FACULTY OF CHEMISTRY
DEPARTMENT OF PHYSICAL CHEMISTRY AND
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SUMMARY OF DOCTORAL DISSERTATION

Modifications of dimethacrylate copolymers with compounds containing quaternary ammonium groups to obtain novel antibacterial dental composite adhesives

A series of monothematic publications

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SCIENTIFIC ACHIEVEMENTS BEING THE BASIS OF THE DOCTORAL DISSERTATION

The doctoral dissertation constitutes a series of eight publications (**P1**–**P8**) published in scientific journals indexed in the Journal Citation Reports (JCR) database between 2020 and 2023. The total impact factor (*IF*) of the listed publications is 39,884, the total MEiN score is 1080, and the citation number (excluding self-citations) is 26.

The doctoral dissertation also includes a series of five national patents (**K1**–**K5**) and one patent application (**Z1**).

SCIENTIFIC PUBLICATIONS

1. **Chrószcz, M.**[*]; Barszczewska-Rybarek, I. Nanoparticles of Quaternary Ammonium Polyethylenimine Derivatives for Application in Dental Materials, *Polymers* **2020**, *12*, 2551, DOI: 10.3390/polym12112551, IF_{2020}: 4,329, MEiN score: 100. **(P1)**

2. Barszczewska-Rybarek, I.M.[*]; **Chrószcz, M.W.**; Chladek, G. Physicochemical and Mechanical Properties of Bis-GMA/TEGDMA Dental Composite Resins Enriched with Quaternary Ammonium Polyethylenimine Nanoparticles, *Materials* **2021**, *14*, 2037, DOI: 10.3390/ma14082037, IF_{2021}: 3,748, MEiN score: 140. **(P2)**

3. **Chrószcz, M.W.**; Barszczewska-Rybarek, I.M.[*] Synthesis and Characterization of Novel Quaternary Ammonium Urethane-Dimethacrylate Monomers—A Pilot Study, *International Journal of Molecular Sciences* **2021**, *22*, 8842, DOI: 10.3390/ijms22168842, IF_{2021}: 6,208, MEiN score: 140. **(P3)**

4. **Chrószcz, M.W.**[*]; Barszczewska-Rybarek, I.M.; Kazek-Kęsik, A. Novel Antibacterial Copolymers Based on Quaternary Ammonium Urethane-Dimethacrylate Analogues and Triethylene Glycol Dimethacrylate, *International Journal of Molecular Sciences* **2022**, *23*, 4954, DOI: 10.3390/ijms23094954, IF_{2022}: 6,208, MEiN score: 140. **(P4)**

5. **Chrószcz-Porębska, M.W.**; Barszczewska-Rybarek, I.M.; Chladek, G[*]. Characterization of the Mechanical Properties, Water Sorption, and Solubility of Antibacterial Copolymers of Quaternary Ammonium Urethane-Dimethacrylates and Triethylene Glycol Dimethacrylate, *Materials* **2022**, *15*, 5530, DOI: 10.3390/ma15165530, IF_{2022}: 3,748, MEiN score: 140. **(P5)**

6. **Chrószcz-Porębska, M.W.**; Barszczewska-Rybarek, I.M.[*]; Chladek, G. Physicochemical Properties of Novel Copolymers of Quaternary Ammonium UDMA Analogues, Bis-GMA, and TEGDMA, *International Journal of Molecular Sciences* **2023**, *24*, 1400, DOI: 10.3390/ijms24021400, IF_{2023}: 6,208, MEiN score: 140. **(P6)**

7. **Chrószcz-Porębska, M.W.**; Barszczewska-Rybarek, I.M.[*]; Kazek-Kęsik, A.; Ślęzak-Prochazka, I. Cytotoxicity and Microbiological Properties of Copolymers Comprising Quaternary Ammonium Urethane-Dimethacrylates with Bisphenol A Glycerolate Dimethacrylate and Triethylene Glycol Dimethacrylate, *Materials* **2023**, *16*, 3855, DOI: 10.3390/ma16103855, IF_{2023}: 3,748, MEiN score: 140. **(P7)**

8. **Chrószcz-Porębska, M.**; Kazek-Kęsik, A.; Chladek, G.; Barszczewska-Rybarek, I.* Novel mechanically strong and antibacterial dimethacrylate copolymers based on quaternary ammonium urethane-dimethacrylate analogues, *Dental Materials*, article in press, DOI: 10.1016/j.dental.2023.05.008, IF_{2023}: 5,687, MEiN score: 140. **(P8)**

* corresponding author

The IF was given in accordance with the publication year. For publications published in 2022 and 2023, IF from 2021 was given. The Minister of Education and Science (MEiN) scores were given in accordance with the latest list dated 21 December 2021.

NATIONAL PATENTS

1. Barszczewska-Rybarek, I.M.; **Chrószcz, M.W.** Urethane-dimethacrylate monomer, its preparation and application, patent no. Pat.242186, registration date: 3.11.2022 (**K1**)
2. Barszczewska-Rybarek, I.M.; **Chrószcz, M.W.** Urethane-dimethacrylate monomer, its preparation and application, patent no Pat.242187, registration date: 3.11.2022 (**K2**)
3. Barszczewska-Rybarek, I.M.; **Chrószcz, M.W.** Urethane-dimethacrylate monomer, its preparation and application, patent no Pat.242188, registration date: 3.11.2022 (**K3**)
4. Barszczewska-Rybarek, I.M.; **Chrószcz, M.W.** Urethane-dimethacrylate monomer, its preparation and application, patent no Pat.242189, registration date: 3.11.2022 (**K4**)
5. Barszczewska-Rybarek, I.M.; **Chrószcz, M.W.** Urethane-dimethacrylate monomer, its preparation and application, patent no Pat.242190, registration date: 3.11.2022 (**K5**)

PATENT APPLICATION

1. Barszczewska-Rybarek, I.M.; **Chrószcz, M.W.** Urethane-dimethacrylate resin exhibiting antibacterial properties, for binders of dental restorative composite materials and method of its preparation, application, application no P.434582, application date: 07.07.2020 (**Z1**)

Authors statements specifying their contribution to the publications, national patents, and patent application are included in the attachments to the doctoral dissertation.

LIST OF ABBREVIATIONS

BG:QAm:TEG	copolymers comprising 40 wt.% Bis-GMA, 40 wt.% QAUDMA-m and 20 wt.% TEGDMA, where m corresponds to the number of carbon atoms in the N-alkyl substituent in QAUDMA-m
BG:TEG	copolymer comprising 60 wt.% Bis-GMA and 40 wt.% TEGDMA
BG:UD:TEG	copolymer comprising 40 wt.% Bis-GMA, 40 wt.% UDMA and 20 wt.% TEGDMA
Bis-GMA	bisphenol A glycerolate dimethacrylate
Cm	length of the N-alkyl chain in QAUDMA-m, where m corresponds to the number of carbon atoms in the N-alkyl chain
DC	degree of conversion
DC_{DSC}	degree of conversion determined by differential scanning calorimetry (DSC)
DC_{IR}	degree of conversion determined by infrared spectroscopy (FT IR)
DCRM	dental composite restorative materials
d_m	monomer density
DLS	dynamic light scattering
d_p	polymer density
DSC	differential scanning calorimetry
E	flexural modulus
η	viscosity
FS	flexural strength. In publication **P2** flexural strength was abbreviated as σ. In order to unify, the abbreviation *FS* was used in the doctoral dissertation.
FT IR	Fourier transform infrared spectroscopy
HAMA	N,N-(2-hydroxyethyl)methylaminoethyl methacrylate
HB	Brinell hardness
MDEA	N-methyldiethanolamine
MMA	methyl methacrylate
MW	molecular weight
QAHAMA-m	2-(methacryloyloxy)ethyl-2-hydroxyethylmethylalkylammonium bromide, where m corresponds to the numer of carbon atoms in N-alkyl chain
QAM	quaternary ammonium methacrylates
QAm:TEG	copolymers comprising 60 wt.% QAUDMA-m and 40 wt.% TEGDMA, where m corresponds to the number of carbon atoms in the N-alkyl substituent in QAUDMA-m
QA-PEI NP	nanoparticles of quaternary ammonium polyethylenimine derivatives
QAUDMA-m	quaternary ammonium urethane-dimethacrylate analogues, where m corresponds to the numer of carbon atoms in N-alkyl chain
RI	refractive index
S	polymerization shrinkage
SD	standard deviation
S_e	experimental polymerization shrinkage. In publications **P2** and **P3** the abbreviation *S* was used. In order to unify, the abbreviation S_e was used in the doctoral dissertation.
SL	leachability of residual monomer

S_t	theoretical polymerization shrinkage calculated assuming 100% conversion of double bonds
TEGDMA	triethylene glycol dimethacrylate
T_g	glass transition temperature
T_{gm}	monomer glass transition temperature
T_{gp}	polymer glass transition temperature
TMDI	2,4,4-trimethylhexamethylene diisocyanate
UDMA	urethane-dimethacrylate monomer
WCA	water contact angle
WS	water sorption
x_{DB}	double bond concentration in methacrylate monomer/monomer composition
x_{UB}	urethane bond concentration in methacrylate monomer/monomer composition
^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon-13 nuclear magnetic resonance

AIM AND SCOPE OF THE DOCRORAL DISSERTATION

The doctoral dissertation aimed to modify dimethacrylate copolymers, used as adhesives of dental composite restorative materials, to give them antibacterial properties without compromising their utility properties. The antibacterial properties of copolymers, the achievement of which was an important goal of the studies, were provided by the utilization of compounds containing quaternary ammonium groups.

The studies conducted within the framework of the doctoral dissertation were bidirectional.

The first of the undertaken research areas involved the physical modification of dimethacrylate copolymers with nanoparticles of quaternary ammonium polyethylenimine derivatives (QA-PEI NP), characterized in the literature by high antibacterial activity. The work carried out in this area can be divided into the following stages:

i) literature review on QA-PEI NP and dental materials enriched with them **(P1)**,
ii) modification of copolymer comprising 60 wt.% bisphenol A glycerolate dimethacrylate (Bis-GMA) and 40 wt.% triethylene glycol dimethacrylate (TEGDMA) by dispersing QA-PEI NP in it and characterization of its physicochemical and mechanical properties **(P2)**.

The second and main area of the research conducted within the framework of the doctoral dissertation concerned the chemical modification of dimethacrylate copolymers with monomers containing quaternary ammonium groups. As a part of these studies, a series of six novel quaternary ammonium urethane-dimethacrylate analogues (QAUDMA-m, where m corresponds to the number of carbon atoms in the N-alkyl substituents) was obtained. Their molecules consisted of a core derived from 2,4,4-trimethylhexamethylene diisocyanate (TMDI) and two methacrylate-terminated wings, each containing a quaternary ammonium group substituted with an N-alkyl chain of 8, 10, 12, 14, 16 or 18 carbon atoms. The presence of a TMDI core in QAUDMA-m allows to classify them as derivatives of commonly used in dentistry urethane-dimethacrylate monomer (UDMA). The work carried out in this area can be divided into the following stages:

i) synthesis and characterization of physicochemical properties of a series of six novel QAUDMA-m monomers **(P3, K1**–**K5, Z1)**,
ii) modification of a copolymer comprising 60 wt.% Bis-GMA and 40 wt.% TEGDMA by complete replacement of Bis-GMA with QAUDMA-m and characterization of the antibacterial, physicochemical, and mechanical properties of the obtained copolymers **(P4, P5)**,
iii) modification of a copolymer comprising 40 wt.% Bis-GMA, 40 wt.% UDMA and 20 wt.% TEGDMA by complete replacement of UDMA with QAUDMA-m and characterization of microbiological, physicochemical, and mechanical properties, as well as cytotoxicity of the obtained copolymers **(P6**–**P8)**.

I. THE SUBJECT OF THE DOCTORAL DISSERTATION

Tooth decay is one of the civilization diseases of the 21st century [1–3]. In 2022, World Health Organisation estimated that 2 billion people worldwide are struggling with dental caries, of which 540 million children suffer from caries of primary teeth. This problem does not only concern the population living in undeveloped countries because 3 in 4 people affected by caries live in economically developed countries [4].

Dental caries constitute a significant health problem. Apart from the pain in the area of infected teeth [5] and the decrease in the esthetic value of the teeth [6], it also causes inflammation of the oral cavity [5] or may even lead to the development of cardiovascular [7], urinary [8], and osteoarticular [9] systems diseases, as well as to the ophthalmological problems [10].

One of the most common and effective ways of treating dental caries is to remove the infected tissues and replace them with an appropriate substitute [11,12]. Photocurable dental composite restorative materials (DCRM) based on dimethacrylate adhesives are commonly used for these purposes [13,14]. The commonness of their use results mainly from their functional properties, high esthetic, and economic reasons [15–17]. However, they do not have antibacterial properties, which is one of their shortcomings [18,19]. The studies conducted in this direction showed that the accumulation of bacteria on the surfaces of dimethacrylate based DCRM occurs to a greater extent than on other dental materials, such as amalgams or ceramics [19–23]. In addition, DCRM curing generates shrinkage in the material. It leads to the formation of marginal gaps between the composite filling and the adjacent tissues [24–26]. Bacteria that occur in the environment of the oral cavity, mainly of the *Streptococcus mutans* strain, accumulate on the surface of such filling and in the resulting marginal gaps, and the acidic products of their metabolism lead to the formation of secondary caries [27,28] and post-reconstructive inflammations of tissues adjacent to the reconstruction site [29].

The limitation of secondary carries and post-reconstructive inflammation occurrence is one of the main goals of modern restorative dentistry. One proposed solution is the modification of DCRM adhesives (physical or chemical) with antibacterial compounds [30].

The simplest and very effective method of giving DCRMs antibacterial properties is to physically modify the polymer adhesive by dispersing particles of antibacterial compounds. This modification involves both inorganic and organic compounds. Inorganic compounds include nanoparticles of silver [31–35], gold [35–38], zinc oxide [20,35,39,40], titanium oxide [35,41,42], and calcium phosphate [43,44]. Organic compounds include antibiotics [45,46], chlorhexidine and its derivatives [47,48], furanone [20], and ursolic acid [20]. The biggest problem related to their use is the lack of their chemical bonding with the polymer adhesive. Due to the small size, particles of those compounds can easily leach from the material structure and therefore cause a decrease in the antibacterial properties and deterioration of its physical and mechanical properties. Moreover, after being released from the material, they may migrate to more distant tissues of the body and cause irritation or exert cytotoxic effects [30,49,50].

Therefore, alternative solutions are being sought. One of the proposals is a physical modification of DCRM adhesives with bioactive quaternary ammonium polyethylenimine nanoparticles (QA-PEI NP) [51]. The innovative nature of this solution results from the high content of quaternary

ammonium groups, which allows for obtaining materials with high antibacterial activity at low concentrations of QA-PEI NP [52].

However, greater attention of scientists focuses on the chemical modification of DCRM adhesives using methacrylate monomers with quaternary ammonium groups (QAM) [53–55]. It is due to the possibility of designing a wide range of monomers of different chemical structures, which allows obtaining materials with high microbiological activity and diverse physicochemical and mechanical properties. As QAMs have methacrylate groups in their chemical structure, they can be chemically incorporated into the DCRM adhesive by copolymerization with other methacrylate monomers [53]. It ensures their firm connection to the DCRM, which results in the stability of its properties throughout the entire period of exploitation and long-term antibacterial activity.

Although the literature data provide a wide range of information on QA-PEI NP, QAM, and DCRM adhesives modified with their addition, obtaining a system with antibacterial activity while maintaining appropriate physicochemical and mechanical properties has not yet been achieved. For this reason, designing polymeric compounds with quaternary ammonium groups is still a new and challenging yet promising task for modern restorative dentistry. It was an inspiration to undertake this research topic.

II. RESULTS AND DISCUSSION

The doctoral dissertation included the modification of dimethacrylate copolymers used as DCRM adhesives to give them antibacterial properties.

The studies conducted within the framework of the doctoral dissertation were bidirectional. The first of the undertaken research area involved the physical modification of dimethacrylate copolymers with quaternary ammonium polyethylenimine nanoparticles (QA-PEI NP). The second area of the research conducted within the framework of the doctoral dissertation concerned the chemical modification of dimethacrylate copolymers with monomers containing quaternary ammonium groups (QAUDMA-m).

1. PHYSICAL MODIFICATION OF COPOLYMERS WITH QUATERNARY AMMONIUM POLYETHYLENIMINE NANOPARTICLES

The research performed using QA-PEI NP (Figure 1) started with a literature review on the QA-PEI NP and dental materials enriched with them. As a result, a review article (**P1**) was published. This article discussed i) the methods of QA-PEI NP synthesis, ii) the relationship between the chemical structure of QA-PEI NP and their antibacterial activity, and iii) the influence of the QA-PEI NP addition on the antibacterial properties and cytotoxicity of DCRM and other classes of dental materials.

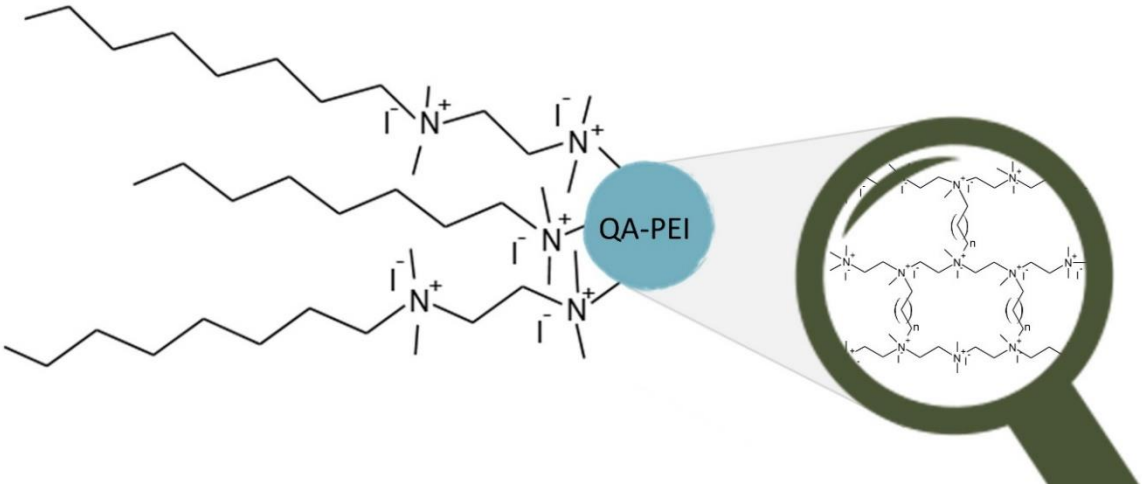

Figure 1. Chemical structure of QA-PEI NP.

The literature data showed that QA-PEI NP and dental materials modified with their use had high antibacterial activity and low cytotoxicity. At the same time, the analysis of the current state of the literature revealed the existence of a research gap relating to the physicochemical and mechanical properties of materials enriched with QA-PEI NP. These properties, although studied to a low extent, are crucial for the proper functioning of dental materials. To fill the research gap indicated in the literature review (**P1**), the research on physical modification aimed to determine the physicochemical and mechanical properties of dimethacrylate copolymers enriched with the QA-PEI NP.

Laboratory studies on the physical modification of dimethacrylate copolymers started with QA-PEI NP synthesis. It was performed according to the procedure described in the literature [51,52] and described in detail in article **P2** (Chap. 2.2).

In the first stage, linear polyethylenimine was synthesized by acidic hydrolysis of poly(2-ethyl-2-oxazoline). It was then subjected to crosslinking with the use of 1,5-dibromopentane, N-alkylation with the use of 1-bromooctane, and quaternization with the use of iodomethane, which led to the formation of QA-PEI NP. The chemical structure of QA-PEI NP was confirmed by proton nuclear magnetic resonance (^{1}H NMR) (**Fig. 4** in **P2**) and infrared spectroscopy (FT IR) (**Fig. 5** in **P2**). The nanometric sizes of QA-PEI NP, which on average were 151 nm (**Fig. 6** in **P2**), were confirmed by dynamic light scattering (DLS) analysis (**Fig. 6** in **P2**)

The obtained QA-PEI NP were then used as physical modifiers of copolymer comprising 60 wt.% Bis-GMA and 40 wt.% TEGDMA, representing the simplest DCRM adhesive system. The modification was performed by dispersing 0.5, 1, and 2 wt.% of QA-PEI NP in the monomer compositions and subsequent photopolymerization. The modification procedure was described in detail in article **P2** (Chap. 2.3.)

Copolymers enriched with QA-PEI NP were then tested for physicochemical (Table 1) and mechanical (Table 2) properties, that was described in article **P2**.

The analysis of the results showed that the introduction of QA-PEI NP into the copolymer in an amount not exceeding 2 wt.% did not adversely affect many of its physicochemical properties.

*Table 1. Experimental polymerization shrinkage (S_e – **Tab. 1** in **P2**), degree of conversion (DC – **Fig. 8** in **P2**), glass transition temperature (Tg_p – **Fig. 9** in **P2**), water contact angle (WCA – **Fig. 12** and **Fig. 13** in **P2**), water sorption (WS – **Fig. 10** in **P2**), and water solubility (SL – **Fig. 11** in **P2**) of copolymers modified with QA-PEI NP.*

	***S_e* (%)**		***DC* (%)**		***Tg_p* (°C)**		***WCA* (°)**		***WS* (μg/mm³)**		***SL* (μg/mm³)**	
	average	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**
0,5% QA-PEI NP	7.37	0.58	70.44	6.25	58.96	4.08	84.26	3.93	36.59	2.52	2.54	0.30
1% QA-PEI NP	8.13	0.72	70.34	5.49	57.12	2.78	85.63	4.10	34.63	2.63	4.05	0.33
2% QA-PEI NP	8.87	0.63	70.77	6.06	55.47	3.05	89.01	4.46	35.16	3.62	10.48	1.08
0% QA-PEI NP	7.37	0.70	68.08	6.27	54.19	3.31	81.08	5.89	31.50	3.78	2.17	0.32

Copolymers enriched with QA-PEI NP were characterized by a slightly higher degree of conversion (*DC*) than the reference copolymer. It resulted in higher values of experimental polymerization shrinkage (S_e) compared to the reference copolymer. The addition of QA-PEI NP also did not negatively affect the copolymer glass transition temperature (Tg_p). Its values were higher than 55°C, ensuring the glassy state of copolymers under the temperature conditions of the oral cavity [56]. The introduction of QA-PEI NP into the copolymer did not also significantly affect the hydrophilicity of its surface. Although a slight increase in surface hydrophobicity was observed with the increasing concentration of QA-PEI NP, the obtained water contact angle (*WCA*) values were lower than 90°. It allowed classifying the surfaces of obtained materials as hydrophilic [57].

On the other hand, the introduction of QA-PEI NP affected the behavior of the copolymers in the aqueous environment, which was manifested by an increase in water sorption (*WS*) and solubility (*SL*). In the case of *WS*, this increase was insignificant, and all of the modified copolymers had *WS* lower than the value of 40 μg/mm^3, specified in the ISO 4049 standard as the maximum for dental materials [58]. In the case of *SL*, the presence of QA-PEI NP caused a significant increase in its value. The largest increase in *SL* was observed for copolymer containing 2 wt.% of QA-PEI NP. It was the only copolymer that exceeded the value of 7.5 μg/mm^3, specified in the ISO 4049 standard as the maximum value for dental materials [58].

*Table 2. Hardness (HB – **Fig. 16** in **P2**), flexural strength (FS – **Fig. 15** in **P2**), and flexural modulus (E – **Fig. 14** in **P2**) of copolymers enriched with QA-PEI NP.*

	***HB* (MPa)**		***FS* (MPa)**		***E* (MPa)**	
	average	**SD**	**average**	**SD**	**average**	**SD**
0,5% QA-PEI NP	111.81	2.31	63.09	3.69	3712.67	203.95
1% QA-PEI NP	110.94	4.08	52.94	6.90	3634.18	297.77
2% QA-PEI NP	109.10	3.94	36.76	5.87	3557.40	290.61
0% QA-PEI NP	106.19	5.14	85.18	7.91	3731.65	321.61

Among the tested mechanical properties, flexural strength (*FS*) showed the highest sensitivity to the presence of QA-PEI NP. The introduction of QA-PEI NP into the reference copolymer at as low content of QA-PEI NP as 0.5 wt.% resulted in a 26% decrease in *FS* value. A downward trend was also observed for the flexural modulus (*E*). However, the scale of changes was not as significant as in the case of *FS*. The introduction of QA-PEI NP in the amount of 0.5 wt.% resulted in a 0.5% decrease in *E* value. On the other hand, the presence of QA-PEI NP exerted an opposite effect on hardness (*HB*), whose values increased due to the introduction of QA-PEI NP, an average of 4.17%.

2. CHEMICAL MODIFICATION OF COPOLYMERS WITH QUATERNARY AMMONIUM URETHANE-DIMETHACRYLATE ANALOGUES

Methacrylate monomers containing quaternary ammonium groups (QAM) have high antibacterial activity against oral bacteria. The literature data show that the chemical modification of dimethacrylate adhesives containing QAM results in materials with high antibacterial activity. However, the introduction of QAM to the DCRM adhesive often caused the deterioration of its physiochemical and mechanical properties. For this reason, QAMs with a chemical structure that allows obtaining DCRM with appropriate physicochemical properties, satisfactory mechanical parameters, high antibacterial activity, and low cytotoxicity are still sought after.

Studies on the chemical modification of dimethacrylate adhesives, performed within the framework of the doctoral dissertation, were based on the utilization of six novel quaternary ammonium urethane-dimethacrylate analogues (QAUDMA-m, where m corresponds to the number of carbon atoms in the N-alkyl substituent) (Figure 2).

The presence of a core derived from 2,4,4 – trimethylhexamethylene diisocyanate (TMDI) in QAUDMA-m allows to classify them as urethane-dimethacrylate derivatives. Their molecules also consist of two methacrylate-terminated wings, each containing a quaternary ammonium group substituted with an N-alkyl chain with 8, 10, 12, 14, 16, or 18 carbon atoms that are linked to the core by a urethane bond.

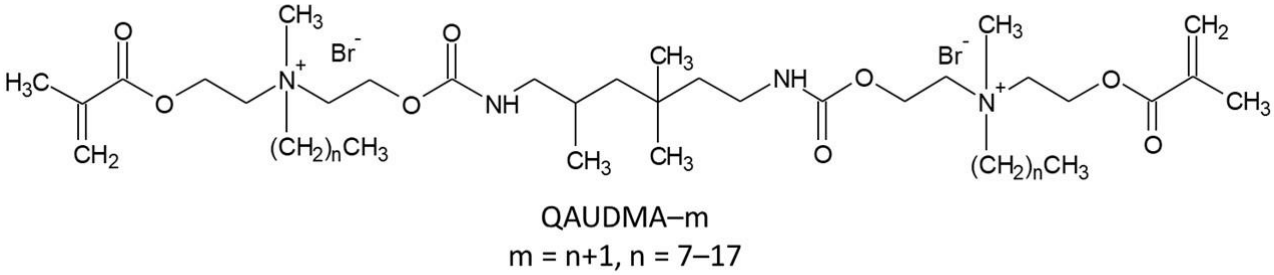

Figure 2. Chemical structure of QAUDMA-m.

2.1. Synthesis and characterization of QAUDMA-m monomers

Laboratory studies on chemical modification of dimethacrylate copolymers started from the synthesis and characterization of physicochemical properties of a series of six QAUDMA-m monomers and their homopolymers, which was described in article **P3**, national patents **K1**–**K5**, and patent application **Z1**.

QAUDMA-m monomers were obtained in a three-stage synthesis process that included:

i) transesterification of methyl methacrylate with N-methyldiethanolamine to obtain N,N-(2-hydroxyethyl)methylaminoethyl methacrylate (HAMA),
ii) N-alkylation of HAMA using alkyl bromides of various chain lengths to obtain [2-(methacryloyloxy)ethyl]-2-hydroxyethylmethylalkylammonium bromide (QAHAMA-m, where m corresponds to the number of carbon atoms in the N-alkyl substituent)
iii) addition of the hydroxyl group of QAHAMA-m to the isocyanate groups of TMDI to obtain QAUDMA-m monomers.

The chemical structure of QAUDMA-m monomers and intermediate products (HAMA, QAHAMA-m) was confirmed by ^{1}H NMR (HAMA – **Fig. 1** in **P3**, QAHAMA-m – **Fig. 3** in **P3**, QAUDMA-m – **Fig. 5** in **P3**), ^{13}C NMR (HAMA – **Fig. 2** in **P3**, QAHAMA-m – **Fig. 4** in **P3**, QAUDMA-m – **Fig. 6** in **P3**), and FT IR (QAUDMA-m – **Fig. 7** w **P3**). Obtained QAUDMA-m monomers were then tested for physicochemical properties (Table 3), which was described in article **P3**.

QAUDMA-m monomers were straw-coloured, highly viscosus liquids. Therefore, they meet the basic condition for monomers for use in DCRM adhesives, which says that the monomer must be in a liquid state. The refractive index (*RI*) of QAUDMA-m monomers was in the range of 1.46 to 1.55, which is adequate for dental adhesive monomers [59]. However, the high viscosity of QAUDMA-m monomers excluded the possibility of their use as standalone components of DCRM adhesives and imposes the need to use them in combination with low viscosity monomers, the so-called reactive diluents, e.g. TEGDMA.

*Table 3. Length of the N-alkyl substituent (Cm), molecular wieght (MW – **Tab. 1** in **P3**), concentration of double bond (x_{DB} – **Tab.1** in **P3**), concentration of urtehane bonds (x_{UB} – **Tab. 1** in **P3**), refractive index (RI – **Tab. 1** in **P3**), viscosity (η – **Tab. 1** in **P3**), density (d_m – **Tab. 1** in **P3**), and glass transition temerature (Tg_m - **Tab. 1** and **Rys. 9** in **P3**) of QAUDMA-m monomers.*

	Cm	***MW*** **(g/mol)**	$x_{DB} = x_{UB}$ **(mol/kg)**	***RI****	***η* (Pa·s) (50°C)**		***d_m* (g/cm^3)**		***Tg_m* (°C)**	
					average	**SD**	**average**	**SD**	**average**	**SD**
QAUDMA-8	C8	970	2.06	1.5161	1.28×10^{3}	0.28×10^{3}	1.199	0.010	-17.25	0.61
QAUDMA-10	C10	1026	1.95	1.5112	1.39×10^{4}	0.14×10^{4}	1.166	0.010	-16.38	0.71
QAUDMA-12	C12	1082	1.85	1.5080	1.14×10^{4}	0.13×10^{4}	1.129	0.010	-15.15	1.03
QAUDMA-14	C14	1138	1.76	1.5054	3.18×10^{3}	0.36×10^{3}	1.101	0.010	-22.39	1.71
QAUDMA-16	C16	1194	1.68	1.5005	2.79×10^{3}	0.39×10^{3}	1.085	0.018	-24.32	1.65
QAUDMA-18	C18	1250	1.60	1.5003	3.33×10^{3}	0.32×10^{3}	1.070	0.010	-31.01	0.92
Bis-GMA	-	512	3.90	1.5493	1.14×10^{3}** 8.36	0.13×10^{3} 0.20	1.150	0.010	-7.31	0.76
UDMA	-	470	4.25	1.4614	9.54**	0.02	1.096	0.010	-35.93	1.24
TEGDMA	-	286	6.99	1.4852	0.011***	-	1.070	0.020	-83.74	1.56

* *SD* for *RI* was 0,0001 in each case
** measured in 25°C
*** as cited in [60]

Subsequently, the QAUDMA-m monomers were polymerized, and the obtained homopolymers were characterized in terms of polymerization shrinkage and the degree of double bond conversion (Table 4), which was described in article **P3**.

QAUDMA-m monomers polymerized to a high degree of conversion (*DC*). Compared to reference monomers, QAUDMA-m monomers polymerized to *DC* higher than Bis-GMA but lower than UDMA and TEGDMA. In addition, QAUDMA-m monomers had lower theoretical (S_t) and experimental (S_e) polymerization shrinkage than dental monomers. It suggests that dimethacrylate copolymers containing QAUDMA-m will be characterized by small marginal gaps and stable physicochemical properties [61,62].

*Table 4. Theoretical (S_t) and experimental (S_e) polymerization shrinkage, degree of conversion determined by infrared spectroscpy (DC_{IR}), and differential scanning calorimetry (DC_{DSC}) of QAUDMA-m homopolymers (**Tab.2** in **P3**).*

	S_t (%)	S_e (%)		DC_{IR} (%)		DC_{DSC} (%)	
		average	SD	average	SD	average	SD
QAUDMA-8	5.56	1.24	0.07	60.39	1.41	61.36	3.90
QAUDMA-10	5.11	1.69	0.16	71.76	2.31	70.76	3.54
QAUDMA-12	4.69	2.25	0.22	73.35	2.49	71.64	4.90
QAUDMA-14	4.35	2.99	0.37	79.42	1.94	78.12	2.59
QAUDMA-16	4.09	1.99	0.10	74.79	2.03	74.27	2.95
QAUDMA-18	3.85	1.47	0.04	53.37	2.67	52.83	2.77
Bis-GMA	10.11	4.72	0.19	32.56	0.92	-*	-*
UDMA	10.65	2.33	0.26	78.92	1.33	-*	-*
TEGDMA	17.12	10.61	0.20	84.23	0.91	-*	-*

* DC_{DSC} was not determined for dental dimethacrylates

The satisfactory physicochemical properties of QAUDMA-m monomers led to further research, in which they were used as chemical modifiers of the two most commonly used DCRM adhesives.

2.2. Characteristics of copolymers comprising 60 wt.% QAUDMA-m and 40 wt.% TEGDMA (QAm:TEG)

A copolymer comprising 60 wt.% Bis-GMA and 40 wt.% TEGDMA (BG:TEG) was first modified. It was achieved by the total replacement of Bis-GMA with QAUDMA-m. The series of six novel QAm:TEG copolymers (where m corresponds to the number of carbon atoms in the N-alkyl substituent in QAUDMA-m) was then characterized for antibacterial, physicochemical, and mechanical properties, which was described in articles **P4** and **P5**.

Firstly, the antibacterial activity of QAm:TEGs against *Staphylococcus aureus* and *Escherichia coli* bacteria was determined (Figure 3, Table 5).

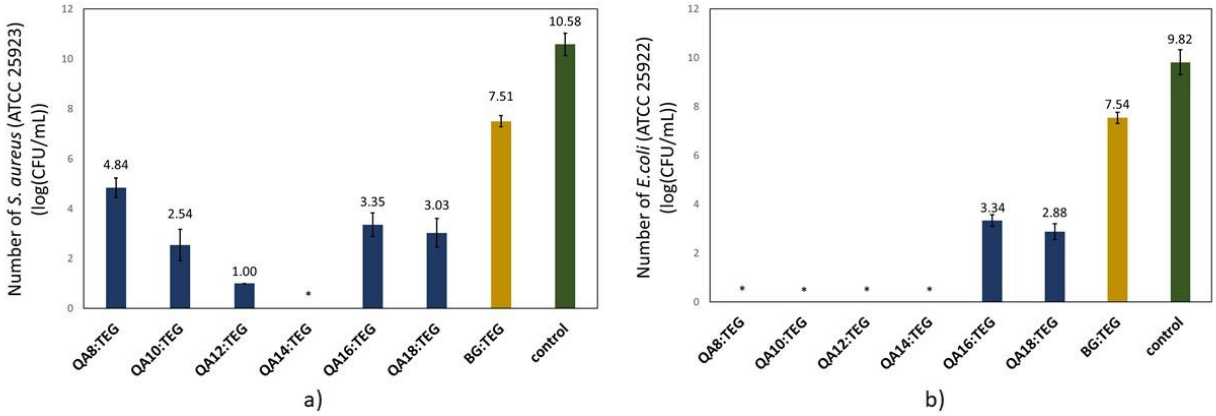

*Figure 3. Number of **a)** S. aureus (ATCC 25923), **b)** E. coli (ATCC 25922) bacteria colonies on QAm:TEG surfaces (**Fig. 6** in **P4**).*
** no bacteria were observed*

Compared to the reference copolymer, the number of bacteria of both strains found on the QAm:TEG surfaces was lower. The highest reduction in the number of bacteria on the copolymer surface was observed for QA14:TEG, on whose surface no bacteria of both strains were observed.

*Table 5. S. aureus (ATCC 25923) and E. coli (ATCC 25922) bacteria growth inhibition zone (**Tab. 4**, **Fig. 7** in **P4**) and bactericidal activity (**Fig. 8** in **P4**) of QAm:TEGs.*

	bacteria growth inhibition zone* (mm)				***bactericidal activity***	
	***S. aureus* (ATCC 25923)**		***E. coli* (ATCC 25922)**		***S. aureus* (ATCC 25923)**	***E. coli* (ATCC 25922)**
	average	**SD**	**average**	**SD**		
QA8:TEG	19	1	10	1	+	+
QA10:TEG	12	1	8	1	+	+
QA12:TEG	13	2	7	1	+	+
QA14:TEG	13	2	6	1	+	+
QA16:TEG	10	1	5	0	+	-
QA18:TEG	6	1	5	0	+	-
BG:TEG	6	1	5	0	-	-

* the value of 5 mm indicates that no inhibition zone was observed
** determined for 25 mg/mL copolymer suspensions

S. aureus growth inhibition zone was observed for all QAm:TEGs. The highest inhibition zone was found for QA8:TEG, and the lowest was found for QA18:TEG. *E. coli* growth inhibition zone was observed for QAm:TEGs with *Cm* lower than C16. The highest inhibition zone was observed for QA8:TEG, and the lowest was found for QA14:TEG. A similar trend was observed for the bactericidal activity of QAm:TEG suspensions. Suspensions of all QAm:TEGs had a bactericidal effect against *S. aureus*, whereas only suspensions of QAm:TEGs with *Cm* lower than C16 had a bactericidal effect against *E. coli*.

Further, QAm:TEGs were tested for physicochemical (Table 6) and mechanical (Table 7) properties.

*Table 6. Theoretical (S_t – **Tab. 3** w **P4**) and experimental (S_e – **Tab. 3** in **P4**) polymerization shrinkage, degree of conversion (DC – **Tab. 3** in **P4**), glass transition temperature (Tg_p – **Tab. 3** and **Fig. 4** in **P4**), water contact angle (WCA – **Fig. 5** in **P4**), water sorption (WS – **Fig. 3** w **P5**), and water solubility (**Fig. 3** in **P5**) of QAm:TEGs.*

	***S_t* (%)**	***S_e* (%)**		***DC* (%)**		***Tg_p* (°C)**		***WCA* (°)**		***WS* (μg/mm³)**		***SL* (μg/mm³)**	
		average	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**
QA8:TEG	10.41	6.62	0.28	84.16	1.18	60.52	0.79	82.05	272	148.31	1.33	52.39	3.58
QA10:TEG	10.05	6.44	0.29	83.96	0.91	60.33	1.37	82.64	2.42	138.42	2.71	32.23	4.67
QA12:TEG	9.66	6.57	0.63	86.06	1.16	63.18	1.43	84.41	1.67	130.67	2.04	24.21	3.33
QA14:TEG	9.43	6.88	0.59	88.67	1.38	64.07	1.15	86.12	1.74	124.89	2.84	19.08	1.01
QA16:TEG	9.23	6.53	0..96	87.41	1.10	65.03	0.73	94.70	2.31	121.21	1.07	15.41	1.41
QA18:TEG	9.04	6.52	0.92	87.11	0.91	66.32	1.23	98.66	2.08	116.08	1.75	12.67	3.27
BG:TEG	12.89	8.38	0.57	64.83	1.57	61.66	0.58	87.85	2.30	27.20	1.36	3.92	0.71

QAm:TEGs were characterized by high degrees of conversion (*DC*), which exceeded the values typical for DCRM adhesives in each case [63]. In addition, QAm:TEGs showed lower polymerization shrinkage (S_e) than the reference copolymer. They were also characterized by glass transition temperature (Tg_p) higher than 60°C, which ensures the stability of their physicochemical properties in the conditions prevailing in the oral cavity [56]. Most of the tested QAm:TEGs had hydrophilic surfaces. It promotes good adhesion to the enamel and increases the affinity to the inorganic filler

particles, which are also hydrophilic [57]. Surfaces of only QA16:TEG and QA18:TEG were hydrophobic. Replacement of the Bis-GMA with QAUDMA-m negatively affected the behavior of copolymers in an aqueous environment. The values of both water sorption (*WS*) and solubility (*SL*) obtained for QAm:TEGs exceeded the maximum value for DCRM specified in the ISO 4049 standard [58].

The replacement of Bis-GMA with QAUDMA-m caused a decrease in the mechanical properties of studied copolymers. A two-fold decrease in hardness (*HB*) and flexural strength (*FS*) and a four-fold decrease in flexural modulus (*E*) were observed. It means that the use of QAm:TEGs as DCRM adhesives would not allow obtaining materials with appropriate mechanical properties. Thus, such materials could not withstand the forces exerted on the filling during chewing and biting.

*Table 7. Hardness (HB), flexural strength (FS), and flexural modulus (E) of QAm:TEGs (**Tab. 2** w **P5**).*

	***HB* (MPa)**		***FS* (MPa)**		***E* (MPa)**	
	average	**SD**	**average**	**SD**	**average**	**SD**
QA8:TEG	51.41	4.32	21.59	0.66	679.04	36.20
QA10:TEG	51.17	6.93	37.37	2.27	851.58	47.41
QA12:TEG	50.87	4.08	34.46	2.18	848.86	24.69
QA14:TEG	41.60	3.63	28.38	1.38	772.34	31.08
QA16:TEG	41.21	2.27	20.13	1.62	753.46	31.79
QA18:TEG	42.17	1.08	21.75	1.90	459.41	34.42
BG:TEG	107.56	5.70	51.63	6.76	2800.87	78.85

2.3. Characteristics of copolymers comprising 40 wt.% Bis-GMA, 40 wt.% QAUDMA-m, and 20 wt.% TEGDMA (BG:QAm:TEG)

In the next step, a copolymer comprising 40 wt.% Bis-GMA, 40 wt.% UDMA, and 20 wt.% TEGDMA (BG:UD:TEG) was subjected to chemical modification. It was achieved by the total replacement of UDMA with QAUDMA-m. This modification aimed to check whether the reduction of QAUDMA-m concentration in copolymers by 20 wt.% and the presence of Bis-GMA will allow obtaining copolymers with satisfactory antibacterial activity and appropriate physicomechanical characteristics. The series of six novel BG:QAm:TEG copolymers (where m corresponds to the number of carbon atoms in the N-alkyl substituent in QAUDMA-m) obtained in that way was characterized for microbiological activity, physicochemical and mechanical properties, and cytotoxicity, which was described in articles **P6**, **P7**, and **P8**.

Firstly, the antibacterial activity of BG:QAm:TEGs against *S. aureus* and *E. coli* bacteria was determined (Figure 4, Table 8).

The number of *S. aureus* and *E. coli* bacteria found on all BG:QAm:TEG surfaces was lower than that found on the surfaces of BG:TEG and BG:UD:TEG reference copolymers. No bacteria of both strains were found on the surfaces of BG:QA8:TEG and BG:QA10:TEG.

S. aureus growth inhibition zone was observed for all BG:QAm:TEGs. The highest growth inhibition zone was found for BG:QA8:TEG, whereas the lowest was found for BG:QA18:TEG. *E. coli* growth inhibition zone was observed for all BG:QAm:TEGs, except the BG:QA18:TEG. The highest growth inhibition zone was found for BG:QA8:TEG, whereas the lowest was found for

BG:QA16:TEG. Bactericidal activity was observed for all BG:QAm:TEG suspensions against both tested bacteria strains.

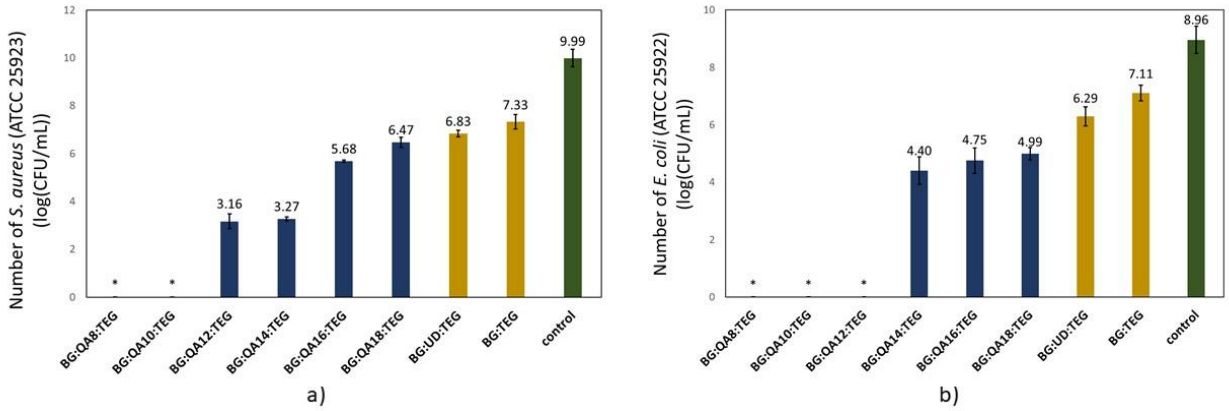

*Figure 4. Number of **a)** S. aureus (ATCC 25923), **b)** E. coli (ATCC 25922) bacteria colonies on BG:QAm:TEG surfaces (**Tab. 3** in **P8**). * no bacteria were observed*

*Table 5. S. aureus (ATCC 25923) and E. coli (ATCC 25922) bacteria growth inhibition zone (**Tab. 3** in **P8**) and bactericidal activity (**Fig. 7b**, **Fig. 7c** in **P7**) of BG:QAm:TEGs.*

	bacteria growth inhibition zone* (mm)				***bactericidal activity*** **	
	***S. aureus* (ATCC 25923)**		***E. coli* (ATCC 25922)**		***S. aureus***	***E. coli***
	average	**SD**	**average**	**SD**	**(ATCC 25923)**	**(ATCC 25922)**
BG:QA8:TEG	23	1	21	1	+	+
BG:QA10:TEG	20	1	20	1	+	+
BG:QA12:TEG	20	1	20	1	+	+
BG:QA14:TEG	18	1	16	1	+	+
BG:QA16:TEG	15	1	12	1	+	+
BG:QA18:TEG	10	1	5	0	+	+
BG:UD:TEG	5	0	5	0	-	-
BG:TEG	6	0	5	0	-***	-***

* the value of 5 mm indicates that no inhibition zone was observed
** determined for 25 mg/mL copolymer suspensions
*** bactericidal activity was not determined for BG:TEG

The BG:QAm:TEGs were also tested for antifungal activity against the *C. albicans* strain (Figure 5, Table 9).

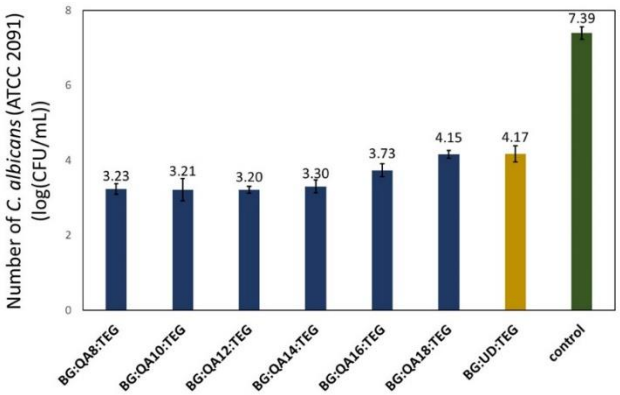

*Figure 5. Number of C. albicans (ATCC 2091) fungi colonies on BG:QAm:TEG surfaces (**Fig. 5** w **P7**). Number of fungi colonies on surface was not determined for BG:TEG.*

The number of fungi colonies observed on all BG:QAm:TEG surfaces, except BG:QA18:TEG, was lower than that on the BG:UD:TEG reference copolymer. BG:QAm:TEGs with *Cm* lower than C16 showed similar and the highest surface antibacterial activity.

*Table 9. C. albicans (ATCC 2091) growth inhibition zone (**Fig. 6** in **P7**) and fungicidal activity (**Fig. 7a** in **P7**) of BG:QAm:TEGs.*

	fungi growth inhibition zone (mm)*		***fungicidal activity***
	average	**SD**	
BG:QA8:TEG	13	1	+
BG:QA10:TEG	11	1	+
BG:QA12:TEG	9	1	+
BG:QA14:TEG	7	1	+
BG:QA16:TEG	5	0	+
BG:QA18:TEG	5	0	+
BG:UD:TEG	5	0	-
BG:TEG	-***	-	-***

* the value of 5 mm indicates that no inhibition zone was observed
** determined for 25 mg/mL copolymer suspensions
*** fungicidal activity was not determined for BG:TEG

C. albicans growth inhibition zone was observed for BG:QAm:TEGs with *Cm* lower than C16. The highest growth inhibition zone was found for BG:QA8:TEG, whereas the lowest was found for BG:QA14:TEG. The fungicidal activity was observed for suspensions of all BG:QAm:TEGs.

Further, the physicochemical (Table 10) and mechanical (Table 11) properties of BG:QAm:TEGs were determined.

*Table 10. Theoretical (S_t – **Tab. 2** in **P6**) and experimental (S_e – **Tab. 2** in **P6**) polymerization shrinkage, degree of conversion (DC – **Tab. 2** in **P8**), glass transition temperature (Tg_p – **Fig. 3** in **P6**), water contact angle (WCA – **Fig. 4** in **P6**), water sorption (WS – **Fig. 5** in **P6**), and solubility (SL – **Fig. 5** in **P6**) of BG:QAm:TEGs.*

	***S_t* (%)**	***S_e* (%)**		***DC* (%)**		***Tg_p* (°C)**		***WCA* (°)**		***WS* (μg/mm³)**		***SL* (μg/mm³)**	
		average	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**	**average**	**SD**
BG:QA8:TEG	9.81	5.08	0.40	59.28	1.51	42.21	1.34	81.41	1.57	68.27	5.36	5.15	0.84
BG:QA10:TEG	9.60	5.48	0.37	60.14	1.97	45.81	1.17	84.68	2.36	48.42	2.90	5.18	0.77
BG:QA12:TEG	9.38	6.07	0.49	61.08	1.57	46.63	1.12	86.32	1.63	35.54	1.49	5.22	0.54
BG:QA14:TEG	9.18	6.14	0.41	63.18	1.72	47.83	1.08	85.52	1.40	34.43	2.23	5.58	0.41
BG:QA16:TEG	9.04	6.24	0.54	66.52	1.31	50.41	1.87	91.05	1.24	32.67	1.13	5.42	0.55
BG:QA18:TEG	8.90	6.40	0.48	68.37	1.97	50.81	0.95	99.53	1.62	25.94	4.00	5.54	0.46
BG:UD:TEG	12.90	8.35	0.23	68.21	0.94	55.90	1.91	80.76	1.99	11.71	1.09	1.12	0.42
BG:TEG	11.61	8.07	0.80	64.62	1.85	61.46	1.42	86.57	1.53	18.31	1.70	1.56	0.25

BG:QAm:TEGs were characterized by a similar degree of conversion (*DC*) and lower polymerization shrinkage (S_e) compared to the BG:TEG and BG:UD:TEG reference copolymers. They had a glass transition temperature (Tg_p) higher than 40°C, which is sufficient to ensure their occurrence in a glassy state under the thermal conditions of the oral cavity [56]. The surfaces of

BG:QAm:TEGs were hydrophilic, except BG:QAm:TEGs with C16 and C18, which were hydrophobic. BG:QAm:TEGs were also characterized by low water sorption (*WS*) and leachability of residual monomer (*SL*). Only BG:QAm:TEGs with C8 and C18 exceeded the *WS* value normalized for dental materials in the ISO 4049 standard [58].

The presence of Bis-GMA and the reduction of QAUDMA-m content significantly improved the mechanical properties of copolymers. The assessment of BG:QAm:TEGs mechanical properties was carried out in relation to the values obtained for BG:TEG and BG:UD:TEG reference copolymers. From the perspective of mechanical properties, BG:QAm:TEGs with a *Cm* from C8 to C12 could serve as substitutes for the BG:TEG. They had higher hardness (*HB*), flexural strength (*FS*), and flexural modulus (*E*). Compared to the BG:UD:TEG, none of the BG:QAm:TEGs achieved similarly good mechanical properties. Among BG:QAm:TEGs, only the BG:QA8:TEG was characterized by higher *HB* and *E* than BG:UD:TEG. Although its *FS* was the highest among the tested BG:QAm:TEGs, its value was still lower by 20% compared to the BG:UD:TEG.

*Table 11. Hardness (HB), flexural strength (FS), and flexural modulus (E) of BG:QAm:TEGs (**Tab. 2** in **P8**).*

	***HB* (MPa)**		***FS* (MPa)**		***E* (MPa)**	
	average	**SD**	**average**	**SD**	**average**	**SD**
BG:QA8:TEG	153.91	5.56	74.47	4.67	3716.68	90.76
BG:QA10:TEG	128.21	8.08	65.25	3.63	3393.39	83.10
BG:QA12:TEG	126.96	3.98	60.01	3.75	2958.97	177.27
BG:QA14:TEG	113.63	5.25	55.58	3.02	2636.08	190.78
BG:QA16:TEG	98.13	3.04	54.75	3.48	2181.70	164.70
BG:QA18:TEG	83.84	2.85	50.81	4.96	1986.74	58.03
BG:UD:TEG	130.81	4.30	95.65	5.19	3701.30	102.99
BG:TEG	101.34	5.93	55.54	1.75	2810.34	248.29

In the last stage, BG:QAm:TEGs were tested for cytotoxicity on L929 mouse fibroblast cell line (Figure 6). It was observed that the reduction in cell viability after being exposed to the BG:QAm:TEG extracts decreased by no more than 30% in comparison to the control. It allowed to classify BG:QAm:TEGs as noncytotoxic [64].

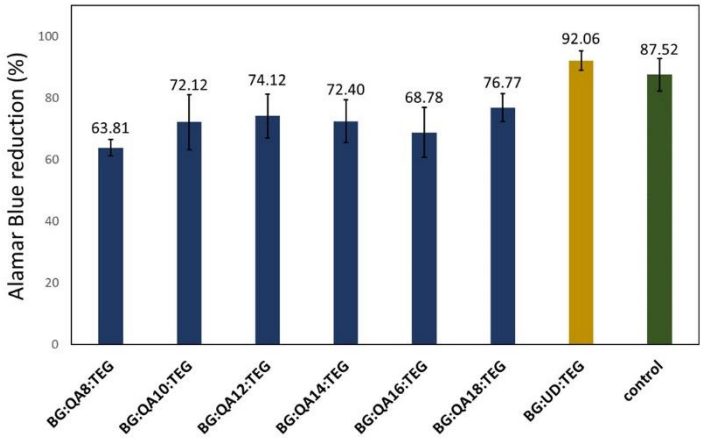

Figure 6. Viability of L929 mouse fibroblast cell line after 24 h of incubation with copolymer extracts.

III. CONCLUSIONS

Within the framework of the doctoral dissertation, studies on physical and chemical modification of dimethacrylate copolymers used as dental composite restorative materials (DCRM) adhesives to give them antibacterial activity without prejudice to their utility properties were conducted. Modifications were performed with the use of compounds containing quaternary ammonium groups.

The results achieved in the doctoral dissertation are summarized in Figure 7.

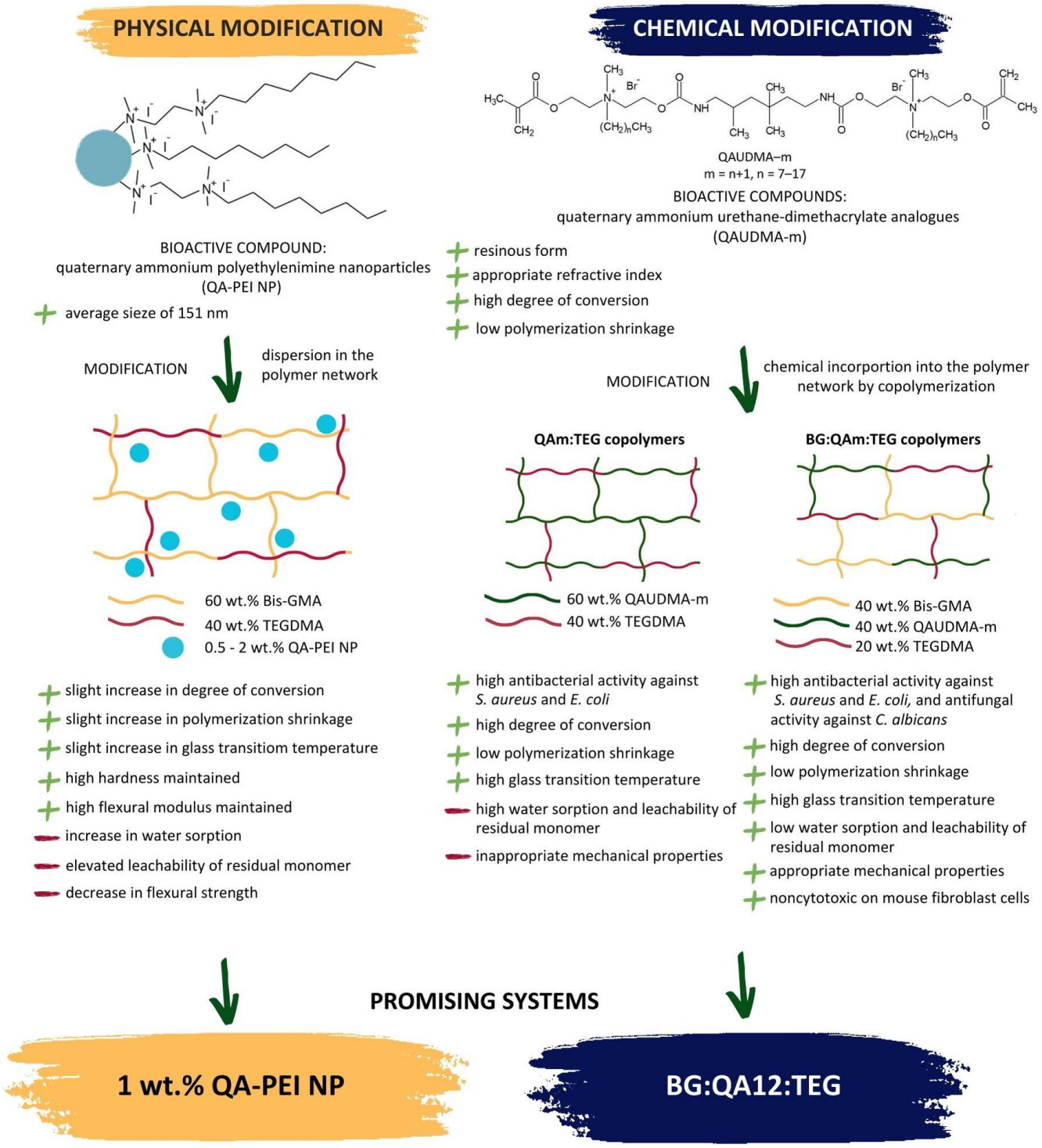

Figure 7.Summary of the results achieved in the doctoral dissertation.

Physical modification

The physical modification of copolymers was based on the utilization of quaternary ammonium polyethylenimine nanoparticles (QA-PEI NP). They were used as modifiers of copolymer comprising 60 wt.% bisphenol A glycerolate dimethacrylate (Bis-GMA) and 40 wt.% triethylene glycol dimethacrylate (TEGDMA).

The obtained results showed that the introduction of QA-PEI NP in the amount of 2 wt.% excludes the possibility of using a modified copolymer as the DCRM adhesive. It is related to the values of flexural strength and leachability of residual monomer, which significantly differ from those acceptable for dental materials. Considering materials containing 0.5 and 1 wt.% of QA-PEI NP, it was observed that they had appropriate physicochemical parameters (including low leachability of residual monomer) and satisfactory values of mechanical properties. In addition, taking into account the literature data on the antibacterial properties of dimethacrylate materials modified with the same amount of QA-PEI NP, it can be seen that the system containing 1 wt.% QA-PEI NP is characterized by the most promising combination of physicomechanical and microbiological properties.

Chemical modification

Chemical modification of copolymers utilized newly obtained dimethacrylate monomers containing quaternary ammonium groups (QAUDMA-m), classified as derivatives of urethane-dimethacrylate monomer (UDMA). Due to the satisfactory physicochemical properties of QAUDMA-m and their homopolymers, they were used as chemical modifiers of the two most commonly used DCRM adhesives.

A copolymer comprising 60 wt.% Bis-GMA and 40 wt.% TEGDMA (BG:TEG) was subjected to modification as a first. The complete replacement of Bis-GMA with QAUDMA-m led to obtaining a series of six QAm:TEG copolymers.

QAm:TEGs were characterized by high antibacterial activity against *S. aureus* and *E. coli*, which was manifested by a reduced number of bacteria observed on their surfaces, the presence of an inhibition zone, and the bactericidal effect of the copolymer suspensions. QAm:TEGs were also characterized by a high degree of conversion, low polymerization shrinkage, and high glass transition temperature. However, they exhibited water sorption and leachability of residual monomer several times higher than that allowed for DCRM adhesives and unsuitable mechanical parameters. It suggests that the chemical composition of QAm:TEGs is inappropriate for dental applications.

The results obtained for QAm:TEGs also provided fundamental knowledge about the relationship between the length of the N-alkyl chain (*Cm*) and the values of studied parameters. It was observed that: i) glass transition temperature and water contact angle increased with the increasing *Cm*, ii) water sorption, water solubility, and the bacteria growth inhibition zone decreased with the increasing *Cm*, iii) flexural strength and flexural modulus initially increased with the increasing *Cm* up to C10, and then decreased, iv) the number of bacteria on copolymer surfaces decreased with the increasing *Cm* up to C14, and then increased, v) polymerization shrinkage and degree of conversion were the only parameters that did not show a correlation with *Cm*.

Results obtained for QAm:TEGs excluded the possibility of their use as DCRM adhesives. However, they revealed that QAUDMA-m monomers could be a promising antibacterial component of DCRM adhesives when used at lower concentrations. For this reason, a copolymer comprising

40 wt.% Bis-GMA, 40 wt.% UDMA, and 20 wt.% UDMA (BG:UD:TEG) was subjected to modification. It was done by the total replacement of UDMA with QAUDMA-m (BG:QAm:TEG).

The reduction of QAUDMA-m content did not negatively affect the copolymers antibacterial activity. BG:QAm:TEGs were characterized by high antibacterial activity against *S. aureus* and *E. coli* bacteria strains. They also showed antifungal activity against *C. albicans*. Both were manifested by the reduced number of microbial colonies on BG:QAm:TEGs surfaces, presence of microbial growth inhibition zone, and a biocidal effect of copolymer suspensions. The BG:QAm:TEGs were also characterized by a high degree of conversion, low polymerization shrinkage, high glass transition temperature, low water sorption, and low leachability of residual monomer (except for BG:QA8:TEG and BG:QA10:TEG), and appropriate mechanical properties. In addition, all of the BG:QAm:TEGs were not cytotoxic to mouse fibroblast cells.

A more detailed analysis of the tested parameters leads to the conclusion that the BG:QA12:TEG seems to be the most suitable for use as a potential DCRM adhesive. It was characterized by satisfactory physicochemical and mechanical properties, high microbiological activity, and did not exert cytotoxic effects on mouse fibroblast cells.

The research carried out within the framework of the doctoral dissertation shows that the use of quaternary ammonium compounds to perform the physical and chemical modification of dimethacrylate copolymers allows to give them high microbiological activity while maintaining appropriate functional properties. Besides selecting copolymers suitable for use as potential DCRM adhesives, the achieved results have a cognitive meaning and constitute an important supplement to the knowledge on the influence of the chemical structure of compounds containing quaternary ammonium groups on the properties of dimethacrylate copolymers modified with their use.

IV. BIBLIOGRAPHY

1. Hayashi, M.; Haapasalo, M.; Imazato, S.; Lee, J. Il; Momoi, Y.; Murakami, S.; Whelton, H.; Wilson, N. Dentistry in the 21st Century: Challenges of a Globalising World. *Int. Dent. J.* **2014**, *64*, 333–342.
2. James, S.L.; Abate, D.; Abate, K.H.; Abay, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdela, J.; Abdelalim, A.; et al. Global, Regional, and National Incidence, Prevalence, and Years Lived with Disability for 354 Diseases and Injuries for 195 Countries and Territories, 1990-2017: A Systematic Analysis for the Global Burden of Disease Study 2017. *Lancet* **2018**, *392*, 1789–1858.
3. Peres, M.A.; Macpherson, L.M.D.; Weyant, R.J.; Daly, B.; Venturelli, R.; Mathur, M.R.; Listl, S.; Celeste, R.K.; Guarnizo-Herreño, C.C.; Kearns, C.; et al. Oral Diseases: A Global Public Health Challenge. *Lancet* **2019**, *394*, 249–260.
4. World Healt Organisation Report. *Global Oral Health Status Report: Towards Universal Health Coverage for Oral Health by 2030*. Geneva: World Health Organization **2022**.
5. Holland, G.R. Dental Pain, Etiology, Pathogenesis and Management. In *Encyclopedia of Pain*, ed. Schmidt, R.; Willis, W, Springer: Berlin, Germany, **2007**, pp. 538–540.
6. Kaur, P.; Singh, S.; Mathur, A.; Makkar, D.K.; Aggarwal, V.P.; Batra, M.; Sharma, A.; Goyal, N. Impact of Dental Disorders and Its Influence on Self Esteem Levels among Adolescents. *J. Clin. Diagn. Res.* **2017**, *11*, ZC05-ZC08.
7. Kim, K.; Choi, S.; Chang, J.; Kim, S.M.; Kim, S.J.; Kim, R.J.; Cho, H.J.; Park, S.M. Severity of Dental Caries and Risk of Coronary Heart Disease in Middle-Aged Men and Women: A Population-Based Cohort Study of Korean Adults, 2002–2013. *Sci. Reports* **2019**, *9*, 10491.
8. Kılıç, F.E.; Almiş, H.; Bucak, I.H.; Turgut, M. Evaluation of the Relationship between Dental Caries and Urinary Tract Infections. *Zeynep Kamil Med. J.* **2022**, *53*, 146–150.

9. Lee, Y.H.; Myong, J.P. Relationship between Bone Mineral Density and Dental Caries in Koreans by Sex and Menopausal State. *Int. J. Environ. Res. Public Health* **2022**, *19*, 6917.
10. Tsai, K.Z.; Liu, P.Y.; Lin, Y.P.; Pao, S.I.; Tai, M.C.; Chen, J.T.; Lin, G.M. Dental Caries and Periodontitis and the Risk of Myopia in Young Adults: CHIEF Oral Health Study. *BMC Oral Health* **2022**, *22*, 384.
11. Cheng, L.; Zhang, L.; Yue, L.; Ling, J.; Fan, M.; Yang, D.; Huang, Z.; Niu, Y.; Liu, J.; Zhao, J.; et al. Expert Consensus on Dental Caries Management. *Int. J. Oral Sci.* **2022**, *14*, 17.
12. Szufnara, A.; Majewska-Beśka, S.; Szczepańska, J. Treatment Methods of Deep Caries in Immanture Permanent Teeth. *New Med.* **2020**, *2*, 76–82.
13. Zheng, L.W.; Wang, J.Y.; Yu, R.Q. Biomaterials in Dentistry. In *Encyclopedia of Biomedical Engineering*, ed. Narayan, R., Elsevier Inc.: Amsterdam, The Netherlands, **2019**, pp. 278–288.
14. Aminoroaya, A.; Neisiany, R.E.; Khorasani, S.N.; Panahi, P.; Das, O.; Madry, H.; Cucchiarini, M.; Ramakrishna, S. A Review of Dental Composites: Challenges, Chemistry Aspects, Filler Influences, and Future Insights. *Compos. Part B Eng.* **2021**, *216*, 108852.
15. Watts, D.C. Adhesives and Sealants. In *Biomaterials Science: An Introduction to* Materials, ed. Ratner, B.; Hoffman, A.; Schoen, F.; Lemons, J., Elsevier Inc.: Amsterdam, The Netherlands, **2013**, pp. 889–904.
16. Lin, G.S.S.; Abdul Ghani, N.R.N.; Ismail, N.H.; Singbal, K.P.; Yusuff, N.M.M. Polymerization Shrinkage and Degree of Conversion of New Zirconia-Reinforced Rice Husk Nanohybrid Composite. *Eur. J. Dent.* **2020**, *14*, 448–455.
17. Pratap, B.; Gupta, R.K.; Bhardwaj, B.; Nag, M. Resin Based Restorative Dental Materials: Characteristics and Future Perspectives. *Jpn. Dent. Sci. Rev.* **2019**, *55*, 126–138.
18. Delaviz, Y.; Finer, Y.; Santerre, J.P. Biodegradation of Resin Composites and Adhesives by Oral Bacteria and Saliva: A Rationale for New Material Designs That Consider the Clinical Environment and Treatment Challenges. *Dent. Mater.* **2014**, *30*, 16–32.
19. Spencer, P.; Ye, Q.; Misra, A.; Goncalves, S.E.P.; Laurence, J.S. Proteins, Pathogens, and Failure at the Composite-Tooth Interface. *J. Dent. Res.* **2014**, *93*, 1243–1249.
20. Ali, S.; Sangi, L.; Kumar, N.; Kumar, B.; Khurshid, Z.; Zafar, M.S. Evaluating Antibacterial and Surface Mechanical Properties of Chitosan Modified Dental Resin Composites. *Technol. Heal. Care* **2020**, *28*, 165–173.
21. Bourbia, M.; Ma, D.; Cvitkovitch, D.G.; Santerre, J.P.; Finer, Y. Cariogenic Bacteria Degrade Dental Resin Composites and Adhesives. *J. Dent. Res.* **2013**, *92*, 989–994.
22. Kuper, N.K.; Van De Sande, F.H.; Opdam, N.J.M.; Bronkhorst, E.M.; De Soet, J.J.; Cenci, M.S.; Huysmans, M.C.D.J.N.M. Restoration Materials and Secondary Caries Using an in Vitro Biofilm Model. *J. Dent. Res.* **2015**, *94*, 62–68.
23. Zhang, N.; Melo, M.A.S.; Weir, M.D.; Reynolds, M.A.; Bai, Y.; Xu, H.H.K. Do Dental Resin Composites Accumulate More Oral Biofilms and Plaque than Amalgam and Glass Ionomer Materials? *Materials* **2016**, *9*, 888.
24. Hamama, H.H. Recent Advances in Posterior Resin Composite Restorations. In *Applications of Nanocomposite Materials in Dentistry*, ed. Abdullah, M.A.; Inamuddin,; Mohammad, A., Elsevier Inc.: Amsterdam, The Netherlands, **2018**, pp. 319-336.
25. Pałka K, Janiczuk P, K.J. Polymerization Shrinkage of Resin Mixtures Used in Dental Composites. *Eng. Biomater.* **2020**, *154*, 16–21.
26. Irie, M.; Suzuki, K.; Watts, D.C. Marginal Gap Formation of Light-Activated Restorative Materials: Effects of Immediate Setting Shrinkage and Bond Strength. *Dent. Mater.* **2002**, *18*, 203–210.
27. Forssten, S.D.; Björklund, M.; Ouwehand, A.C. Streptococcus Mutans, Caries and Simulation Models. *Nutrients* **2010**, *2*, 290–298.
28. Halpin, R.M.; O'Connor, M.M.; McMahon, A.; Boughton, C.; O'Riordan, E.D.; O'Sullivan, M.; Brady, D.B. Role of Streptococcus Mutans in Human Dental Decay. *Eur. food Res. Technol.* **2008**, *227*, 353–380.
29. Sirajuddin, S.; Narasappa, K.N.; Gundapaneni, V.; Chungkham, S.; Walikar, A.S. Iatrogenic Damage to Periodontium by Restorative Treatment Procedures: An Overwiev. *Open. Dent. J.* **2015**, *9*, 217-222.
30. Sun, Q.; Zhang, L.; Bai, R.; Zhuang, Z.; Zhang, Y.; Yu, T.; Peng, L.; Xin, T.; Chen, S.; Han, B. Recent Progress in Antimicrobial Strategies for Resin-Based Restoratives. *Polymers* **2021**, *13*, 1590.

31. Cheng, L.; Weir, M.D.; Xu, H.H.K.; Antonucci, J.M.; Lin, N.J.; Lin-Gibson, S.; Xu, S.M.; Zhou, X. Effect of Amorphous Calcium Phosphate and Silver Nanocomposites on Dental Plaque Microcosm Biofilms. *J. Biomed. Mater. Res. B. Appl. Biomater.* **2012**, *100*, 1378–1386.
32. Cheng, L.; Weir, M.D.; Xu, H.H.K.; Antonucci, J.M.; Kraigsley, A.M.; Lin, N.J.; Lin-Gibson, S.; Zhou, X. Antibacterial Amorphous Calcium Phosphate Nanocomposites with a Quaternary Ammonium Dimethacrylate and Silver Nanoparticles. *Dent. Mater.* **2012**, *28*, 561–572.
33. Durner, J.; Stojanovic, M.; Urcan, E.; Hickel, R.; Reichl, F.X. Influence of Silver Nano-Particles on Monomer Elution from Light-Cured Composites. *Dent. Mater.* **2011**, *27*, 631–636.
34. Corrêa, J.M.; Mori, M.; Sanches, H.L.; Cruz, A.D. Da; Poiate, E.; Poiate, I.A.V.P. Silver Nanoparticles in Dental Biomaterials. *Int. J. Biomater.* **2015**, *2015*, 485275.
35. Allaker, R.P.; Memarzadeh, K. Nanoparticles and the Control of Oral Infections. *Int. J. Antimicrob. Agents* **2014**, *43*, 95–104.
36. Sokołowski, J.; Szynkowska, M.I.; Kleczewska, J.; Kowalski, Z.; Sobczak-Kupiec, A.; Pawlaczyk, A.; Sokołowski, K.; Łukomska-Szymańska, M. Evaluation of Resin Composites Modified with Nanogold and Nanosilver. *Acta Bioeng. Biomech.* **2014**, *16*, 51–61.
37. Dadkan, S.; Khakbiz, M.; Ghazanfari, L.; Chen, M.; Lee, K.B. Evaluation of Antibacterial and Mechanical Features of Dental Adhesives Containing Colloidal Gold Nanoparticles. *J. Mol. Liq.* **2022**, *365*, 119824.
38. Bapat, R.A.; Chaubal, T. V.; Dharmadhikari, S.; Abdulla, A.M.; Bapat, P.; Alexander, A.; Dubey, S.K.; Kesharwani, P. Recent Advances of Gold Nanoparticles as Biomaterial in Dentistry. *Int. J. Pharm.* **2020**, *586*, 119596.
39. Ferrando-Magraner, E.; Bellot-Arcís, C.; Paredes-Gallardo, V.; Almerich-Silla, J.M.; García-Sanz, V.; Fernández-Alonso, M.; Montiel-Company, J.M. Antibacterial Properties of Nanoparticles in Dental Restorative Materials. A Systematic Review and Meta-Analysis. *Med.* **2020**, *56*, 55.
40. Wang, Y.; Hua, H.; Li, W.; Wang, R.; Jiang, X.; Zhu, M. Strong Antibacterial Dental Resin Composites Containing Cellulose Nanocrystal/Zinc Oxide Nanohybrids. *J. Dent.* **2019**, *80*, 23–29.
41. Dias, H.B.; Bernardi, M.I.B.; Bauab, T.M.; Hernandes, A.C.; de Souza Rastelli, A.N. Titanium Dioxide and Modified Titanium Dioxide by Silver Nanoparticles as an Anti Biofilm Filler Content for Composite Resins. *Dent. Mater.* **2019**, *35*, e36–e46.
42. Esteban Florez, F.L.; Hiers, R.D.; Larson, P.; Johnson, M.; O'Rear, E.; Rondinone, A.J.; Khajotia, S.S. Antibacterial Dental Adhesive Resins Containing Nitrogen-Doped Titanium Dioxide Nanoparticles. *Mater. Sci. Eng. C* **2018**, *93*, 931–943.
43. Zhou, W.; Peng, X.; Zhou, X.; Weir, M.D.; Melo, M.A.S.; Tay, F.R.; Imazato, S.; Oates, T.W.; Cheng, L.; Xu, H.H.K. In Vitro Evaluation of Composite Containing DMAHDM and Calcium Phosphate Nanoparticles on Recurrent Caries Inhibition at Bovine Enamel-Restoration Margins. *Dent. Mater.* **2020**, *36*, 1343–1355.
44. Li, Y.; Hu, X.; Ruan, J.; Arola, D.D.; Ji, C.; Weir, M.D.; Oates, T.W.; Chang, X.; Zhang, K.; Xu, H.H.K. Bonding Durability, Antibacterial Activity and Biofilm PH of Novel Adhesive Containing Antibacterial Monomer and Nanoparticles of Amorphous Calcium Phosphate. *J. Dent.* **2019**, *81*, 91–101.
45. Zhang, R.; Jones, M.M.; Moussa, H.; Keskar, M.; Huo, N.; Zhang, Z.; Visser, M.B.; Sabatini, C.; Swihart, M.T.; Cheng, C. Polymer–Antibiotic Conjugates as Antibacterial Additives in Dental Resins. *Biomater. Sci.* **2018**, *7*, 287–295.
46. Colton, M.B.; Ehrlich, E. Bactericidal Effect Obtained by Addition of Antibiotics to Dental Cements and Direct Filling Resins. *J. Am. Dent. Assoc.* **1953**, *47*, 524–531.
47. Boaro, L.C.C.; Campos, L.M.; Varca, G.H.C.; dos Santos, T.M.R.; Marques, P.A.; Sugii, M.M.; Saldanha, N.R.; Cogo-Müller, K.; Brandt, W.C.; Braga, R.R.; et al. Antibacterial Resin-Based Composite Containing Chlorhexidine for Dental Applications. *Dent. Mater.* **2019**, *35*, 909–918.
48. Gilbert, P.; Moore, L.E. Cationic Antiseptics: Diversity of Action under a Common Epithet. *J. Appl. Microbiol.* **2005**, *99*, 703–715.
49. Zhang, J.F.; Wu, R.; Fan, Y.; Liao, S.; Wang, Y.; Wen, Z.T.; Xu, X. Antibacterial Dental Composites with Chlorhexidine and Mesoporous Silica. *J. Dent. Res.* **2014**, *93*, 1283-1289.
50. Ceci, M.; Viola, M.; Rattalino, D.; Beltrami, R.; Colombo, M.; Poggio, C. Discoloration of Different Esthetic Restorative Materials: A Spectrophotometric Evaluation. *Eur. J. Dent.* **2017**, *11*, 149–156.

51. Domb, A.J.; Weiss, E.I.; Beyth, N.; Farber, I.; Davidi, M.P. Antimicrobial Nanoparticulate Additives Forming Non-Leachable Sustained Antimicrobial Polymeric Compositions. Patent no CA2594216A1, **2006**.
52. Beyth, N.; Yudovin-Farber, I.; Bahir, R.; Domb, A.J.; Weiss, E.I. Antibacterial Activity of Dental Composites Containing Quaternary Ammonium Polyethylenimine Nanoparticles against Streptococcus Mutans. *Biomaterials* **2006**, *27*, 3995–4002.
53. Makvandi, P.; Jamaledin, R.; Jabbari, M.; Nikfarjam, N.; Borzacchiello, A. Antibacterial Quaternary Ammonium Compounds in Dental Materials: A Systematic Review. *Dent. Mater.* **2018**, *34*, 851–867.
54. Ge, Y.; Wang, S.; Zhou, X.; Wang, H.; Xu, H.H.K.; Cheng, L. The Use of Quaternary Ammonium to Combat Dental Caries. *Materials* **2015**, *8*, 3532–3549.
55. Imazato, S.; Chen, J. hua; Ma, S.; Izutani, N.; Li, F. Antibacterial Resin Monomers Based on Quaternary Ammonium and Their Benefits in Restorative Dentistry. *Jpn. Dent. Sci. Rev.* **2012**, *48*, 115–125.
56. Moraes, J.C.S.; Sostena, M.M.D.S.; Grandini, C.R. The Glass Transition Temperature in Dental Composites. In *Metal, Ceramic and Polymeric Composites for Various Uses*, Cuppoletti, J. ed., IntechOpen: London, United Kingdom, **2011**.
57. Commentary, G. Definitions for Hydrophilicity, Hydrophobicity, and Superhydrophobicity: Getting the Basics Right. *J. Phys. Chem. Lett.* **2014**, *5*, 686–688.
58. PN-EN ISO 4049:2019 - Dentistry – Polymer Based Restorative Materials. International Standard Organisation: London, United Kingdom, **2019**.
59. Manappallil, J.J. Basic Dental Materials. Jaypee Brothers Medical Publishers: New Delhi, India, 2015.
60. Sideridou, I.; Tserki, V.; Papanastasiou, G. Effect of Chemical Structure on Degree of Conversion in Light-Cured Dimethacrylate-Based Dental Resins. *Biomaterials* **2002**, *23*, 1819–1829.
61. Al Sunbul, H.; Silikas, N.; Watts, D.C. Polymerization Shrinkage Kinetics and Shrinkage-Stress in Dental Resin-Composites. *Dent. Mater.* **2016**, *32*, 998–1006.
62. Abbasi, M.; Moradi, Z.; Mirzaei, M.; Kharazifard, M.J.; Rezaei, S.; Rezaei, S. Polymerization Shrinkage of Five Bulk-Fill Composite Resins in Comparison with a Conventional Composite Resin. *J. Dent.* **2018**, *15*, 365–374.
63. Alshali, R.Z.; Silikas, N.; Satterthwaite, J.D. Degree of Conversion of Bulk-Fill Compared to Conventional Resin-Composites at Two Time Intervals. *Dent. Mater.* **2013**, *29*, e213–e217.
64. PN-EN ISO 10993-5:2009. Biological Evaluation of Medical Devices – Part 5: Tests for in Vivo Cytotoxicity. International Standard Organisation: London, United Kingdom, **2009**.