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Synthesis of di- and triacrylamides with tertiary amine cores and their evaluation as monomers in dental adhesive interfaces

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Abstract

Purpose/aim: In an attempt to increase the service life of dental adhesive interfaces, more hydrolytically and enzymatically-stable methacrylate alternatives, such as methacrylamides, have been proposed. The aim of this study was to investigate polymerization behavior, as well as mechanical and biological properties of experimental adhesives containing multi-functional acrylamides.

Materials and methods: Multi-functional acrylamides (N,N-Bis[(3-methylaminoacryl)propyl]methylamine - BMAAPMA, Tris[(2-methylaminoacryl)ethyl]amine - TMAAEA, N,N'-bis(acrylamido) 1,4-diazepane - BAADA, N,N-Diethyl-1,3-bis(acrylamido)propane - DEBAAP) or HEMA (2-Hydroxyethyl methacrylate - control) were added at 40 wt% to UDMA. 0.2 wt% DMPA and 0.4 wt% DPI-PF6 were used as initiators. Polymerization kinetics was followed in real-time in near-IR during photoactivation (320–500 nm, at 630 mW/cm²). Water sorption/solubility and flexural strength/modulus were measured according to ISO 4049. ¹H NMR was used to assess monomer degradation kinetics. MTT assay was used to assess cytotoxicity against OD-21 and DPSC cells. Biofilm formation and adhesion were assessed by Luciferase Assay and Impingement technique, respectively. Solvated adhesives (40 vol% ethanol) were used to test interfacial adhesion strength. The results were analyzed by ANOVA/Tukey's test ($\alpha = 0.05$).

Results: In general, the pure methacrylate mixture had higher rate of polymerization ($R_{p_{max}}$), degree of conversion (DC) at $R_{p_{max}}$, and final DC than the acrylamides. Flexural properties after

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Author Contributions

APP Fugolin: conceptualization, investigation, data curation, formal analysis, writing original draft; O Navarro: investigation, writing original draft; M Logan: investigation; V Huynh: investigation; CM França: investigation; JL Ferracane: conceptualization, review and editing of manuscript, funding acquisition; CS Pfeifer: conceptualization, review and editing of the manuscript, supervision, project administration and funding acquisition.

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Supporting Information. ¹H, ¹³C NMR and IT-MS spectra for the new compounds. ¹H NMR spectra of degradation studies for new compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

water storage decreased between 11 and 65%, more markedly for acrylamides. Interfacial bond strength was greater and more stable long-term for the newly synthesized acrylamide formulations (less than 4% reduction at 6 months) compared to the methacrylate experimental control (42% reduction at 6 months). HEMA degraded by almost 90%, while the acrylamides showed no degradation in acidic conditions. Cytotoxicity and biofilm formation, in general, were similar for all groups.

Conclusions: Despite demonstrating high water sorption, the acrylamide-containing materials had similar mechanical and biological properties and enhanced interfacial bond strength stability compared to the methacrylate control.

Graphical Abstract

Keywords

Acrylamide; Methacrylate; Adhesive interface; Dental restorations; Micro-tensile bond strength

1. Introduction

Dental adhesives are widely used in restorative dentistry to provide micromechanical and chemical retention between restorative resin-based composites and the dental substrate. Despite the significant advancements achieved for dental adhesive systems, the degradation of the hybrid layer remains a challenge for the longevity of the bond at the dentin-adhesive interface. This degradation process is complex and multifactorial, and involves both the hybrid layer constituents (mainly the collagen fibers) and the adhesive polymer [1].

Though not without controversy, the collagen degradation has been hypothesized to be due to proteolytic activity of host-derived matrix metalloproteinases (MMPs) [1–3] and cysteine cathepsins (CPs), which together have a synergistic collagenolytic/gelatinolytic activity [1, 4, 5]. The adhesive polymer layer may be degraded by water percolation, in addition to the action of proteolytic enzymes capable of hydrolyzing the ester bonds in the methacrylate-

based materials [6–8]. Ester groups have shown accelerated degradation in the presence of esterases such as cholesterol esterase (CE) and pseudocholinesterase (PCE) [9]. In addition, the presence of residual water compromises the polymerization of adhesive monomers, leading to phase separation and loss of adhesive integrity early in the bonding process [10, 11]. Additionally, the copolymerization of HEMA with a hydrophobic difunctional methacrylate (such as BisGMA and UDMA) forms a loosely cross-linked network, with hydrophilic HEMA-rich domains [12] that are easily plasticized by swelling with water. Also, due to its low molecular weight and the tendency to partition in water, HEMA is easily extracted from the polymer network, with consequent biological repercussions, such as suppression of expression of certain genes associated with protection against excessive cell proliferation, apoptosis and cell mutation [13]. Other than HEMA, the dimethacrylates, commonly used as base monomers can also pose concern in terms of cytotoxicity. Once hydrolyzed, BisGMA may result in the formation of several by-products, including bis-HPPP - a toxic dialcohol, and methacrylic acid [8, 14]. However unlikely, according to several recent studies [15, 16], the potential release of estrogenic BPA (bisphenol A) from the BisGMA degradation is a persistent concern.

Considering the shortcomings of current materials, the use of methacrylamides, in particular multifunctional crosslinkable amides, have been proposed as components of adhesives [17]. For example, one diacrylamide (DEBAAP - *N,N*-Diethyl-1,3-bis(acrylamido)propane) was proposed as a hydrolytically-stable monomer as a replacement for methacrylates in self-etching adhesives and composites [18]. When used in the formulation of an adhesive, this monomer produced shear bond strength approximately 20% higher than the pure methacrylate control. When used in the formulation of a composite, the material had comparable flexural strength/modulus, with the advantage of a 35-fold decrease in cytotoxicity compared with TEGDMA-containing formulations. Another recent study on the use of acrylamides and methacrylamides in etch-and-rinse dental adhesives demonstrated increased stability of the dentin bond strength, despite experiencing a decrease in mechanical properties after storage in water [17]. In this same study, the monomer structure was systematically varied to highlight the effect of steric hindrance and electronic factors, and the results showed that tertiary methacrylamides, although more hydrolytically and enzymatically stable than the acrylamides, presented markedly lower reactivity [17]. Taken together, these results demonstrate that, in spite of the greater susceptibility to water sorption, the use of (meth)acrylamides as ester-free monomers to totally or partially replace methacrylates is a promising strategy to tune polymer properties, and ultimately produce dental adhesive systems with longer clinical service.

Using analog monomers of similar functionality is paramount to understand the influence of the methacrylamide on the function of these adhesives. This was indeed the topic of a previous study, where systematically modified mono-acrylamides and methacrylamides are compared with HEMA and HEA (2-hydroxyethyl acrylate). The goal of the present study was to move the field one step further, by adding multi-functional, crosslinkable monomers to the composition, and compare those to the gold standard in dental adhesive formulations. *Tert*-amine core-bearing multi-functional acrylamides were designed and synthesized, and used in BisGMA-free dental adhesive formulations. Kinetics of polymerization, water stability, mechanical properties, dentin bond strength, cytotoxicity and biofilm formation

were evaluated. The tested hypothesis was that the newly synthesized multi-functional acrylamides would provide greater hydrolytic stability, translating into enhanced bond stability while maintaining other properties comparable to the methacrylate control.

2. Materials And Methods

Experimental adhesives were formulated with 60 wt% UDMA and 40 wt% of one of the monomers shown in Figure 1. The rationale for the selection of these specific monomers was a combination of the greater reactivity of acrylamides in comparison with methacrylamides [17], and the potential for increased crosslinking with the multi-functional monomers. Therefore, three novel *tert*-amine multi-functional acrylamides, *N,N*-bis[(3-methylaminoacryl)propyl]methylaniline (**BMAAPMA**), tris[(2-methylaminoacryl)ethyl]amine (**TMAAEA**) and *N,N*-bis(acrylamido) 1,4-diazepane (**BAADA**) were synthesized *de novo*, as described below. *N,N*-Diethyl-1,3-bis(acrylamido)propane (**DEBAAP**) was synthesized as described previously [18] to serve as the experimental acrylamide control. HEMA-containing materials were tested as methacrylate controls. The photoinitiator system was composed by 0.2 wt% 2,2-dimethoxy-2-phenylacetophenone (DMPA) and 0.4 wt% diphenyl iodonium hexafluorophosphate (DPI-PF6). This initiator system was based in a previous study [19], which demonstrated its better efficiency compared with a conventional camphorquinone/amine system. Butylated hydroxytoluene (BHT, 0.1 wt%) was added to each formulation as an inhibitor to improve shelf life.

2.1 Monomers Synthesis

Reagents for the synthesis reactions were used as received (obtained from Sigma Aldrich, Milwaukee, WI, unless otherwise noted), except Acetonitrile (ACS grade), which was dried over 3 Å molecular sieves for at least 24 h prior to use.

Reactions were carried out in anhydrous MeCN and on a blanket of N₂. Acryloyl chloride was freshly distilled before use. ¹H and ¹³C NMR spectra were acquired with a Bruker AMX (400 and 100 MHz, respectively). ¹H NMR chemical shifts are reported as values in ppm relative to CHCl₃ (7.26) in CDCl₃, H₂O (4.79) in D₂O and Me₄Si (TMS) was used as internal standard. Chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (J) are reported in hertz (Hz).

***N,N*-Bis[(3-methylaminoacryl)propyl]methylaniline (BMAAPMA):** Acryloyl chloride (90.0 mmol, 7.35 mL) and MEHQ (2.0 mg) were dissolved in 150 mL of anhydrous MeCN. The flask was placed in an ice bath between −5 °C and −10 °C before dropwise addition of *N,N*-bis[3-(methylamino)propyl]methylaniline (90.0 mmol, 15.6 g) dissolved in 45 mL of MeCN. After 48 hours, the excess amine formed an insoluble hydrochloride salt and was filtered. The filtrates were concentrated in vacuo and the remaining oil was re-dissolved in DCM and washed in sequence with 0.1 M HCl aqueous solution, saturated NaHCO₃ aqueous solution and water. The remaining organic layer was dried over MgSO₄ and concentrated in vacuo, yielding 6.99 g of a slightly yellow oil (24.8 mmol, 55.1% yield). ¹H NMR (400 MHz, Deuterium Oxide) δ 6.81 – 6.60 (m, 2H), 6.21 – 6.06 (m, 2H), 5.86 – 5.70 (m, 2H), 3.56 – 3.34 (m, 4H), 3.11 (s, 3H), 2.97 (s, 3H), 2.48 – 2.30 (m, 3H), 2.28 –

2.12 (m, 3H), 1.88 – 1.67 (m, 4H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 169.01, 168.77, 128.36, 128.28, 127.70, 127.40, 53.56, 53.50, 53.17, 53.10, 48.39, 46.28, 46.24, 40.90, 40.82, 35.59, 33.91, 24.74, 24.72, 23.40, 23.34. ITMS m/z Calcd ($\text{C}_{15}\text{H}_{28}\text{N}_3\text{O}_2^+$): 282.22; experimental 282.21 (Figure S1).

Tris[(2-methylaminoacryl)ethyl]amine (TMAAEA): Acryloyl chloride (40.0 mmol, 3.24 mL) and MEHQ (1.0 mg) were dissolved in 75 mL of anhydrous MeCN. The flask was placed in an ice bath between -5°C and -10°C before dropwise addition of tris[2-(methylamino)ethyl]amine (26.5 mmol, 5.00 g) dissolved in 20 mL of MeCN. After 48 hours, the excess amine formed an insoluble hydrochloride salt and was filtered. The filtrates were concentrated in vacuo and the remaining oil was re-dissolved in DCM and washed in sequence with 0.1 M HCl aqueous solution, saturated NaHCO_3 aqueous solution and water. The remaining organic layer was dried over MgSO_4 and concentrated in vacuo, yielding 3.05 g of a colorless oil (8.70 mmol, 65.7% yield). ^1H NMR (400 MHz, Chloroform- d) δ 6.73 – 6.45 (m, 3H), 6.39 – 6.09 (m, 3H), 5.79 – 5.54 (m, 3H), 3.61 – 3.29 (m, 6H), 3.15 – 2.92 (m, 9H), 2.81 – 2.56 (m, 6H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 169.15, 168.86, 128.55, 128.42, 127.62, 127.40, 52.05, 51.74, 51.33, 50.35, 50.17, 49.92, 48.28, 48.07, 47.88, 45.67, 45.56, 45.48, 36.14, 34.42. ITMS m/z Calcd ($\text{C}_{18}\text{H}_{31}\text{N}_4\text{O}_3^+$): 351.24; experimental 351.23 (Figure S2).

N,N'-bis(acrylamido) 1,4-diazepane (BAADA): Acryloyl chloride (50.0 mmol, 4.04 mL) and MEHQ (1.0 mg) were dissolved in 75 mL of MeCN. The flask was placed in a water bath between -5°C and -10°C before the dropwise addition of 1,4-diazepane (50.0 mmol, 5.01 g) dissolved in 20 mL of MeCN was attached. After 48 hours, the excess amine formed an insoluble hydrochloride salt and was filtered. The filtrates were concentrated in vacuo resulting in 2.99 g of white crystalline solid (14.3 mmol, 57.3% yield). ^1H NMR (400 MHz, Deuterium Oxide) δ 6.82 – 6.61 (m, 2H), 6.28 – 6.05 (m, 2H), 5.91 – 5.71 (m, 2H), 3.88 – 3.71 (m, 4H), 3.72 – 3.56 (m, 4H), 1.97 – 1.73 (m, 2H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 168.96, 168.86, 168.76, 168.73, 129.15, 129.10, 128.79, 128.79, 127.25, 127.17, 127.02, 126.92, 48.43, 48.06, 47.08, 46.96, 46.42, 46.03, 45.55, 45.47, 27.78, 26.11, 24.34. Multiplets in the ^1H spectra and duplicate peaks in the ^{13}C spectra were observed suggesting multiple isomeric forms due to the ring structure of BAADA (Figure S3).

In addition, the diacrylamide BMAAPMA was also quaternized with side chains of 4, 6 and 14 carbons in an attempt to render the materials antimicrobial. The effect of side chain length in the antimicrobial potential has been demonstrated previously [20]. The synthesis procedure was the same as described above, with an additional quaternization step which can be found in supplementary information. ^1H spectra and duplicate peaks in the ^{13}C spectra are shown in Figures S4–6.

In the instances where these monomers were tested as part of the composition, they were added at 10 wt% to the monomer matrix, with 60 wt% UDMA + 30 wt% BMAAPMA. Control formulations (UDMA + HEMA and UDMA + BMAAPMA, 60:40, respectively), photoinitiator system, and photocuring conditions were the same as described throughout the Materials and Methods section.

2.2 Polymerization Kinetics

Materials (10 mm in diameter and 0.8 mm in thickness, n=3) were sandwiched between two glass slides, then placed in the chamber of an Fourier Transform Infra-red spectrometer (Nicolet 6700, ThermoScientific, USA) and irradiated for 300s with a mercury arc lamp (Acticure, EXFO Acticure 4000 UV Cure; Mississauga, Ontario, Canada) filtered to 320–500 nm and delivering 630 mW/cm² (irradiance reaching the surface of the sample from a distance of 1.5 cm, imposed by the IR set up). Spectra were collected in real-time during photoactivation, with 2 scans per spectrum at 4 cm⁻¹ resolution for 5 min. The degree of conversion was calculated based on the area of the methacrylate (6165 cm⁻¹) and acrylamide (6139 cm⁻¹) vinyl overtones, and the rate of polymerization was calculated as the first derivative of the conversion versus time curve [21].

2.3 Water Sorption and Solubility

The same samples used in the polymerization kinetics tests were subjected to water sorption (WS) and solubility (SL) tests, according to ISO 4049 [22]. The initial “dry” mass (M1) was recorded before samples were immersed in 5 ml of Millipore water for one week. At the end of this time, they were carefully wiped off with absorbent paper, and weighed to record their mass after immersion (M2). Samples were then placed in a desiccator under vacuum until mass stabilization (M3). Water sorption (WS) and solubility (SL) were calculated according to Equations 1 and 2, respectively (V is the volume of the cylindrical specimen), reported in µg/mm³:

$$WS = 1000 \times \frac{M2 - M3}{V} \quad (1)$$

$$SL = 1000 \times \frac{M1 - M3}{V} \quad (2)$$

2.4 Flexural Strength (FS) and Elastic Modulus (E)

For the flexure tests, twelve beam-shaped specimens (2.0 × 2.0 × 25.0 mm) per group were produced by filling silicone molds placed between two glass slides and photocuring for 120 s on each side with the mercury arc lamp at 630 mW/cm². Samples were tested in three-point bending after dry storage at room temperature for 24 hours and after 7 days water immersion (n=6). Crosshead speed of 0.5 mm/min until fracture was applied, according to ISO 4049.

2.5 Dentin microtensile bond strength

For bonding procedures, 40 vol% of ethanol was added to the resin formulations. Adper Single Bond 2 (3M ESPE) was tested as a methacrylate-based commercial control. According to the Safety Data Sheet [23], Single Bond is composed of BisGMA (10–20 wt %), HEMA (5–15 wt%), glycerol 1,2 dimethacrylate (5–10wt%), UDMA (<5wt%), ethyl alcohol (25–35 wt%), water (<5%), diphenyliodonium hexafluorophosphate (<0.5 wt%), methacrylate functional copolymer of polyacrylic and poly(itaconic) acids (5–10 wt%), and 5-nm-diameter spherical silica particles (10–20 wt%).

As substrate, caries-free extracted human third molars ($n = 6$) were collected, cleaned and kept in 0.5% chloramine T. This study was cleared by the Oregon Health & Science University IRB (IRB00012056).

After enamel removal by cutting on saw, a flat dentin surface was wet polished with 600-grit silicon-carbide paper for 30 s to standardize the smear layer. The dentin surface was etched with 37% phosphoric acid (Scotchbond Universal Etchant, 3M ESPE) for 15 s, rinsed with water 15 s and carefully dried with cotton pellets in order to maintain the surface slightly wet. The first adhesive coat was vigorously applied for 20 s, the solvent evaporated by a gentle air stream for 10 s and a second adhesive coat was applied for 10 s and photocured for 60 s using the mercury arc lamp. This lamp was used for this layer to overlap the absorption spectrum of the initiator (DMPA - $\lambda_{\max} = 365\text{nm}$). The overall irradiance was 630 mW/cm^2 , and the irradiance for the peak at 365 nm was 330 mW/cm^2 , as determined using a calibrated bench-top UV-Vis spectrometer (MARC Resin Calibrator, Blue Light analytics, Halifax, NS, Canada) [24]. Therefore, the effective radiant exposure delivered to the adhesive was approximately 20 J/cm^2 . Adper Single Bond (3M ESPE) was applied and photocured according to the manufacturer's instructions. In summary, after phosphoric etching for 15s, rinsing and drying, a first layer of the adhesive was actively applied for 15 s and the solvent evaporated. A second layer was applied and after solvent evaporation, it was photoactivated by a curing light (Demi™ Plus; Kavo Kerr) at 550 mW/cm^2 for 10 s (radiant exposure of 5.5 J/cm^2). It is recognized that the radiant exposures were different for the materials manipulated in house and the commercial adhesive. This is an additional reason for the use of an "experimental control", using similar initiator concentrations, and cured under identical conditions as the novel materials. We opted for keeping the commercial adhesive, cured under commercially recommended conditions as a benchmark. For either adhesive, resin blocks (Filtek Supreme, shade body A2) were built on the bonded surfaces in two-2 mm thick increments each photocured for 20 seconds (Demi™ Plus; Kavo Kerr) at 550 mW/cm^2 .

The teeth were immersed in distilled water and kept at $37\text{ }^{\circ}\text{C}$ for 24 h, and then sectioned perpendicular to the interface using a water-cooled diamond saw (Accutom-50; Struers) to obtain resin-dentin sticks with rectangular cross-sectional area of approximately 1.0 mm^2 . The sticks were immersed in distilled water at $37\text{ }^{\circ}\text{C}$ and tested after 24 hours, 3 weeks and 6 months.

The sticks were loaded in tension to failure in a universal testing machine (Criterion, MTS Systems Co., Eden Prairie, MN, USA) at a crosshead speed of 0.5 mm/min . The bonded surface area was calculated after measuring each stick with a digital caliper (Mitutoyo, Tokyo, Japan). Each stick was attached to the grips of a microtensile jig ensuring uniaxial alignment of the stick with the loading device (Odeme Equipamentos, Sao Paulo, SP, Brazil) with a cyanoacrylate adhesive (Zap It Super Glue; Henkel/Loctite, Westlake, Ohio, USA). The results were recorded in MPa and sticks for each tooth were averaged to provide one value per tooth.

2.6 Hydrolytic Degradation

Neat monomers were subject to the hydrolytic degradation kinetics assay. An aqueous solution (pH = 1) was prepared using HPLC grade water and adjusted using 1.0 M HCl. A 50 mM solution of each monomer (n = 3) was prepared using 1.0 mL of the acidic aqueous solution. A capillary tube was filled with a 50 mM solution of tetramethylammonium bromide dissolved in D₂O and flame sealed. The capillary tube was placed at the bottom on the NMR tube to allow the “locking on” of the instrument and to act as an internal standard. ¹H NMR spectra were obtained using a water suppression by excitation sculpting experiment. After the initial reading, the NMR tubes were flame sealed and incubated at 37 °C. During the first week and after 12, 19, and 30 days, the samples were removed from incubation to obtain water suppressed ¹H NMR spectra. To determine the amount of monomer degradation, spectra were first aligned using the ITSD singlet peak and then an integration region unique to a monomer vinyl proton and an integration region unique to a degradation product (methacrylic or acrylic acid) vinyl proton were used to calculate the ratio of monomer to degradation product. An integration region where a vinyl peak is found in both TMAAEA and acrylic acid was used to normalize the integration values. Additional integration regions on either side of the vinyl region were used to determine the average amount of noise in each spectra. Degradation product values with a signal to noise ratio of less than 10 were considered to show no measurable amount of degradation.

2.7 Cytotoxicity

For the cytotoxicity assay, two cell types were tested - odontoblast-like cell line (OD-21) and human dental pulp stem cells (hDPSC) (cat # PT5025, Lonza). OD-21 cells were cultured in high glucose Dulbecco's Modified Eagle's medium without phenol red (DMEM, cat # 21063–029, Thermo Fisher Scientific, Grand Island, NY, USA) and hDPSCs were cultured in alpha Minimal Eagle's medium (α-MEM) (Gibco, Waltham, MA, USA), both supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution. Cells were kept in an incubator at 37°C, under humidified atmosphere with 5% CO₂ for 2–3 days until confluence was reached. Next, cells were washed with Dulbecco's phosphate buffered saline solution (PBS - Thermo Fisher Scientific) and detached from the culture flasks using 0.05% trypsin/ 0.02% EDTA (Sigma). Cells were counted using a hemocytometer, then 10,000 cells per well were seeded in 96-well plates for 24 hours (n = 6).

In order to obtain 1M stock solutions, each neat monomer used in the adhesive formulations: UDMA (urethane dimethacrylate), HEMA, DEBAAP, BMAAPMA, TMAAEA, and BAADA), was dissolved in dimethyl sulfoxide (DMSO). From these stocks, six different concentrated solutions (0.05, 0.1, 0.5, 1, 5 and 10 mM) were prepared in cell culture medium (DMEM for OD-21 and α-MEM for DPSC) and 100 µl per well was used to incubate the cells.

After cell exposure to the monomer solutions for 24 hours, 10 µl of 0.083% diphenyltetrazolium bromide (MTT) prepared in PBS (v/v) was added to each well and the cells were incubated for 4 hours. Next, 200 µl of DMSO was added to each well, plates were gently agitated and the optical density was read at 570 nm and 630 nm in a

spectrophotometer (Epoch Microplate Spectrophotometer, BioTek Instruments Inc, Winooski, VT, USA). OD-21 and DPSCs cultured in the medium free from the monomers were used as negative controls. Additionally, since monomer fluorescence could affect the readings, 10 mM samples of each tested monomer were incubated without cells and their absorbance measured to provide a second control (i.e. medium control). The percentage of cell viability was calculated according to the equations:

$$OD_{cells} = (OD_{570\text{ exper}} - OD_{630\text{ exper}}) - (OD_{570\text{ med contr}} - OD_{630\text{ med contr}}) \quad (3)$$

where: OD_{cells} is the absorbance of the remaining cells after exposure; OD_{exper} is the absorbance of the experimental specimens at 570 nm and 630 nm; $OD_{med\ contr}$ is the absorbance of the 10 mM solution of each monomer without cells at 570 nm and 630 nm.

$$CV(\%) = \left(\frac{OD_{cells\ neg\ contr} - OD_{cells\ exper}}{OD_{cells\ neg\ contr}} \right) \times 100 \quad (4)$$

where: CV (%) is percentage of cell viability in relation to the negative control (cells + medium free from the monomers); $OD_{cells\ neg\ contr}$ is the cells absorbance of the negative control; $OD_{cells\ exper}$ is the cells absorbance of the experimental samples.

2.8 Luciferase Assay and Impingement Test

A derivative of wild type UA159, the bioluminescent *S. mutans* strain IdhRenGSm [25] was selected for evaluating the antimicrobial potential of the adhesive resins. From a frozen stock, the bacteria was streaked onto an agar plate and grow out in an incubator at 37°C under humidified atmosphere of 5% CO₂ in air for 1 day. At the end of this period, planktonic cultures were grown for 16 hours in TH culture medium supplemented with 10% yeast extract under the same conditions described above.

For antimicrobial activity evaluation, six discs (6 mm diameter × 2 mm thick) were prepared in silicone molds, sandwiched between two glass slides covered with mylar strips, and photoactivated for 120 s on each side at 630 mW/cm². After 24 hours, the top surface was ground with 600-grit silicon-carbide paper in order to obtain a surface roughness standardized between 0.2 and 0.3 μm (SurfTest Mitutoyo, cut-off length = 0.25 mm, tracing speed = 1.0 mm/s). Before incubation with the bacteria, the discs were sterilized in isopropyl alcohol for 20 minutes. It is important to note that the use of isopropanol has the potential to extract unreacted monomers from the specimens; therefore exposure time was limited to the minimum necessary to obtain sterile surfaces, as demonstrated in a previous study [26]. At the end of this period, specimens were rinsed vigorously to remove any remnant alcohol and then immersed in 48-well plates containing 1 ml of TH medium supplemented with 1% (w/v) sucrose and 1:500 dilution of the inoculum. One disc for each group was incubated in medium without inoculum as the sterility control. The plates were kept for 24 hours under 40 rpm agitation (Scilogex MX-M Microplate Mixer, Rocky Hill, CT, USA) in the CO₂ incubator.

The discs were then moved to 24-well black plates (Black Visiplate TC, Wallac, Finland) containing 0.5 ml of fresh TH medium per well and kept at 37°C for 1 hour. Immediately, 5

μ l of Coelenterazine-h ethanol solution was added to each well and the bioluminescence measured immediately by a spectrophotometer at 480 nm (GloMax Discover Multimode Microplate Reader, Promega Corporation). Details specific to the testing of the materials formulated with the quaternized monomers are described in the supplemental information.

2.9 Statistical analysis

Data were tested for normality (Anderson-Darling) and homocedasticity (Bartlett/Levene), then analyzed with one-way ANOVA and Tukey's test. Student's t-test was performed for comparison between storage conditions (dry versus wet). An overall significance level of $\alpha = 0.05$ was adopted for all tests.

3. Results

Results for degree of conversion, maximum rate of polymerization and conversion at rate maxima (used as a proxy for the onset of vitrification) values are shown in Table 1. Rate of polymerization as a function of degree of conversion for each of the formulations is shown in Figure 2A. In general, the HEMA-containing formulation showed the highest values of final degree of conversion (DC) (90.4%), maximum rate of polymerization ($R_{p_{max}}$) ($17.1\% \cdot s^{-1}$) and degree of conversion at maximum rate of polymerization (DC at $R_{p_{max}}$) (44.7%). Among the tested acrylamides, TMAAEA formulation showed the lowest final DC (66.4%) and DEBAAP the lowest $R_{p_{max}}$ ($2.4\% \cdot s^{-1}$).

In terms of water sorption (WS), after 7 days water immersion, BMAAPMA and BAADA presented the highest values (184.3 and $181.9 \mu g/mm^3$, respectively) and DEBAAP the lowest ones ($78.0 \mu g/mm^3$) (Figure 2B). Solubility (SL) results ranged between -4.17 and $24.6 \mu g/mm^3$, with DEBAAP and BMAAPMA presenting the lowest values and BAADA the highest ones (Figure 2C). In general, for both WS and SL, there was not a marked difference between methacrylate and acrylamide performance.

In relation to flexural strength (FS), there was a significant difference between the groups for both tested storage conditions ($p < 0.001$). After 24h dry storage, HEMA, DEBAAP and BMAAPMA formulation showed the highest results (127.9 , 144.2 , and 148.3 MPa, respectively) and TMAAEA the lowest ones (69.1 MPa). After 7 days water storage, all groups showed a decrease in FS, with DEBAAP showing the lowest reduction (24%), and HEMA, BMAAPMA, TMAAEA and BAADA formulations showing larger reductions of 37%, 60%, 64%, and 34%, respectively. A similar trend was seen for the elastic modulus (E) results, where HEMA, DEBAAP, BMAAPMA, and BAADA presented the highest results after dry storage (3.7 , 3.5 , 4.0 , and 3.8 GPa, respectively) and TMAAEA the lowest in both storage conditions (2.0 and 0.7 GPa - dry and wet, respectively). DEBAAP was the most resistant formulation to the water storage and showed a reduction of only 11% in E. On the other hand, for HEMA, BMAAPMA, TMAAEA and BAADA more pronounced decreases were found (37%, 50%, 65% and 57%, respectively).

Dentin microtensile bond strength (MTBS) results are presented in Table 2. Significant differences were found among the materials for each storage period. At 24 hours, DEBAAP, TMAAEA and BAADA presented the highest results (33.0 , 33.5 and 33.8 MPa,

respectively) and HEMA the lowest (21.6 MPa). After 3-weeks storage, all groups showed comparable performance. After 6 months, TMAAEA and BAADA presented the highest results (36.4 and 32.5 MPa, respectively), and HEMA the lowest (12.4 MPa). With the exception of the HEMA-formulation, the MTBS within the same material was statistically similar for all storage periods ($p < 0.05$). In general, a few of the newly synthesized acrylamides showed MTBS comparable to the commercial control at 24h and 3-week storage times, but higher MTBS at 6 months.

Figure 3 shows a representative example of the NMR spectra and integration regions used to calculate monomer degradation. The proposed mechanism for the acid catalyzed hydrolysis of the different monomers is shown for 2-hydroxyethyl methacrylate (HEMA) in Figure S7 in the supplemental materials.

The degradation kinetics over one month as well as the final degradation percentage for each monomer in acidic aqueous conditions ($\text{pH} = 1$) are shown in Figure 4 and Table S1 (supplemental information). The methacrylate control HEMA degraded 89.38%, whereas the multifunctional acrylamides showed markedly less degradation, with TMAAEA degrading the most (6.29%). BMAAPMA, BAADA, and DEBAAP showed very little or no measurable degradation, averaging 1.27%, 1.13%, 0% respectively. HEMA and TMAAEA were fit to exponential decay curves to calculate monomer half-lives of 9.5 and 350 days. The stacked ^1H NMR spectra highlighting the vinyl protons of each monomer and methacrylic acid in acidic water ($\text{pH} = 1$) taken at time points of 0, 7, and 30 days are shown in Figures S8–12 in the supplemental materials.

Cytotoxicity results are shown in Figure 5. The percentage of cell viability for both cell lines decreased nearly monotonically with the increase in monomer concentration. Overall, for OD-21, the acrylamides showed biocompatibility comparable to UDMA and HEMA, with cell viability ranging between 41% and 59% in 10 mM concentration (Figure 5A). The only exception was BMAAPMA, which showed 12% and 24% of remaining cells for 10 and 5 mM concentrations, respectively. The DPSC cell line was more sensitive to the monomers, and even in the lowest concentration the cell viability ranged between 59% and 86% (Figure 5B). Half Maximal Inhibitory Concentration (IC_{50}) and derived pK_a can be found in the supplemental information (Table S2).

The Luciferase assay did not show any antimicrobial/antifouling effect for any of the non-quaternized monomer formulations tested (Figure 6A). The relative light unit (RLU) values ranged between $2.80\text{E}+07$ and $4.63\text{E}+07$ and, despite the statistical difference ($p < 0.001$), the values are within the same order of magnitude. The biofilm adhesion strength test did not show statistical difference among the groups, with only 1.4% being the highest percentage of biofilm totally removed from the surface among the tested groups. The results and pertinent discussion on the materials formulated with the quaternized monomers can be found in the supplemental information (Figure S14).

4. Discussion

Since the hydrolytic and the enzymatic degradation of the polymer are identified as contributors to the reduced service life of traditional methacrylate-based dental materials, this study designed, synthesized and tested polyacrylamides as alternative co-polymers for dental adhesive formulations. This approach has shown positive results in previous studies in terms of resistance to degradation, though in most cases, the accompanying reduction in mechanical properties precluded the use of those formulations in load-bearing applications such as restorative composites [27]. In this study, we focused on the use of multifunctional acrylamides that are liquids at room temperature as components of the dental adhesive, which is a topic that remains largely under-explored, although some recent reports demonstrate growing interest [28]. In general, multi-acrylamide formulations showed lower $R_{p_{max}}$, DC at $R_{p_{max}}$ and final DC compared to the methacrylate control HEMA (Figure 2A and Table 1). Despite its lower molecular weight and viscosity compared to the multi-functional acrylamides, the greater reactivity of HEMA was somewhat unexpected, since monofunctional monomers have demonstrated slower rates of polymerization compared to multi-functional counterparts, at least when sharing the same polymerizable functionality (i.e., methacrylates) [29]. In multi-methacrylates, the reaction of one vinyl bond reduces the likelihood of reaction of the other vinyl(s) on the same molecule, due to the diffusion limitation imposed by the attachment to the growing polymer chain [30]. In a study evaluating monofunctional monomers with systematically varied backbone structures, and including (meth)acrylates and (meth)acrylamides, the differences in reactivity were not as marked within the same polymerizing functionality, but were very obvious when comparing methacryl and acryl counterparts with analog backbones and substitutions [17]. In any case, monomethacrylates in general react slower because diffusional limitations that lead to auto-acceleration only occur much later in conversion - therefore, it is expected that monofunctional monomers achieve higher conversion, but at a lower rate [30]. Indeed, for HEMA, the rate maxima was observed at much higher conversion (close to 50%) compared to the acrylamides (around 15–19%), confirming that the onset of vitrification only happens later in conversion for the mono-functional monomer. The higher rate of polymerization for HEMA compared to the acrylamides is then strongly related to the greater reactivity of the methacrylate group compared to the resonance-stabilized acrylamide [31]. Indeed, among the multi-acrylamides, the trifunctional TMAAEA showed the highest maximum rate of polymerization and the lowest DC (66%), likely due to early gelation which led to autoacceleration earlier in the conversion [30]. The fact that the three functional groups are closely linked by short six-carbon arms, in association with the relatively high molecular weight ($MW = 350.46 \text{ g/mol}$), further contributes to the steric hindrance and diffusion limitations for this molecule.

Conversely, the di-functional DEBAAP showed high DC (74.4%), and markedly lower rate of polymerization ($2.4\% \cdot s^{-1}$). In comparison to the other tested compounds, in this molecule the two polymerizable functionalities are physically much closer together, which may cause the molecule to behave more like a mono-functional monomer, since access to the second vinyl would be more challenging once the first was anchored to the growing polymer chain. This slows down the propagation rate of the polymerization reaction. BAADA is also a short

polyacrylamides - BMAAPMA and TMAAEA presented a significant drop in mechanical properties (60 and 64% reduction on FS, respectively), which is likely due to the higher flexibility of these molecules and the less densely packed network formed after polymerization (for BMAAPMA), and the lower overall DC (for TMAAEA) in conjunction with the generally higher hydrophilicity of methacrylamides, as discussed previously. It is noteworthy that the solubility reflects the dissolution and leaching of various components, particularly unreacted monomers. For some of the groups, negative values of solubility resulted, which in practice means that the sample gained mass during the test. This rather unintuitive outcome is the result of some mass of the water being entrapped into the polymer network, and that mass being actually greater than the unreacted monomer mass lost. The ISO 4049 standard does not prescribe lyophilization of the samples to completely remove the entrapped water (it is important to highlight that the final mass (m_3) is obtained after stabilization in a desiccator for at least 3 days). In any event, the negative values, in this case, mean that the solubility was very low.

All tested multi-acrylamides presented higher microtensile bond strength results at 24 h compared to the HEMA and commercial controls (the exception was BMAAPMA, which had statistically similar MTBS compared to the commercial control). In addition, all multi-acrylamides led to stable dentin bonding after 6-months storage, unlike the methacrylate experimental control (HEMA), which showed a 42% decrease over time. The bonding stability of the amides may be related, in part, to the high resistance to hydrolytic degradation (Figure 4). Even though this was expected given the absence of ester groups and the strong resonance stabilization making the carbonyl carbon less polarized and consequently less susceptible to nucleophilic attack, the magnitude of the difference in performance of the traditional methacrylate diluent HEMA and the experimental multifunctional acrylamides was surprising. Of additional interest is the fact that the results suggest that the bond stability is not only a product of the material's mechanical properties, but is highly dependent on the quality of the interaction between the adhesive layer and the dental substrate. Several studies have shown that amides can form hydrogen-bond interactions with the carboxylic acids of the side-chain of aspartic and/or glutamic acids in the dentinal collagen [37–39]. In natural collagen, this leads to a triple helix structure formed via hydrogen bonding between the $-C=O$ of the proline residue and the $-NH$ of the glycine residue [37–39]. In theory, any amide could establish hydrogen bonds with collagen fibrils, which is likely to translate into stronger and more stable bond strength. In addition, other studies on cancer-targeting drugs have demonstrated that amides may potentially function as metalloproteinase (MMP) inhibitors [40, 41]. Even though the specific MMP enzymatic activity was not tested in this study, the more stable behavior of the amide-based adhesives compared to the methacrylate control, in the absence of significant differences in mechanical properties after wet storage, may be indicative that some effect at an enzymatic level is occurring. These aspects will be evaluated in detail in a separate investigation.

Dental adhesives intimately interact with vital dentine and, in many cases, are placed in close contact with the pulp chamber, which facilitates the diffusion of leachates and unreacted monomers through the dentinal tubules into the pulp cavity at toxic concentrations [42]. It has been claimed that the release of certain adhesive components can cause genotoxic, allergic, cytotoxic, mutagenic, and estrogenic effects [42]. Despite the extensive

use of acrylamides in wastewater treatment, general polymer industry, cosmetics and foods, they are identified as potentially cytotoxic for certain cell lines [43]. This is related to the fact that acrylamide functionality is highly electrophilic due to the presence of a lone pair of electrons in the nitrogen molecular orbital, which increases the susceptibility to reacting with biological nucleophilic molecules such as proteins and DNA, causing cell disruption [44, 45]. Thus, the biocompatibility of the compounds was evaluated with undifferentiated pulp cells (OD-21) and human dental pulp stem cells (h-DPSC).

OD-21 is an immortalized cell line genetically engineered from mouse models, which are a reliable source for screening and repeatable tests due to their homogeneous morphology and growth characteristics [46]. On the other hand, h-DSPC is a finite life span cell line that plays a crucial role on human pulp repair process, migrating toward the injury site and differentiating into odontoblast-like cells to produce reparative dentin [47, 48]. The results showed that h-DPSC cells were more sensitive to the presence of monomers than OD-21, which resulted in lower percentages of cell viability (Figure 5B). This was expected as the immortalized cells are more robust due to their ability to continually undergo cell division. Significant differences were found between the monomers at higher mM concentrations for the OD-21 cell line. At 10 and 5 mM, BMAAPMA showed the lowest cell viability, HEMA the highest and UDMA, DEBAAP, TMAAEA and BAADA with similar intermediate results. Interestingly, at lower mM concentrations, the novel designed multi-acrylamides were more biocompatible than HEMA. These results were somewhat unexpected due to the reported cytotoxicity of some acrylamides. It has been shown that acrylamides can form adducts with the reduced glutathione (an important antioxidant - GSH) and increase the production of hydrogen peroxide, which may increase the level of lipid peroxidation (LPO) and carbonyl content, and decrease the enzymatic and non-enzymatic antioxidant activity [49, 50]. In summary, this makes the cells more vulnerable to free radical damage. In addition, amides can bind to sulfhydryl groups to inactivate proteins/enzymes involved in DNA repair and other critical cell functions [49]. Since the toxic mechanisms involve the interaction with the amide functionality site, it is expected that the more flexible BMAAPMA would be more toxic. Also, for analogue monomers bearing methacryl or acryl functionalities, the presence of the methyl group in the former is expected to render the molecule less prone to nucleophilic attack, and therefore, less likely to disturb cellular anti-oxidative mechanisms. However, the comparison between methacrylates and acrylamides is not as straightforward as that between methacrylates and acrylates, for example. Methacrylates are less susceptible to nucleophilic attack in comparison to acrylates for the reasons already mentioned. However, for acrylamides, particularly tertiary acrylamides such as those used in this study, resonance stabilization in the amide carbonyl renders this molecule more stable than the ester-containing methacrylate, as shown by the degradation data (Figure 4). In addition, compared with the mono-functional HEMA, the tertiary acrylamides used here had higher molecular weight (about 2–3 fold higher) and greater degree of functionality (di- or tri-functional), which may have partially prevented their extraction to the culture medium. Indeed, the hydrolytic degradation data shows that the concentration of intact HEMA significantly decreased in the first 24 h, so it is reasonable to assume that the presence of methacrylic acid, a by-product of ester degradation, significantly contributed to the biological toxicity of this group [51]. Based on that and the similarity

between the tested concentrations, it is possible to assume that, despite not being lethal at high concentrations, even small amounts of HEMA can irreversibly injure the cells.

DPSC results showed a different trend. In general, acrylamides and methacrylates showed similar results with few exceptions: [5 mM] BMAAPMA and [0.5 mM] TMAAEA were less biocompatible than the tested methacrylates. Since DPSC are highly sensitive, methacrylates and acrylamides were equally able to damage the cells, though likely through different mechanisms, overshadowing differences between the compounds such as the higher electrophilicity of the amide functionality in comparison with the ester group. In general, all monomers exhibited dose-dependent cytotoxic effects, which was expected [14]. However, at the highest concentrations - 10 and 5 mM, for all tested groups, the monomers induced notable cytotoxic effects, but with no statistical difference between them. This may indicate that there is a threshold in concentration above which the cells become saturated, and no additional cytotoxicity is recorded with the methods used here.

It is important to highlight that the neat monomers were tested instead of the copolymers in order to evaluate the potential toxicity of the leached unreacted monomers. The leaching process is dependent on polymer degree of conversion, molecular weight and hydrophilicity of the monomer, and extraction solvent [52]. One previous study has shown that hydrophilic low-molecular weight HEMA can be released from polymerized dental adhesives from 1.5 to 8 mM [53], while the highest amount of the hydrophobic high-molecular weight BisGMA was 425µg/ml (0.83mM) from an orthodontic adhesive [54]. Therefore, it is difficult to predict a clinically-relevant concentration of leached monomers for the novel multi-acrylamides, but it is possible to assume that they will behave closer to BisGMA than HEMA and, at these concentrations, the results of the present study indicate that the amides and the traditional methacrylates are equally biocompatible.

The upregulating role of some dental monomers on the growth and proliferation of microorganisms involved in the caries development process has been demonstrated [55, 56]. Among other effects, these monomers can also potentially increase the activity of glycosyltransferase, which would increase bacterial extra-cellular matrix formation, therefore maximizing bacterial adhesion and mature biofilm formation [57–59]. In this study, the alternative adhesive formulations were subjected to a biofilm growth assay, based on a previously described renilla-reporter method [25]. Overall, the results showed that the multi-acrylamide formulations were statistically similar to the control group (Figure 6A). The only exception was BAADA, which showed significantly higher values, though still within the same order of magnitude. BAADA is a solid powder and did not form a homogenous mixture with UDMA, which can potentially explain this difference. It is possible that some level of porosity (not noticeable with the naked eye) would provide a favorable surface for *S. mutans* growth. This argument, however, is somewhat negated by the fact that the initial surface roughness of the discs had been standardized before incubation. Interestingly, this slight increase in the biofilm growth did not translate into stronger biofilm adhesion as measured by the impingement test, which was similar to other tested groups.

In summary, this work was focused on the maintenance of the bond strength over time, and tested selected formulations for potential antimicrobial activity. From that standpoint,

BMAAPMA, TMAAEA and BAADA stood out. The control (HEMA) showed more than 40% reduction in bond strength after 6 months, while those monomers were able to maintain the initial results. This was true in spite of the drop in mechanical properties. The antimicrobial activity of these materials was assessed in this study to provide a thorough evaluation, and to ensure that there was no obvious up or down regulating effect on biofilm formation - and that was confirmed. Several other applications where adhesion to mineralized tissues are required can potentially benefit from this technology, since the polyacrylamides already in use present several drawbacks [60].

5. Conclusion

The incorporation of the newly synthesized multi-acrylamides in dental adhesive formulations led to higher and more stable dentin bonding strength with marked resistance to hydrolytic degradation, representing a suitable alternative to the ester containing monomers. Screening biocompatibility, biofilm formation and the other assessed properties did not indicate limiting issues for the clinical applicability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of significance

The study reports on the dental adhesive interface stabilization with the use of novel multi-acrylamide monomers. Our study shows that replacing the conventional 2-hydroxyethyl methacrylate monomer in those formulations increases the interfacial bond strength at 24 h, and also prevents degradation of that interface after long-term storage. A novel method for monomer degradation analysis using proton NMR demonstrates the virtually 0% degradation of those monomers in acidic environments. Cytotoxicity and mechanical properties are similar to conventional materials. Structure-property relationships and potential interface reinforcing mechanisms are discussed as a function of the composition of multiacrylamide-based materials, which find relevance in biomaterials and beyond.

Figure 1:
Chemical structure, acronyms, molecular weight, and log P of the multifunctional tertiary acrylamides proposed as alternative diluent monomers for dental adhesive formulations and the methacrylate control (HEMA).

Figure 2. (.

A) Polymerization rate ($\% \cdot s^{-1}$) as a function of degree of conversion (%) for non-solvated UDMA-containing adhesives. Vinyl conversion was followed in real-time as the materials were photocured (630 mW/cm^2 for 300 seconds). The same kinetics samples were subjected to WS/SL assay and the results ($\mu\text{g/mm}^3$) are shown in the bottom graphs (B) and (C), respectively (the same data is presented as box plots in the supplemental information, Figure S13). Different uppercase letters indicate statistically significant differences between the tested diluent monomers ($p < 0.05$).

Figure 3.

Degradation of TMAAEA in acidic water (pH = 1) at 37 °C, over 30 days. An integration region unique to a TMAAEA vinyl proton (A) and an integration region unique to an acrylic acid vinyl proton (B) was used to calculate monomer degradation. An integration region (C) where a vinyl proton is found in both TMAAEA and acrylic acid was used to normalize the integration values.

Monomer	% Degradation in 30 days	Half-life (days)	R ²
HEMA	89.4 (0.3) a	9.5	0.998
DEBAAP	0.0 (0.0) d	350	0.972
BMAAPMA	1.3 (0.4) c	-	-
TMAAEA	6.3 (0.4) b	-	-
BAADA	1.1 (0.1) c	-	-

Figure 4.

Degradation of monomers (n=3) in acidic water (pH = 1) at 37°C over 30 days. The values shown in the graph were determined based on ¹H NMR spectra integration for the intact monomer and associated degradation product (methacrylic acid). Data were fit to an exponential curve to determine monomer half-life (table insert - similar letters indicate statistical similarity, $\alpha=5\%$). The raw data is presented in Table S1 of the supplemental information.

Figure 5.

Cell viability (%) for OD-21 (A) and DPSC (B) cell lines after stimulation with different concentrations of monomers (10, 5, 1, 0.5, 0.1, and 0.05 mM) after an incubation period of 24 hours in MTT assay. Different uppercase letters indicate significant differences between the monomers at the same mM concentration ($p < 0.05$). Bars indicate statistically significant differences between the tested mM concentrations ($p < 0.001$).

Figure 6.

(A) *S. mutans* bioluminescence activity measured by Luciferase Assay after 24 hours of incubation. Relative light unit (RLU) indicates metabolic activity in the biofilms adherent on the surface composite discs and is calculate based on the luciferase reaction and it is proportional to the expression of the luciferase gene, which produces a bioluminescence according to the amount of adenosine triphosphate (ATP). (B) Biofilm area removed (%) from the discs surface after microjet impingement test are shown (data obtained via imaging of the crystal violet-stained specimens). Bars with the same letter indicate statistically similar values ($p < 0.001$).

Table 1.

Mean and standard deviation of degree of conversion (DC in %), maximum rate of polymerization (R_{pmax} in $\% \cdot s^{-1}$), DC at R_{pmax} (in %), flexural strength (FS, MPa), and elastic modulus (E, GPa). Values followed by the same lowercase letter on the same column and uppercase letter on the same row are statistically similar. The one-way ANOVA probability values (p) for each assessed experimental parameter are depicted on the last line.

	DC (%)	R_{pmax} ($\% s^{-1}$)	DC at R_{pmax} (%)	FS, dry (MPa)	FS, wet (MPa)	% Reduction	E, dry (GPa)	E, wet (GPa)	% Reduction
HEMA	90.4 (0.9) a	17.1 (0.9) a	44.7 (1.0) a	127.9 (11.4) abA	80.6 (3.7) bB	37	3.7 (0.4) aA	2.3 (0.1) bB	38
DEBAAP	74.4 (1.7) c	2.4 (0.02) d	14.5 (1.8) b	144.2 (10.5) aA	110.5 (10.8) aB	23	3.6 (0.1) aA	3.2 (0.1) aB	11
BMAAPMA	79.8 (0.3) b	12.5 (1.3) b	18.9 (1.2) b	148.3 (38.5) aA	59.0 (9.0) cB	60	4.0 (0.5) aA	2.0 (0.2) cB	50
TMAAEA	66.4 (0.4) d	13.1 (0.9) b	16.4 (0.7) b	69.1 (14.7) cA	24.8 (8.2) dB	64	2.0 (0.1) bA	0.7 (0.1) eB	65
BAADA	78.8 (1.4) b	7.31 (1.5) c	16.1 (1.7) b	99.7 (26.7) bcA	65.2 (6.8) cB	35	3.8 (0.8) aA	1.6 (0.08) dB	58
P	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	-

Table 2.

Mean and standard deviation of microtensile bond strength tests (MPa) for adhesive formulations after storage in distilled water at 37 °C for 24 hours, 3 weeks and 6 months. Values followed by the same lowercase letter in the same column are statistically similar, and values followed by the same uppercase letter in the same row are statistically similar. The one-way ANOVA probability values of the comparison among the groups within the same storage time (p) and the comparison among the storage times within the same experimental group (p^*) are presented.

	Single Bond	HEMA	DEBAAP	BMAAPMA	TMAAEA	BAADA	p
24 h	23.7 (1.4) aBC	21.6 (4.4) aC	33.0 (4.5) aA	30.2 (5.5) aAB	33.5 (4.2) aA	33.8 (2.3) aA	<0.001
3 weeks	25.7 (4.5) aAB	23.0 (7.1) aB	34.7 (7.2) aA	27.9 (6.2) aAB	33.3 (3.6) aA	32.7 (5.9) aA	0.001
6 months	23.3 (1.4) aC	12.5 (3.6) bD	27.0 (4.6) aBC	29.5 (2.6) aB	36.4 (4.3) aA	32.5 (3.3) aAB	<0.001
p^*	0.315	0.003	0.072	0.729	0.371	0.849	-