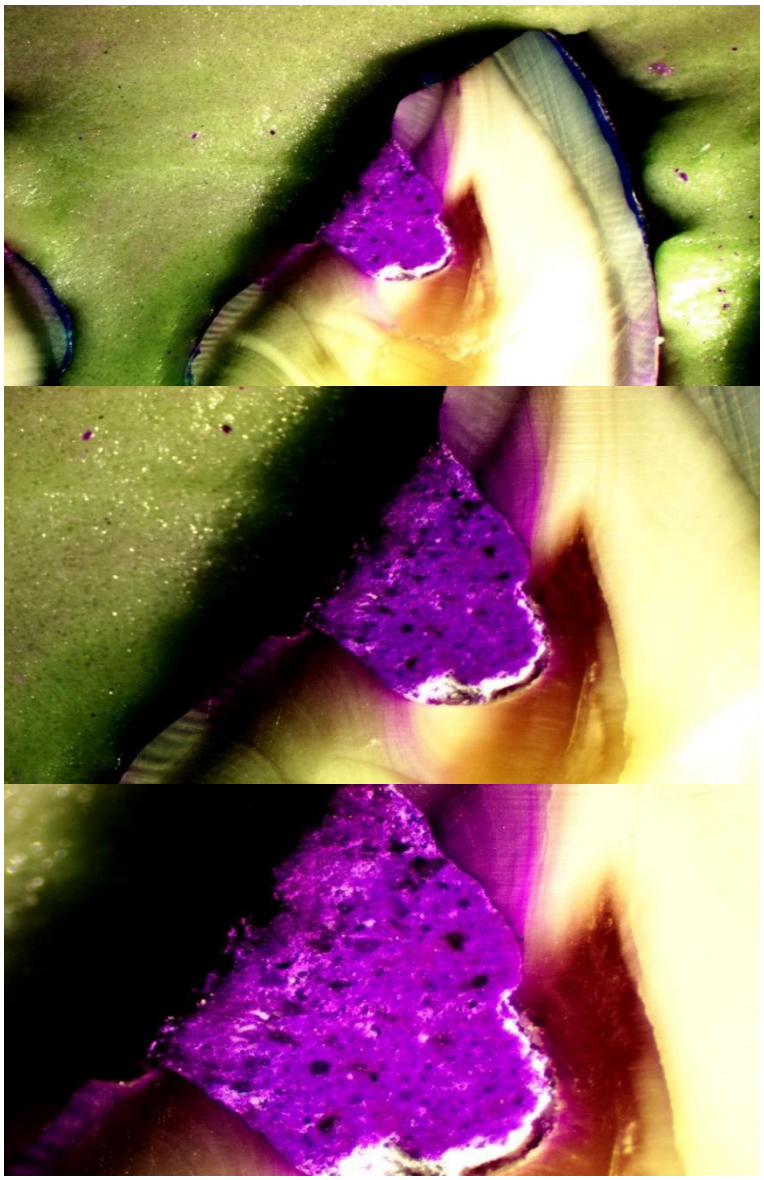

Figure 25: Tooth cavity obturated with Citodur (magnifications: a. x8, b. x15 and c. x25)

- In the second group, the cavities were obturated with zinc oxide eugenol cement IRM, Dentsply. The average depth of the cavities in the different groups of teeth was measured and calculated – 2.85 mm for incisors, 2.86 mm for canines and 2.93 mm for premolars, and the overall average depth of cavities was 2.88 mm.

The depth of dye penetration in mm was examined in two sections (Table 13) – the average vestibular microleakage was 1.96 mm, and the average lingual microleakage was 1.66 mm. The overall average microleakage was 1.81 mm. There aren't any teeth without microleakage.

Table 13: Mean microleakage values in mm for cavities obturated with IRM

Tooth type	**Incisors**		**Canines**		**Premolars**	
Microleakage	Vestibular	Lingual	Vestibular	Lingual	Vestibular	Lingual
Average values (mm)	1.9663	1.4450	1.7717	1.6467	2.1300	1.8800
N	4	4	3	3	3	3
Standard deviation	.25636	.32838	.02255	.10214	.26963	.29816

The relative microleakage M rel for the whole group, calculated by formula (1) is 97%. Fig. 26 shows the penetration of the dye.

- In the third group, the cavities were obturated with photopolymerizing material for temporary obturation i-PRO LC, i-dental. An average cavity depth of 2.5 mm for incisors, 2.9 mm for canines and 2.9 mm for premolars was calculated and the overall average cavity depth for the group was 2.7 mm.

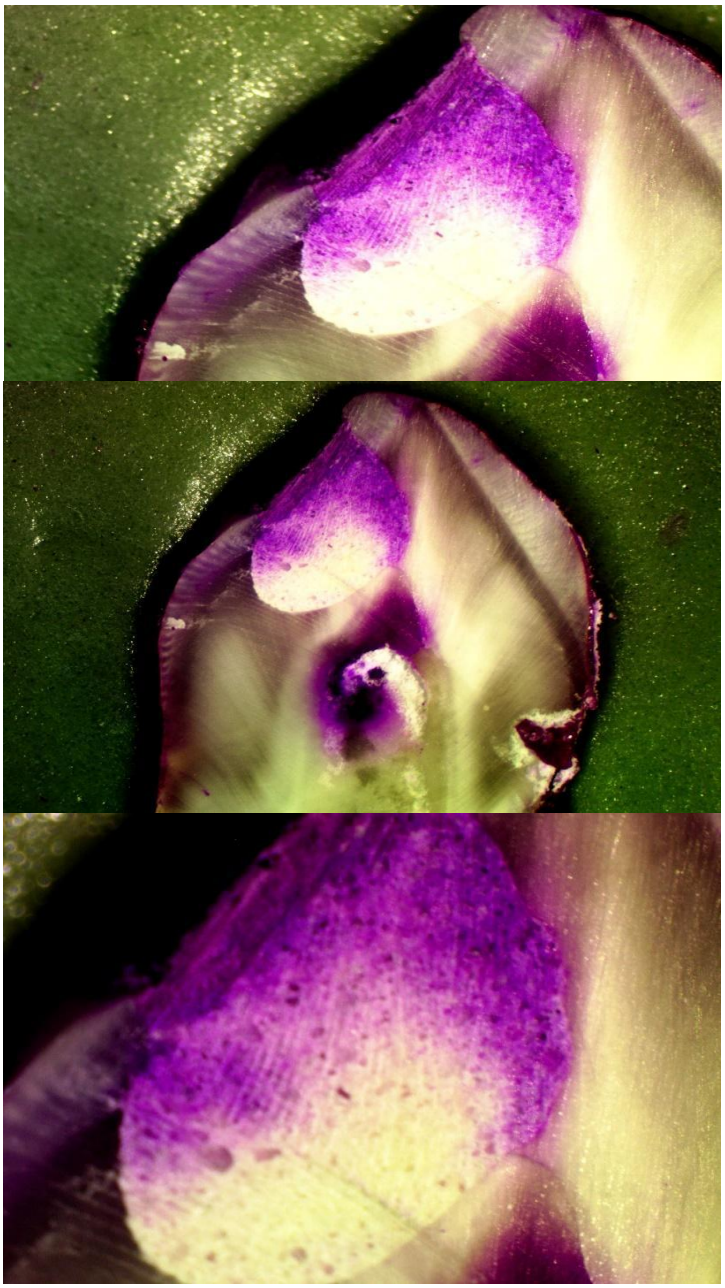

Figure 26: Tooth cavity obturated with IRM (magnifications: a. x8, b. x15 and c. x25)

The depth of penetration of the dye in mm was examined in two sections (Table 14) – an average vestibular microleakage of 1.85 mm and an average lingual microleakage of 1.25 mm were found. The overall average microleakage was 1.55 mm. No teeth were identified without any microleakage (Fig. 27).

Table 14: Average microleakage values in mm for cavities obturated with i-PRO

Tooth type	Incisors		Canines		Premolars	
Microleakage	Vestibular	Lingual	Vestibular	Lingual	Vestibular	Lingual
Average value (mm)	1.4563	.9563	1.7450	1.2550	2.3600	1.5400
N	4	4	3	3	3	3
Standard deviation	.60942	.20589	.49616	.38089	.27622	.60399

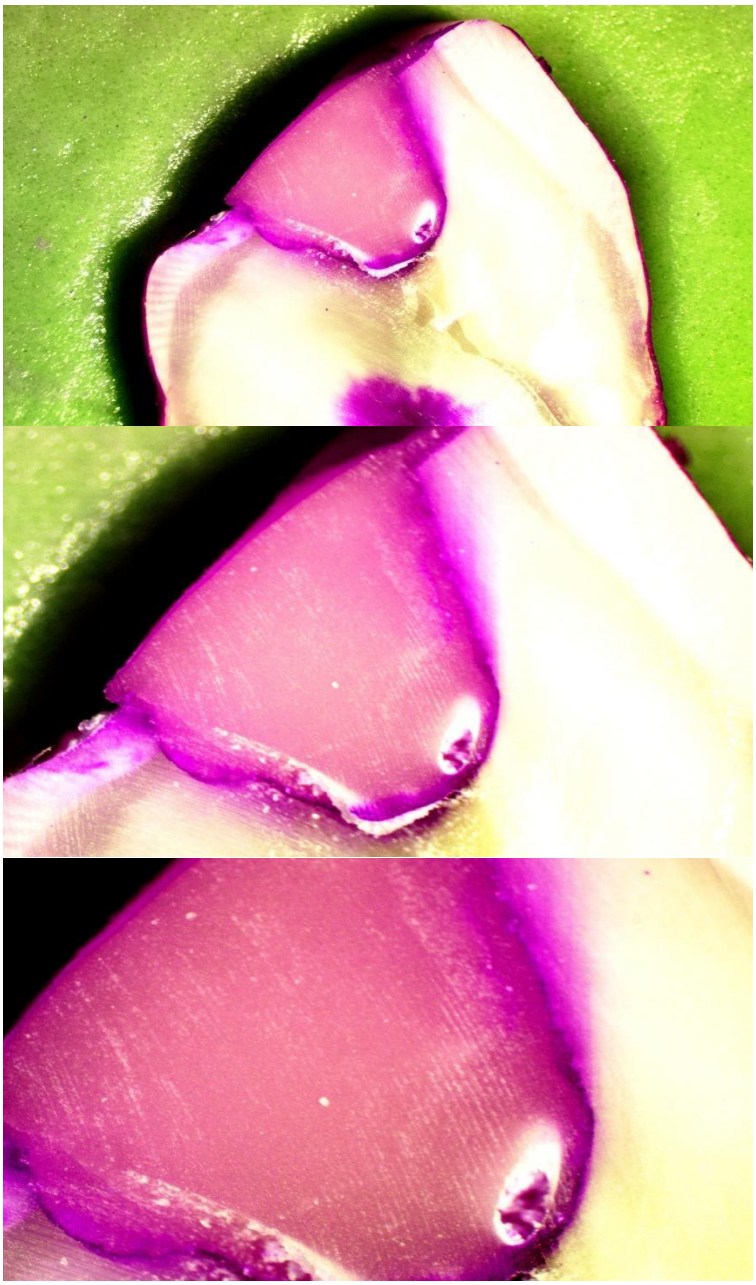

Figure 27: Tooth cavity obturated with i-PRO (magnifications: a. x8, b. x15 and c. x25)

The relative microleakage for the whole group, calculated by formula (1), is 84.52%. It is the lowest compared to the two previous groups – 84.52%.

The microleakage values of the cavities obturated with the different types of materials show the greatest leakage with IRM, while those with i-PRO and Citodur are close. In all three groups, increased microleakage was observed for the period of 14 days compared to the 2-day period (Fig. 28, Fig. 29).

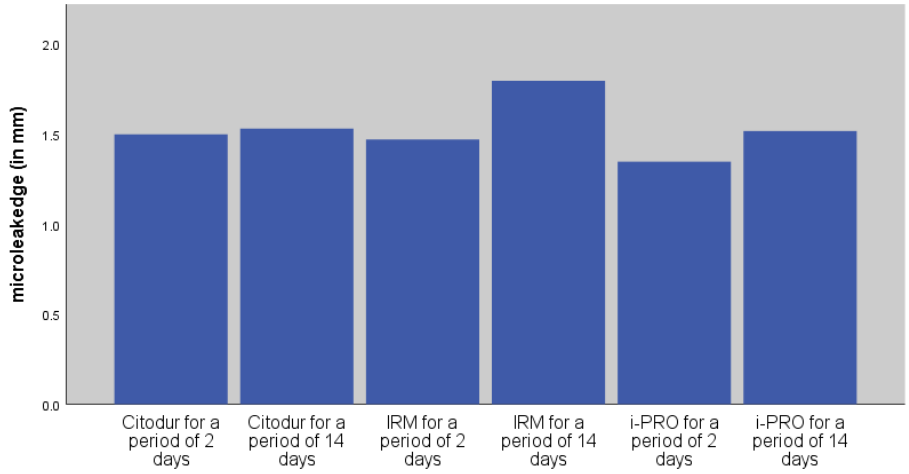

Figure 28: Average microleakage in individual groups

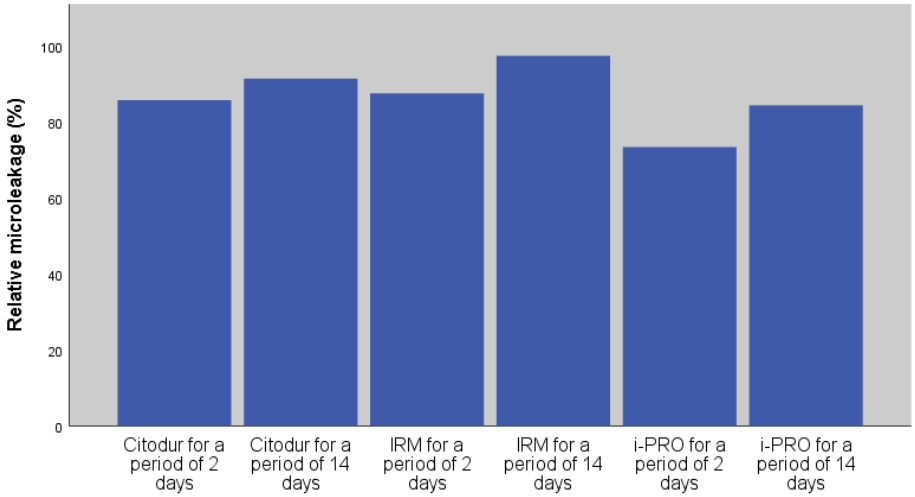

Figure 29: Relative microleakage in individual groups of cavities obturated with different materials

Results and discussion for task 3

724 of the patients who passed through the Dental Allergology office underwent skin allergy testing (SAT) – patch tests to confirm sensitization to dental materials (components of temporary filling materials). From them, 600 (82.9%) were women and 124 (17.1%) were men (Fig. 30). Females significantly dominated over males in number – p=0.000.

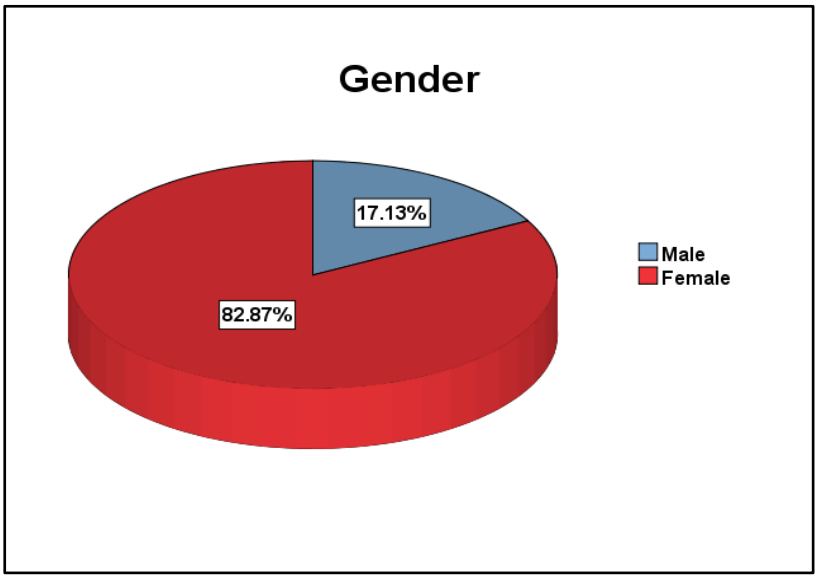

Figure 30: Distribution by gender of participants in SPT (n=724)

The average age of the participants was 56.25±17.61 years. Between the average age of the women in the study (57.71±16.35 years) and that of the men (49.13±21.46 years), there was a significant difference (Mann-Whitney U, p=0.000).

Distribution by age groups was uneven – W=0.917, p=0.000 (Fig. 31). This unevenness was due to both female participants – W=0.911, p=0.000 and male participants – W=0.952, p=0.000.

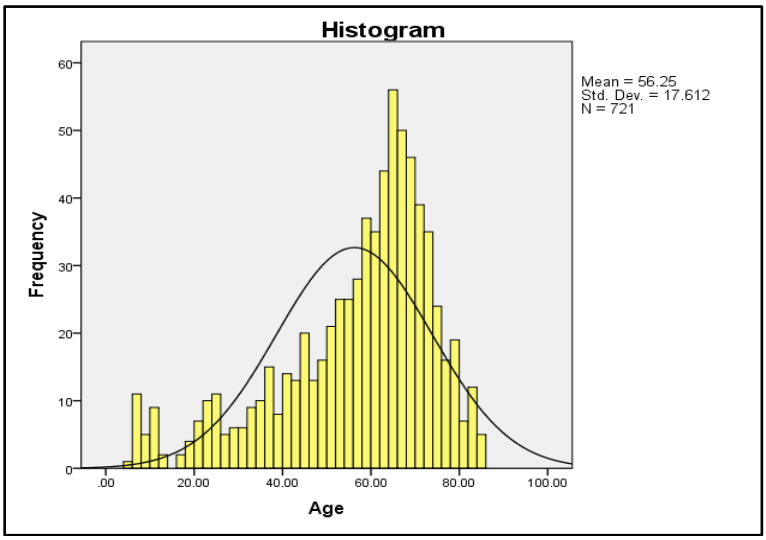

Figure 31: Age distribution of the participants (n=721)

79 (10.9%) of the patients reported suffering from some form of allergy (with an emphasis on drug, pollen or food allergy). The relative proportion of women with a history of allergy (11.4%) was greater than that of men (8.9%), but gender was not a determinant of the presence of concomitant allergies (φ=0.030, p=0.420) (Fig. 32).

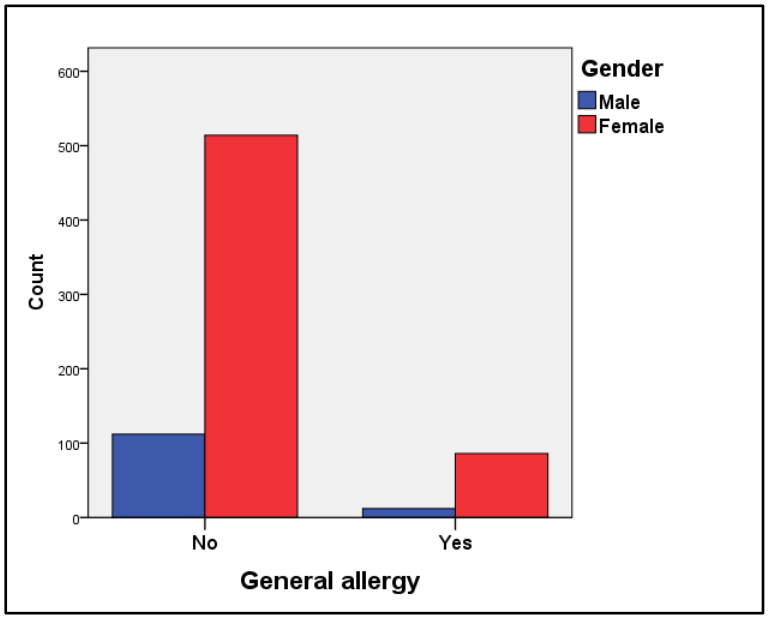

Figure 32: Influence of the gender factor on concomitant allergies(n=724)

With regard to individual allergic diseases – in relative shares, women more often declared the presence of drug and food allergies compared to men, and pollen allergy less often than them (Table 15). These differences were not statistically significant, i.e. gender did not determine the prevalence of individual allergies in this group.

Table 15: Distribution of patients with a history of allergic disease and influence of the gender factor

Indicator	Total number of patients	Men	Women	φ	p
DA	52 (7,2)	5 (4,0%)	47 (7,8)	0,055	0,136
PA	26 (3,6%)	5 (4,0%)	21 (3,5%)	-0,011	0,772
FA	42 (5,8%)	6 (4,8%)	36 (6,0%)	0,019	0,615
CA	29 (4,0%)	2 (1,6%)	27 (4,5%)	0,055	0,136

Only 29 of the patients (4.0%) also mentioned a previously diagnosed allergy to dental materials. Women were more often sensitized, but again gender was not a determining factor (Table 15).

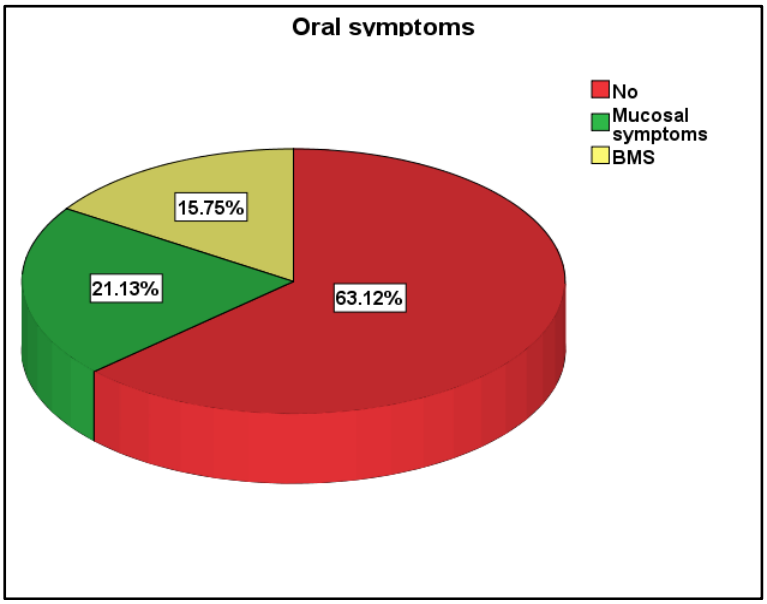

Figure 33: Distribution of patients based on oral symptoms (n=724)

Some of the patients (457) were referred for testing prior to dental treatment, and the rest (267) were referred because of oral symptoms – mucosal manifestations or BMS (burning mouth syndrome). The exact distribution in the group is presented in Fig. 33, and by gender – in Fig. 34.

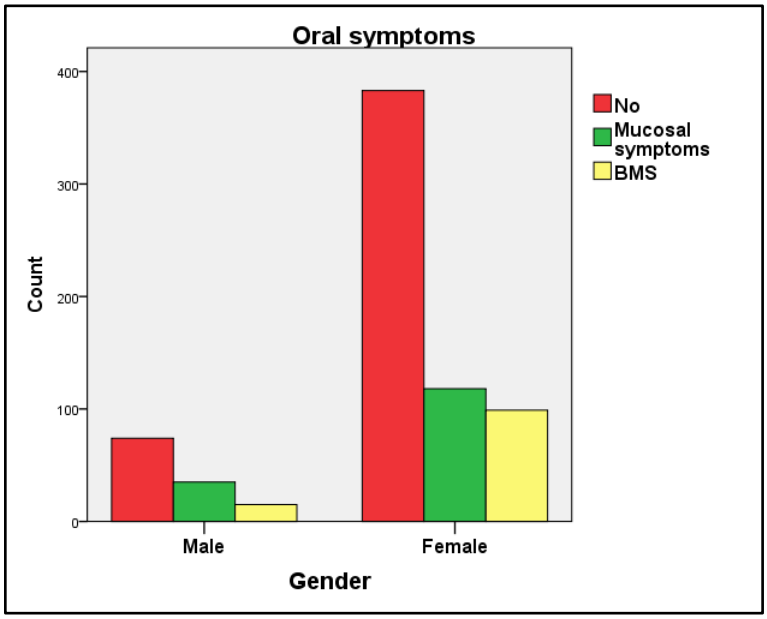

Figure 34: Gender distribution of patients with oral symptoms (n=724)

We investigated the possibility that the oral symptoms were provoked by the accompanying allergic diseases (Table 16). It was logical that the data in the last column of the table was zero – after hypersensitivity to a given material was established, it was no longer used in the treatment of the patient.

Table 16: Correlation of present oral symptoms with concomitant allergies (n=267)

Symptoms	DA	PA	FA	CA
Mucous (153)	2 (1,3%)	0 (0,0%)	0 (0,0%)	0 (0,0%)
BMS (114)	1 (0,9%)	1 (0,9%)	1 (0,9%)	0 (0,0%)

Patch tests were performed on patients to demonstrate sensitization to selected components of the temporary filling materials. At least one positive reaction was found in 19 (2.65%) of them – 16 patients were sensitized to one ingredient, two to 2 ingredients and one to more than 2 ingredients (Fig. 35).

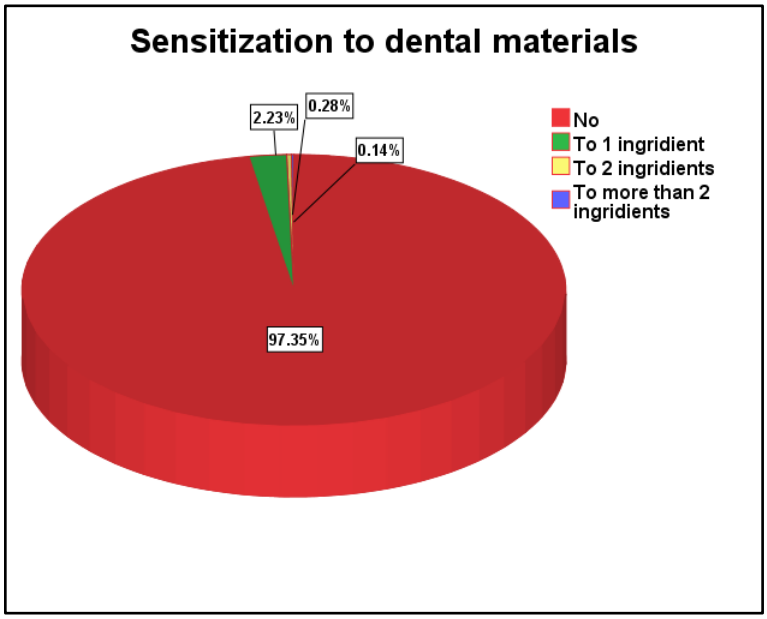

Figure 35: Distribution of participants according to the number of positive reactions in one individual (n=724)

Among the women, 17 reacted positively to at least one allergen, and among men – only 2. As shares, these were 2.9% and 1.6%, respectively – the difference was not statistically significant (p=0, 380) (Table 17).

Table 17: Distribution by gender and number of positive epicutaneous tests (n=724)

Gender	Sensitization to dental materials			
	Absent	Up to 1	Up to 2	Up to more than 2
Men	122 (98,4%)	1 (0,8%)	1 (0,8%)	0 (0,0%)
Women	583 (97,1%)	15 (2,5%)	1 (0,2%)	1 (0,2%)

No statistically significant differences were found in the relative share of patients with established sensitization to one or more components of the temporary

filling materials (positive patch test) depending on the anamnestic data on existing allergy to other dental materials – $\chi 2=0.079$, $p=0.545$ (Table 18). The reported extremely weak positive correlation was not statistically significant ($\varphi=0.010$, $p=0.778$).

Table 18: Correlation between history of allergy to dental materials and patch test results (n=724)

		Patch test results		p
		Negative	Positive	
Data on allergy to dental materials	No	677 (97,4%) (96,0%)	18 (2,6%) (94,7%)	0,545
	Yes	28 (96,6%) (4,0%)	1 (3,4%) (5,3%)	

No statistically significant differences were found in the relative share of patients with established sensitization to one or more components of the temporary filling materials (positive patch test) depending on the clinical data on oral symptoms – $\chi 2=3.728$, $p=0.057$ (Table 19). There was an extremely weak negative correlation ($\varphi=-0.072$, $p=0.054$).

Table 19: Correlation between clinical oral symptom data and patch tests results (n=724)

		Patch test results		p
		Negative	Positive	
Clinical data on oral symptoms	No	441 (96,5%) (62,6%)	16 (3,5%) (84,2%)	0,057
	Yes	264 (98,9%) (37,4%)	3 (1,1%) (15,8%)	

We have presented in Table 20 the patch test results for all allergens of the series, reported according to the requirements of the ICDRG (Table 3).

Table 20: Patch test results (n=724)

Allergen	Result			
	Negative –	Weak positive +	Strong positive ++	Extreme positive +++
MMA	529 (98,7%)	5 (0,9%)	1 (0,2%)	1 (0,2%)
TEGDMA	221 (98,7%)	2 (0,9%)		1 (0,4%)
EGDMA	55 (100,0%)			
BisGMA	188 (97,9%)	4 (2,1%)		
HEMA	29 (96,7%)	1 (3,3%)		
UDMA	4 (80,0%)		1 (20,0%)	
Eugenol	162 (98,8%)	2 (1,2%)		
Zn	31 (88,57%)	3 (8,57%)		1 (2,86%)
Epoxy resin	49 (98,0%)	1 (2,0%)		

We also investigated the gender distribution of the reported results, looking for relation (Table 21). It turned out that for all of the allergens tested, except for HEMA, women were more often sensitized and tested positive. But the frequency was low and the results did not have statistical significance – gender was not a determining factor in this case.

The following figures (36 – 39) visualize the results for some allergens from the series we selected to establish hypersensitivity to the ingredients of the temporary filling materials.

Table 21: Gender distribution of patch test results (n=724)

Allergen	Gender	Result				p
		Negative –	Weak positive +	Strong positive ++	Extreme positive +++	
MMA	m	86 (98,9%)	1 (1,1%)			0,932
	w	443 (98,7%)	4(0,9%)	1 (0,2%)	1 (0,2%)	
TEGDMA	m	41 (100,0%)				0,711
	w	180 (98,4%)	2 (1,1%)		1 (0,5%)	
EGDMA	m	14 (100,0%)				
	w	41 (100,0%)				
BisGMA	m	38 (100,0%)				0,411
	w	150 (97,4%)	4 (2,6%)			
HEMA	m	10 (90,9%)	1 (9,1%)			0,367
	w	19 (100,0%)				
UDMA	m	1 (100,0%)				0,800
	w	3 (75,0%)		1 (25,0%)		
Eugenol	m	34 (97,1%)	1 (2,9%)			0,382
	w	128 (99,2%)	1 (0,8%)			
Zn	m	4 (100,0%)				0,747
	w	27 (87,1%)	3 (9,7%)		1 (3,2%)	
Epoxy resin	m	9 (100,0%)				0,820
	w	40 (97,6%)	1 (2,4%)			

Methyl methacrylate (MMA) was the only allergen tested, for which we reported positive results from all degrees of manifestation (Fig. 36).

For triethylene glycol-dimethacrylate (TEGDMA) we reported weak positive and strong positive reactions (Fig. 37), and for ethylene glycol-dimethacrylate (EGDMA) there were no positive results.

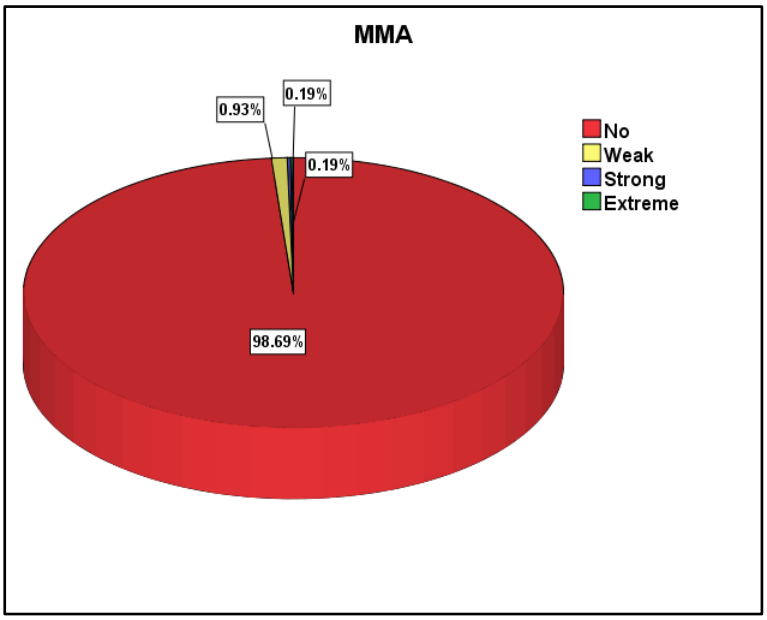

Figure 36: MMA patch test results (n=536)

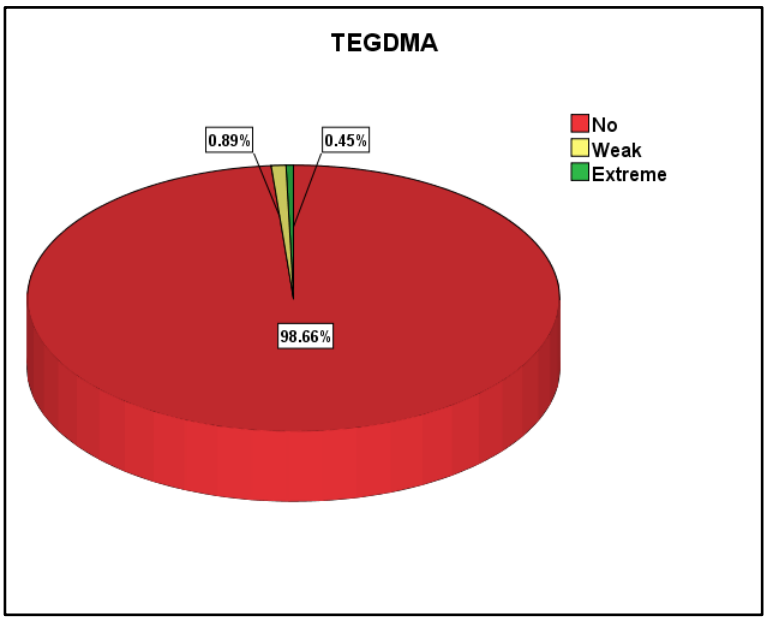

Figure 37: TEGDMA patch test results (n=224)

Among the results for bisphenol A glycerol-dimethacrylate (BisGMA) we recorded weak positive reactions. Among the acrylates, we recorded the most frequent positive reactions to hydroxyethyl methacrylate (HEMA). A surprise for us was the strong positive reaction to urethane dimethacrylate (UDMA), which was considered a weak allergen (Fig.38).

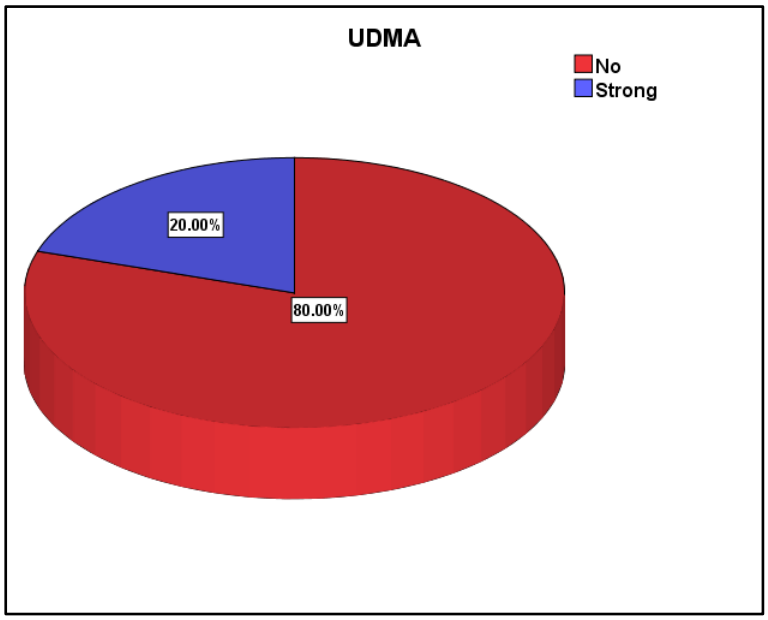

Figure 38: UDMA patch test results (n=4)

We reported one case of cross-sensitization to three acrylate allergens – MMA, TEGDMA and UDMA. Considering the report of only 14 cases with positive results, this explained the calculated correlation in sensitization between these allergens (Table 22).

Table 22: Degree of correlation between acrylate allergens

Indicators	n	τ	p
MMA+TEGDMA	223	**0,283**	**0,000**
MMA+BisGMA	91	-0,028	0,793
MMA+HEMA	26	-0,040	0,841
TEGDMA+BisGMA	44	-0,023	0,879
TEGDMA+ HEMA	25	-0,042	0,838
TEGDMA+ UDMA	2	**1**	**0,01**
BisGMA+ HEMA	28	-0,037	0,847

Statistically significant was the correlation between MMA and TEGDMA (weak positive) and that between TEGDMA and UDMA (complete, but in a small number of cases studied and with a significance level of p=0.01).

We also investigated the relationship between history of oral symptoms (OS) and sensitization to individual acrylate allergens. We found an extremely weak negative correlation, the data being without statistical significance in all cases (Table 23).

Table 23: Correlation between oral symptoms and sensitization to acrylate allergens

Indicators	n	τ	p
OC+MMA	536	-0,080	0,054
OC+TEGDMA	224	-0,075	0,244
OC+BisGMA	192	-0,066	0,342
OC+HEMA	30	-0,072	0,695

The results of our research showed that the share of those sensitized to zinc was the highest – 11.5% of those tested had weak or extreme positive results (Fig. 39). This share was higher with UDMA, but the number of those tested was very small and the result could be ignored.

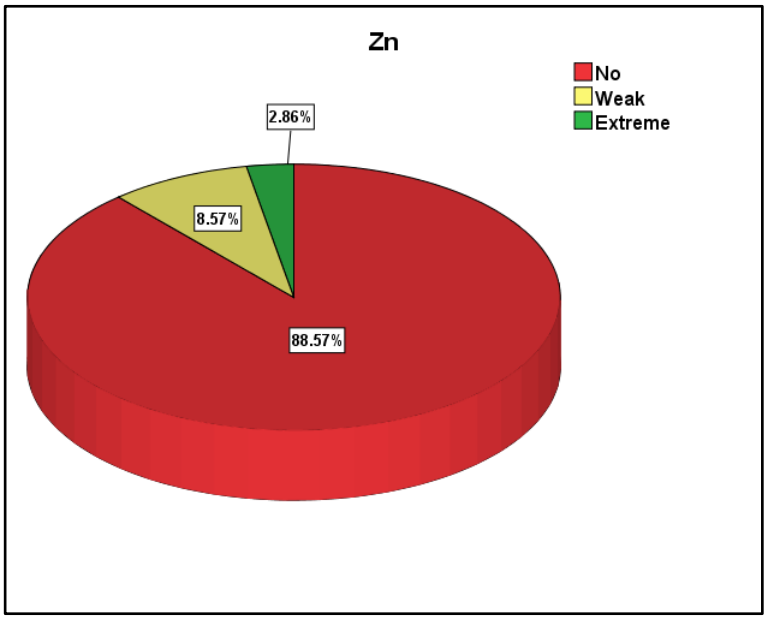

Figure 39: Zinc patch test results (n=35)

Only weak positive results were recorded for eugenol and for epoxy resin.

Two cases of simultaneous sensitization had been registered – to MMA and eugenol, as well as to BisGMA and zinc. The calculated strong positive correlation confirmed these facts (Tab. 24).

Table 24: Correlation in sensitization to allergens from different groups

Indicators	n	τ	p
MMA+Eugenol	54	**0,566**	**0,000**
MMA+Zn	17	-0,063	0,803
BisGMA+Eugenol	82	-0,018	0,874
BisGMA+Zn	17	**0,685**	**0,006**

We also investigated the relationship between history of oral symptoms (OS) and sensitization to the last three allergens included in the series. We found a weak positive correlation with statistical significance only with the epoxy resin (Table 25).

Table 25: Correlation between oral symptoms and sensitization to other allergens

Indicators	n	τ	p
OS+ Eugenol	164	0,049	0,519
OS+ Zn	35	-0,142	0,395
OS+Epoxy resin	50	**0,289**	**0,039**

Women were predominant in the studied group – the fact is not surprising, since the group was composed of patients who had consecutively passed through the allergology office and women are more likely to search for, confirm and treat their various diseases.

The age distribution in the group was uneven with the histogram looking similar to that of the female age histogram – again this was due to the predominance of participants of that gender.

Almost 11% of the study participants reported a concomitant allergy – this was in line with other studies showing even higher levels of sensitization in the population. Regarding dental materials – about 4% of our patients shared that they had confirmed such sensitization. Other researchers analyzed the literature and results from epidemiological studies and skin samples, presenting similar data. Women with sensitization to dental materials predominated over men – our result was also confirmed by other researchers. The difference in our study was the proportion of tested patients with oral manifestations – only about 27% versus more than 52% in other studies. This leads to the conclusion that it was likely that in our country many cases of allergy remain undiagnosed and untreated, or patients neglect their condition. Only 4% of the available oral symptoms were associated with the already confirmed concomitant allergy.

The acrylate allergens MMA and TEGDMA were defined as strong allergens – in our study they were the only ones with registered strong positive reactions. On the other hand, BisGMA and UDMA were considered allergens with low sensitizing potential, but we reported a high proportion of positive results. We believe that the result for UDMA was not significant due to the small number of cases.

Similarly, Lyapina et al. found in their study the most pronounced sensitization to BisGMA and 2-HEMA in the patient group. They attributed it to multiple contacts with acrylate materials and suspected/confirmed allergy to other dental materials as well.

The calculated correlation between some acrylate allergens – especially between MMA and TEGDMA – was also expected. It was also confirmed by the results of other authors. Some works also commented on the possibility of cross-sensitization between acrylates and other materials such as epoxy resin, but this was not confirmed in our case, most likely due to the small number of recorded positive reactions to the resin.

Regarding the other allergens, the share of positive reactions to zinc was the largest, and extreme positive reaction was also recorded. Our results were in sync with what other authors had commented, namely that most often people were sensitized to the metals used in dental work.

Our study data showed a significant correlation only between oral symptoms and sensitization to epoxy resin.

It is difficult to compare our results regarding sensitization to the constituents of temporary filling materials with those of other authors because the subject is rarely exploited. Usually acrylates, metals and others are considered individually, not in the combination we offer. There are earlier Bulgarian studies, but they used final-product allergens ("Bulgarian school") and some of these materials are no longer in use.

V. CONCLUSIONS

From the microbiological study:

1. Eugenol has an antimicrobial effect against all tested strains.
2. When eugenol was combined with ZnO, the results overlapped to some extent with eugenol alone.
3. ZnO shows antimicrobial activity against *E. coli, C. albicans* and *K. pneumoniae*.

From the microleakage study:

1. The lowest microleakage values are observed around the light-curing temporary filling materials, both for the short-term and after a longer period.
2. All three groups show increased microleakage at a 14-day period compared to a 2-day period.

From the allergological study:

1. Sensitization and polysensitization to the examined dental materials is registered more often in women.
2. Zinc is the most common sensitizer among the ingredients of temporary filling materials.
3. Acrylates and methacrylates often cause cross-sensitivity.
4. There is an extremely small risk for the ingredients of the temporary filling materials to cause oral symptoms.

VI. CONCLUSION

Although modern dentistry prioritizes one-stage treatment of dental caries and its complications, more than one visit is often required.

Temporary filling materials serve to seal cavities in multistage treatment. Although they do not possess the mechanical and esthetic qualities of definitive obturation materials, they have a number of functions, which include tight sealing of the cavity, satisfactory mechanical strength, supporting the normal functioning of the tooth, good attachment to hard dental tissue, low sensitivity to moisture, antibacterial properties, protecting hard dental tissue from fracture and protecting periodontal tissues. One of their main disadvantages is the increased levels of microleakage, the lowest being observed in the photopolymerizing representatives.

Various materials are available, and the choice of a specific one depends on a number of factors such as cavity size, tooth vitality, type of definitive restoration and dwell time.

It is extremely important for dental practitioners to know the advantages and disadvantages of different temporary obturation materials in order to select the most appropriate one for each clinical case.

VII. SELF-ASSESSMENT OF DISSERTATION CONTRIBUTIONS

Original scientific and applied contributions:

1. The antibacterial properties of temporary filling materials were investigated for the first time.
2. Sensitization to temporary filling materials was investigated for the first time with the proposed panel of diagnostic allergens.

Confirmatory scientific and applied contributions:

1. We confirmed the antimicrobial action of eugenol, both alone and in combination with ZnO.

2. We confirmed a high degree of microleakage around all types of temporary obturation, especially after a longer period.

3. We confirmed the high risk of developing cross-hypersensitivity between different acrylates and methacrylates in dental practice.

VIII. PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS (ON THE SUBJECT)

1. Publications

- Valkov B, Balcheva M. Temporary filling materials in endodontics – a literature review. Scripta Scientifica Medicinae Dentalis. 2022;8(1):13-7
- Valkov B, Balcheva M, Ermenlieva N. Microorganisms colonizing dental structures and antibacterial effect of temporary obturation materials. Varna Medical Forum. 2023;12(2):168-73 (Article in Bulgarian)
- Valkov B, Balcheva M, Zaneva-Hristova Z. Microleakage of temporary filling materials used in endodontics. Scripta Scientifica Medicinae Dentalis. 2024;10(1):(in print)

2. Participation in scientific forums

- Valkov B, Balcheva M. Properties and application of temporary filling materials in operative dentistry and endodontics. Jubilee Symposium "10 years Alumni club and Friends – Medical University – Varna", 01-02.04.2022. (In Bulgarian)
- Valkov B, Balcheva M, Zaneva-Hristova D. Microleakage of temporary filling materials used in endodontics. 8th Black Sea Symposium for Young Scientists in Biomedicine (BSYSB), Varna 09-12 November 2023