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New Dental Composites Containing Multimethacrylate Derivatives of Bile Acids: A Comparative Study with Commercial Monomers

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ABSTRACT We have prepared multifunctional methacrylate derivatives of bile acids as cross-linkable monomers for use in dental composites. By modifying the chemical structure of the monomers, we were able to vary the viscosity, hydrophobicity, and reactivity and have studied the effect of these parameters on the conversion of the monomers, the shrinkage during polymerization, and the mechanical properties of the resulting polymers and composites. Materials containing these new monomers generally had physical, thermal, and mechanical properties comparable to those containing the commonly used dental monomers BisGMA or UDMA and had lower polymerization shrinkage. The multimethacrylate derivatives of cholic acid, which are known to be less cytotoxic than BisGMA and UDMA, are shown to be promising materials for dental applications.

KEYWORDS: bile acids • dental monomers • dental composites • methacrylates • mechanical properties • polymerization shrinkage

INTRODUCTION

Mercury-containing dental amalgams (silver fillings) are being replaced with dental composites (white fillings) as the common restorative materials mainly because of the inherent esthetic appeal of the latter and the long-standing controversy related to the toxicity of the former (1). Dental composites contain both inorganic filler particles and an organic matrix. This organic matrix is typically a mix of two or more dimethacrylate monomers, of which the most common are 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane (BisGMA, **1**), 1,6-bis-(methacryloyloxy-2-ethoxycarbonylamino)-2,4,4-trimethylhexane (UDMA, **2**), and triethylene glycol dimethacrylate (TEGDMA, **3**) (Figure 1). Despite the common use of dental composites containing these monomers, numerous problems still exist, of which polymerization shrinkage is of great importance because of the development of contraction stress, which can lead to marginal leakage, potentially increasing the risk of recurrent caries and reducing restoration longevity (2, 3). Moreover, the organic matrix is known to not fully polymerize and has been shown to leach a variety of cytotoxic, estrogenic, and/or mutagenic molecules (monomers and monomer degradation products) in in vitro experiments (4−6). It is thus desirable to develop monomers that are less viscous and more hydrophobic (less water absorption (7)) and that shrink to a lesser extent, while maintaining higher levels of conversion and mechanical properties (8). To this end, various substitutes for BisGMA (9−12), branched, dendritic, or liquid-crystalline monomers (13, 14), fluorinated monomers (15, 16), reactive diluents for reducing the TEGDMA content (17), and alternative polymerization methodologies (i.e., ring-opening polymerizations) (18−20) have been explored.

Bile acids are natural amphiphilic compounds that exist in the body. Biocompatible by nature, they have been used for various therapeutic or pharmaceutical applications (21−25) and in some cases are generally recognized as safe (cholic acid) or approved for applications (deoxycholic acid and ursodeoxycholic acid) by the American Food and Drug Administration. Also, various polymers have been made from derivatives of bile acids for potential biomedical applications (26, 27). Their polar groups, good for adhesion on solid substrates, and their rigid steroid backbone, which contains only cyclic C−C single bonds (therefore, hard and UV-transparent), also make them ideal starting materials for deep-UV photoresist materials (28). In fact, the same characteristics (adhesiveness and mechanical properties) are also required for the organic matrices of dental composites. In addition, the high molecular weights of bile acids should help in reducing polymerization shrinkage; the possibility of adding multiple double bonds (methacrylates) should help ensure their incorporation in the polymer matrix and avoid leaching after polymerization; their biological origin and abundant occurrence in the gastrointestinal tract (29) should help to ease concerns of toxicity and biocompatibility, even in the case of incomplete polymerization and/or (bio)deg-

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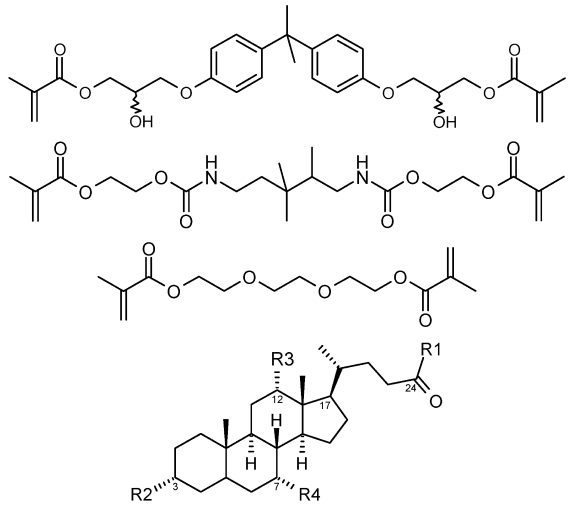

Molecule	R1	R2	R3	R4
4 (Cholic Acid)	HO–	HO–	HO–	HO–
5	H_3CO–	HO–	HO–	HO–
6	H_3CO–	**M**O–	**M**O–	**M**O–
7	$HOCH_2CH_2O$–	HO–	HO–	HO–
8	**M**OCH_2CH_2O–	**M**O–	HO–	HO–
9	**M**OCH_2CH_2O–	**M**O–	**M**O–	HO–
10	**M**OCH_2CH_2O–	**M**O–	**M**O–	**M**O–

M: Methacrylate Group

FIGURE 1. Chemical structures of the commercial monomers (1−3), cholic acid (4), and its derivatives (5−10).

radation of such materials in vivo. The cytotoxicity of the dental monomers derived from bile acids has been evaluated in a preliminary study (30). Concurrently, a study showed that polymers containing a tetramethacrylate derivative of cholic acid (compound **10** in this study) absorbed significantly less water than analogues containing BisGMA (31).

It is thus important to assess and optimize the physical properties of monomer mixtures, polymers, and composites containing bile acid derivatives for use in dental composites, prior to extensive biological evaluation. We have reported the synthesis of a variety of methacrylate derivatives of cholic acid (**4**) (32). Herein we have measured the viscosity, extent of conversion, and optical properties of these monomers (Figure 1) and evaluated the effects of these parameters on the final degree of conversion, thermal/mechanical properties, and polymerization shrinkage of the resulting polymers/composites. All results are compared to those of equivalent control materials containing the common monomers BisGMA and UDMA.

RESULTS AND DISCUSSION

Physical Properties of the Neat Monomers and Monomer Mixtures. For dental monomers, a relatively low viscosity is desired for easy handling. All monomer mixtures behaved like Newtonian fluids, and their viscosities varied greatly depending on the structure of the monomers (Table 1). The decrease of the viscosity from di-, tri-, to tetramethacrylate compounds **8**−**10**, which have two, one, and zero hydroxyl groups, respectively, is an obvious result of stronger intermolecular interactions resulting from hydrogen bonding. Although increased viscosity is undesirable from a handling perspective, and because more viscous formulations will have a greater difficulty conforming to irregularities in the surfaces of teeth, these interactions also favor greater cohesiveness, which manifests itself by the decrease of the static modulus and yield strength for polymers containing **8** versus **9** or **10** (Table 1). In comparison to BisGMA, which has two free hydroxyl groups, the cholic acid dimethacrylate **8**, which also presents two hydroxyl groups, has a significantly greater viscosity, likely indicating that this monomer has a greater hydrodynamic radius than BisGMA according to the Stokes−Einstein equation, which would contribute to the observed lower polymerization shrinkage of the monomer mixture containing **8** versus that containing BisGMA. A small difference in the viscosity may not influence the maximum filler loading or handling characteristics of uncured composite pastes because these parameters are more influenced by the filler shape and content than the viscosity of the unfilled mixture (based on the Krieger−Dougherty equation, which describes the rheological properties of highly concentrated dispersions (33)).

The hydrophobicity of all neat compounds was studied by measuring their octanol−water partition coefficients (30), a commonly used parameter for comparing the hydrophobicity of molecules. In comparison to BisGMA, all monomers derived from bile acids had similar or higher hydrophobicities (30), which is advantageous because of the potentially reduced water sorption and leaching from resulting materials after polymerization (34). The same characteristic may, however, have a negative impact on the bonding of the

Table 1. Comparison of the Viscosity, Degree of Conversion (DC), and Polymerization Shrinkage (ΔV) of the Different Monomer Mixtures after Light Curing[a]

		DC_{NIR} (%)[b]			postcured polymer		postcured composite	
monomer mixture	viscosity at 1 Hz (Pa · s)	light cure	postcure	ΔV (%) (ΔV_{100} (%))[c]	flexural modulus (GPa)	yield strength (MPa)	flexural modulus (GPa)	yield strength (MPa)
BisGMA/TEGDMA	0.88 ± 0.01^{d}	72 ± 1^{b}	89 ± 1^{b}	8.1 ± 0.9^{b} (12 ± 1)	3.1 ± 0.2^{a}	$114 \pm 27^{a,b}$	10.3 ± 0.3^{b}	$88 \pm 18^{a,b}$
UDMA/TEGDMA	0.27 ± 0.02^{e}	79 ± 1^{a}	94 ± 1^{a}	9.4 ± 0.5^{a} (11.8 ± 0.7)	$2.5 \pm 0.1^{b,c}$	124 ± 16^{a}	$9.3 \pm 0.4^{b,c}$	107 ± 32^{a}
6/TEGDMA	0.25 ± 0.01^{e}	55 ± 3^{e}	70 ± 2^{e}	9 ± 1^{a} (17 ± 2)	$2.31 \pm 0.06^{c,d}$	41 ± 9^{e}	8.7 ± 0.3^{d}	69 ± 9^{b}
8/TEGDMA	16.0 ± 0.2^{a}	66 ± 2^{c}	$77 \pm 2^{c,d}$	5.7 ± 0.3^{d} (8.7 ± 0.5)	$2.8 \pm 0.1^{a,b}$	$86 \pm 21^{b,c}$	10.0 ± 0.2^{a}	69 ± 14^{b}
9/TEGDMA	2.77 ± 0.03^{b}	$60 \pm 1^{d,e}$	$76 \pm 4^{d,e}$	$5.8 \pm 0.7^{c,d}$ (10 ± 1)	$2.4 \pm 0.2^{c,d}$	$74 \pm 6^{c,d}$	$9.2 \pm 0.3^{b,c,d}$	109 ± 10^{a}
10/TEGDMA	1.66 ± 0.01^{c}	48 ± 1^{f}	$76 \pm 1^{d,e}$	$6.6 \pm 0.8^{c,d}$ (14 ± 2)	2.1 ± 0.5^{d}	$55 \pm 13^{d,e}$	9.4 ± 0.3^{b}	63 ± 11^{b}

[a] The static mechanical properties of polymers and composites prepared from these monomer mixtures are reported after postcuring. Values are presented as mean (SD). Superscripts denote homogeneous subsets (Tukey, $p < 0.05$). [b] Measured by NIR spectroscopy after visible-light curing and after postcuring. [c] ΔV_{100} (%) represents the volumetric shrinkage extrapolated to 100% polymerization conversion ($\Delta V_{100} \approx \Delta V \times 100/DC_{NIR\ light\ cure}$).

materials to the wet or moist tooth, and this issue may be circumvented by the use of dentin adhesives containing hydrophilic comonomers (some of the polar groups of bile acids can be left free and nonfunctionalized) or by the development of alternative hydrophobic dentin bonding techniques (35). The increased hydrophobicity of polymers/composites made from these monomers (as was already observed for **10** (31)) may therefore help to improve their usefulness in a humid environment.

The di-, tri-, and tetramethacrylate bile acid derivatives were combined with equimolar amounts of the diluant TEGDMA, as is common for dental materials. The refractive index of the monomer mixtures is an important parameter to measure for composite translucency and esthetic matching with teeth. Mixtures containing cholic acid derivatives had refractive indexes of between 1.48 and 1.49, which were intermediate to those containing the model commercial monomers UDMA and BisGMA (1.48 and 1.51, respectively, and comparable to values reported in the literature at 22.5 °C (11)), implying that they are acceptable from an esthetic standpoint.

Extent of Conversion of Neat Monomers and Curing of the Monomer Mixtures. The extent of conversion of the neat monomers was measured by thermal analysis and is presented in Table 2 as both the percentage of methacrylate groups polymerized (DC_{DSC}) and the average number of methacrylate groups reacted per monomer molecule (N_m). The use of a thermal polymerization initiator (benzoyl peroxide) ensured that the onset of polymerization occurred at the same temperature (~80 °C), which helps in the comparison of the curing behaviors of the neat monomers among themselves. The extent of conversion measured by this method allows an evaluation of the relative efficiency of the monomers as cross-linking agents in an environment that disfavors their polymerization, that is, in the absence of diluent monomers, which would reduce the viscosity and T_g of the curing mixture. Among the dimethacrylates, TEGDMA polymerized to a greater extent than BisGMA, UDMA, and **8**, likely because of the greater flexibility of the oligo(oxyethylene) units of TEGDMA. The degrees of conversion observed for the commercial monomers **1**–**3** here were slightly higher than those reported in the literature (36).

Table 2. Comparison of the Hydrophobicity [$\log(K_{OW})$], Degree of Conversion (DC_{DSC}), and Average Number of Methacrylate Groups To Polymerize per Monomer (N_m)[a]

neat monomer	$\log(K_{OW})$[b]	DC_{DSC} (%)[c]	$N_m \pm 0.1$[d]
BisGMA (**1**)	6.6 ± 0.2	$75 \pm 7^{d,e}$	$1.5^{d,e}$
UDMA (**2**)	5.0 ± 0.2	$85 \pm 5^{a,b,c,d}$	$1.7^{b,c,d,e}$
TEGDMA (**3**)	2.8 ± 0.1	100 ± 7^{a}	2.0^{b}
4	4.9 ± 0.1		
5	6.1 ± 0.1		
6	7.9 ± 0.2	47 ± 4^{g}	1.4^{e}
7	6.0 ± 0.2		
8	7.3 ± 0.1	$83 \pm 8^{b,c,d}$	$1.7 \pm 0.2^{c,d,e}$
9	7.8 ± 0.1	$64 \pm 3^{e,f}$	$1.9^{b,c}$
10	7.2 ± 0.4	$58 \pm 4^{f,g}$	2.3^{a}

[a] Measurements were performed on neat monomer, without TEGDMA as the diluent. Superscripts denote homogeneous subsets (Tukey, $p < 0.05$). [b] Logarithm of the octanol–water partition coefficient (30). [c] Measured for the neat monomer by a DSC. [d] $N_m = DC_{DSC}/100 \times$ number of methacrylate groups on the monomer.

On a per molecule basis (N_m in Table 2), increasing the number of polymerizable methacrylate groups improves the chances of the monomer being covalently linked to the organic matrix. When polymerized in the neat form, 2.3 of the 4 methacrylate groups on the tetramethacrylate **10** participated in polymerization, which was higher than that for all other monomers and points to the potential use of this monomer for improving the cross-linking density. The degrees of conversion (percentage of the double bonds reacted) of the tri- and tetramethacrylate derivatives of cholic acid (**7**, **9**, and **10**), however, are lower than those of the dimethacrylates. If we suppose that the methacrylate groups on positions 3 and 24 participate in the polymerization reaction in the same manner as those on the dimethacrylate **8**, the incomplete conversion observed for the tri- and tetramethacrylate monomers can be accounted for by the steric hindrance of the steroid backbone (all hydroxyl groups are on the same side of the molecule) (26) rather than the diffusion limitation of conversion or the effects of the polymer T_g. This also explains the lower limiting conversion of the trimethacrylate **6**, for which all three polymerizable groups are on the steroid structure (~1.4 methacrylate

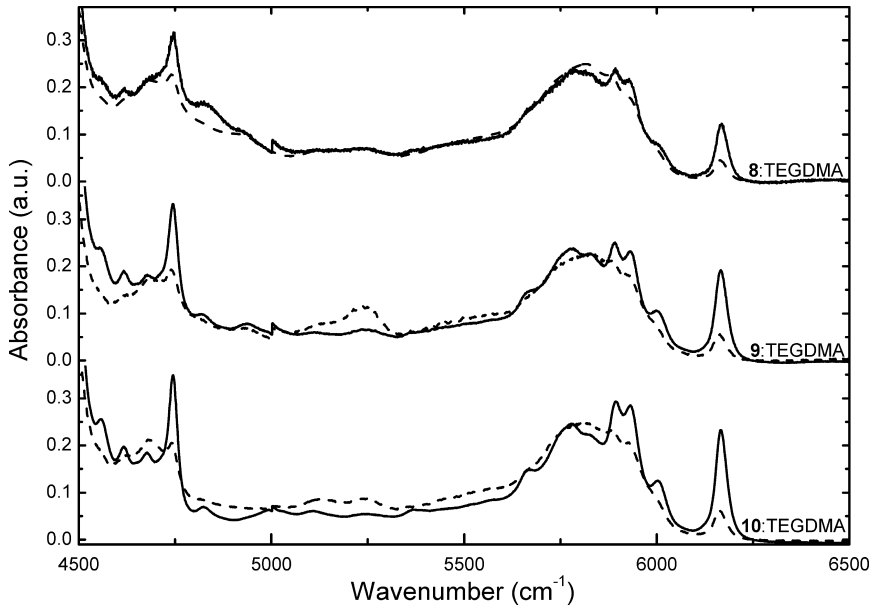

FIGURE 2. NIR spectra before (solid line) and after (dashed line) polymerization of monomer mixtures 8/TEGDMA, 9/TEGDMA, and 10/TEGDMA. Spectra are offset for clarity.

groups reacted per molecule) when compared to the trimethacrylate **9** (~1.9 methacrylate groups reacted per molecule). The lower overall extent of conversion for the polymers containing cholic acid monomers may ultimately affect the long-term durability of these materials. One possible way of further increasing the conversion of the bile acid monomers may be to change the configuration of the 3α-hydroxyl group to 3β (37) so that the α face of the steroid structure will be less crowded, as in the case of ursodeoxycholic acid, which has a 7β-hydroxyl group (26).

The degree of conversion of the monomers diluted with the comonomer TEGDMA after visible-light curing and after postcure heating was measured by near-infrared (NIR) spectroscopy and reported as DC_{NIR} *light cure* and DC_{NIR} *postcure* in Table 1, respectively. The NIR spectra of selected monomer mixtures and their corresponding polymers (after postcuring) are shown in Figure 2. These spectra present peaks at 5895, 5930, 6000, and 6165 cm^{-1} that are representative of the methacrylate group and whose intensity is proportional to the concentration of these groups within the sample. Postcuring was performed to accelerate the process of aging of the polymers (and avoid artifacts in their subsequent mechanical analysis), which can take place over a 120 h period at 37 °C following photopolymerization (38).

Polymerization Shrinkage. Polymerization shrinkage (ΔV) is a highly undesirable characteristic associated with methacrylate dental monomers because of the buildup of contraction stresses within the matrix and internal stress and deformation in the surrounding tooth structure (39). In this study, only the shrinkage of unfilled resins was measured in order to highlight the differences between the formulations and to adequately interpret the results as a function of the degree of conversion (which is more difficult to measure for composites). The results are listed in Table 1. The method used to measure shrinkage involves the direct measurement of the volumetric change of a droplet of

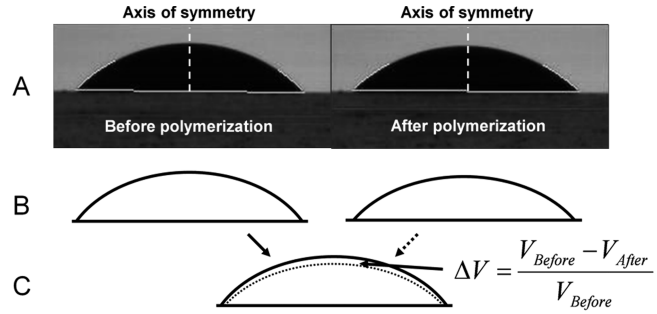

FIGURE 3. Representative illustration of volumetric shrinkage by axiosymmetric droplet image analysis: (A) images of axiosymmetric droplets; (B) estimation of the cross section using image analysis software; (C) calculation of the volume (V) for each droplet by integration of the cross section around the axis of symmetry and of the volumetric shrinkage (ΔV) from the equation shown.

monomer formulation concurrent with photoirradiation from images of the droplet (Figure 3). Shrinkage values obtained for the formulations containing BisGMA ($\Delta V = 8.1 \pm 0.9$) were comparable to values measured with alternative methods [$\Delta V = 8.31 \pm 0.43$ for a 1:1 (w/w) BisGMA/TEGDMA mixture by mercury dilatometry, and $\Delta V = 6.43 \pm 0.04$~7.43 ± 0.08 for a 1:1 (mol/mol) BisGMA/TEGDMA mixture by a water displacement technique] (9, 40). The mixtures containing **7**, BisGMA, and UDMA exhibited the highest shrinkage values of all, while those containing bile acid derivatives **8**−**10** showed lower polymerization shrinkage. In order to remove the bias on these values caused by the lower conversion of the cholic acid monomers, all values of shrinkage were extrapolated to 100% polymerization conversion (ΔV_{100}) using the degrees of conversion measured by NIR spectroscopy for each polymer after visible-light curing (Table 1). This, purely illustrative, parameter represents a worst-case scenario shrinkage value should conversion reach 100% during polymerization. This value is also an overestimation of the maximum shrinkage attainable because methacrylate groups polymerizing in the late

stages of polymerization, when conversion is already high, are less likely to induce large volume changes of the matrix owing to its already rigid nature. Furthermore, because the heat-transfer rates and sample geometries used for measuring conversion by NIR spectroscopy differ from those used to polymerize the monomer droplets within the shrinkage apparatus, it is possible that the conversion values in the latter case may be different. After extrapolation, it was found that the number of methacrylate groups on compounds **8**−**10** significantly affected shrinkage because this parameter correlates with the actual concentration of groups susceptible to shrink. The generally accepted trend of shrinkage versus the ratio of the number of polymerizable groups (methacrylate) to the molecular weight of the monomer remained in effect (41). All monomers based on cholic acid had ΔV_{100} values lower than or equivalent (**10**) to that of BisGMA or UDMA with the exception of **6**. It seems clear that the location of the double bonds on the steroid skeleton affected the shrinkage of the monomers during polymerization. Additional experiments geared toward determining the equilibrium three-dimensional structure of these monomers may provide answers to these questions.

In comparison to the tetramethacrylate **10**, which has a methacryloylethyloxy group, the trimethacrylate **6** with a methoxy group had a significantly lower viscosity. Also, the monomer mixture containing **6** had displayed anomalously higher polymerization shrinkage in comparison to that of the mixture containing **10**. Both of these results seem to point to the influence of the group at position 24 on the hydrodynamic radius of the monomer prior to polymerization. More specifically, the group at position 24 can influence the hydrodynamic radius of the molecule in two ways. First, it is clear that the methacryloylethyloxy group on monomer **10** is larger than the methoxy group on **6** and thus the volume occupied by **10** should be larger. Second, the polarity of the methacryloylethyloxy group may induce an intramolecular folding of this group onto the hydrophilic α face of the steroid molecule, as illustrated in Figure 4. This would create an empty cavity, which would contribute to the monomer having a greater than expected volume before polymerization and also following polymerization should this folding be maintained. This would contribute in a beneficial manner to lower polymerization shrinkage and would potentially explain the anomalously high polymerization shrinkage of the monomer mixture containing **6** versus those containing **8**−**10**. With this understanding, the design of the bile acid based monomers can be further improved for optimal performance.

Dynamic Mechanical Properties. The dynamic mechanical properties of the polymers were measured between −50 and +150 °C (Figure 5) based on the relevance of this extended temperature range for biomedical applications. The thermomechanical spectra of polymers containing BisGMA and UDMA (Figure 5A,B) are quite simple and differ slightly from those previously reported by Lee et al. and Emami and Söderholm for polymers containing either of these compounds (38, 42). For instance, in both of these reports, large transitions within the 50−100 °C range have been observed and attributed to the thermal reactivation of polymerization. Trapped radicals (associated with the highly glassy nature of these cross-linked polymers) become more mobile at temperatures beyond the cure temperature (the temperature that is achieved in the polymer during photopolymerization). The enhanced mobility of radicals and residual methacrylate groups at higher temperatures then promotes continued reaction. The assignment of this transition was based on the observation that the storage (or elastic) modulus increased with temperature during this transition (38) and that the presence of this transition is conversion-dependent (42). Interestingly, Lee et al. noticed that this thermal transition disappeared upon prolonged storage in air at room temperature, to be replaced by smaller transitions also within this temperature range (38). In the present study, we do observe transitions between 50 and 100 °C (Figure 5B), which may be due to this thermal reactivation of polymerization, though we observe no increase of the storage modulus with temperature (Figure 5A). Because thermal postcuring of the polymers was carried out in order to reduce/eliminate conversion-dependent peaks of this type, we believe that the transitions observed at 45 and 95 °C for the polymers containing UDMA and BisGMA (Figure 5B), respectively, are not due to thermal reactivation of polymerization but rather a subglass (or β) transition, generally defined as the activation of a local molecular movement on one type of the monomeric units. It should also be mentioned that thermograms recorded for five polymer samples of a given composition (i.e., $n = 5$) and taken over a period of 1 week were highly reproducible, indicative that the postcuring procedure employed was efficient in eliminating variability related to aging. Attempts to prepare master curves from multifrequency analysis (i.e., G'' vs T^{-1} and solicitation frequency) of a given sample were unsuccessful because they were not superimposable by translation along the T^{-1} axis, indicating that this transition may result from more than a single local motional process,

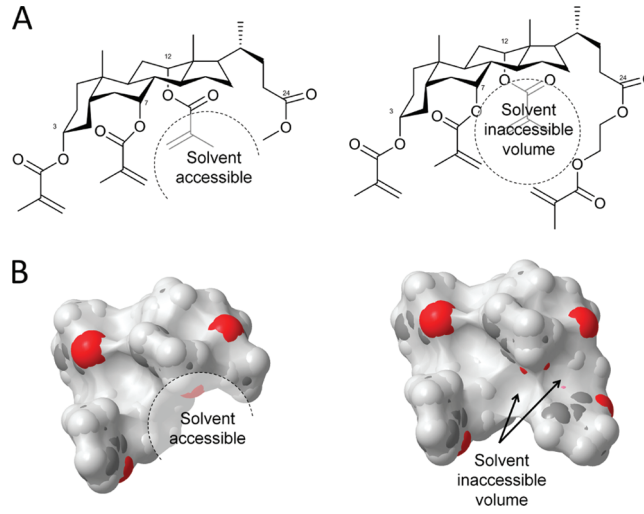

FIGURE 4. Schematic illustration of possible folded structures of monomers 6 (left) and 10 (right). The presence of a void in monomer 10 may be responsible for greater viscosity and lower polymerization shrinkage compared to 6.

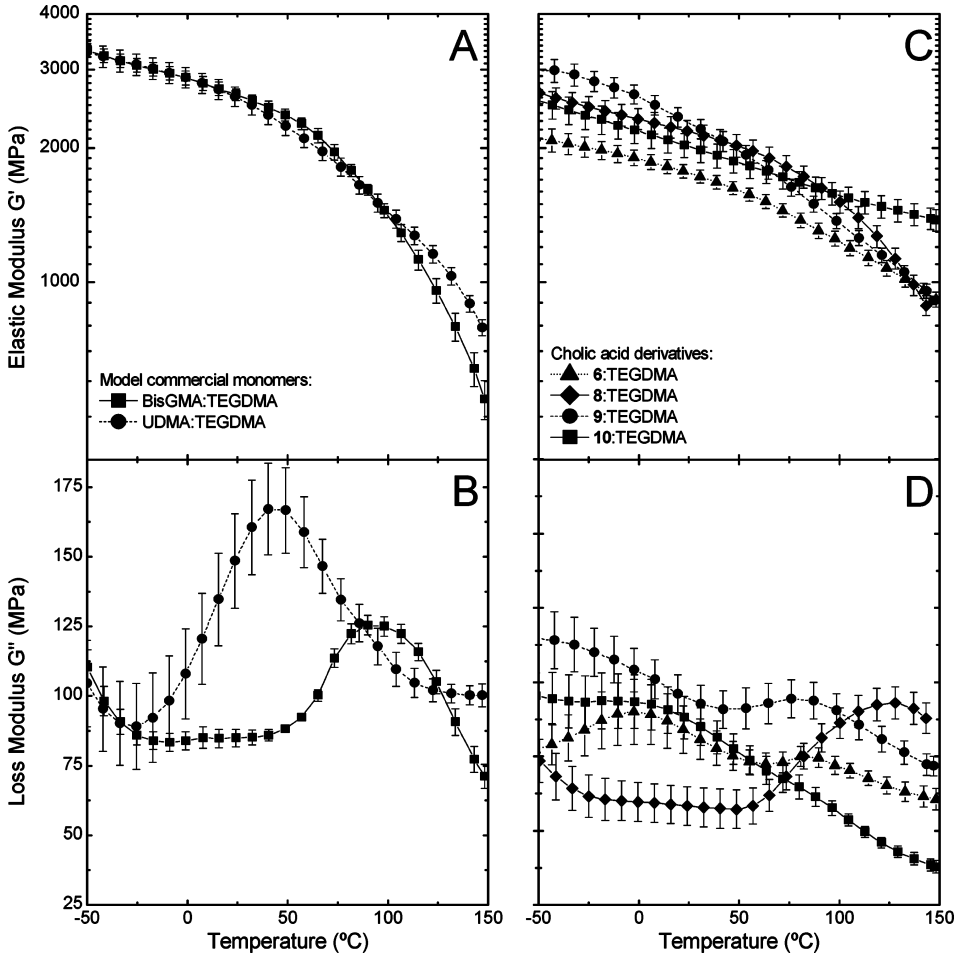

FIGURE 5. Dynamic mechanical properties of unfilled, postcured polymers prepared from equimolar mixtures of TEGDMA with BisGMA and UDMA or of TEGDMA with cholic acid derivatives 6 and 8−10, as indicated in the legends. Elastic moduli are shown in parts A and C, while loss moduli are shown in parts B and D (solicitation frequency 1 Hz; mean ± SD; $n = 5$).

thus increasing the difficulty in assigning its origin(s) (43). Spatial heterogeneity may also contribute to this phenomenon. While, arguably, performing a thermal postcure in this manner is not clinically relevant, it is our belief that the elimination of conversion-dependent peaks and/or relaxation of the polymer network with aging is essential for a better comparison of the differences in thermomechanical spectra, which result from structural differences between the cholic acid derived monomers and the controls.

In comparison to the two commercial controls, the polymers containing the cholic acid monomers have a different thermomechanical spectrum (Figure 5C,D), likely resulting from their more complex chemical structure. Also, the intensity of the subglass (or β) transition(s) observed for these polymers is less intense, which results in greater thermal stability of these polymers, as manifested by their elastic moduli (Figure 5C). For instance, while the storage moduli of the polymers containing UDMA and BisGMA decrease by 4−7-fold between −50 and +150 °C, over the same temperature range the elastic moduli of the polymers containing the cholic acid derivatives only decrease by a factor of 1.7−3 (Figure 5C). There are no clearly observable trends between the number and/or position of methacrylate groups on the monomer and the thermal transitions observed, and therefore assignment of these is difficult and may require elaborate experimentation. The breadths of the observed transitions were comparable for all polymers, indicative that the relative heterogeneities among the samples were comparable. Furthermore, as for the polymers containing BisGMA and UDMA, attempts to prepare master curves from multifrequency analysis were unsuccessful, indicative that there are many overlapping transitions within the temperature range investigated.

On the basis of the presence of this β transition, a distinction between low- and high-temperature mechanical properties should be made. In the −50 to +75 °C region, the polymer containing the trimethacrylate **9** had the highest elastic moduli of all polymers containing cholic acid derivatives, pointing to the importance of noncovalent interactions (i.e., hydrogen bonding) to G'. Consequently, the better dynamic mechanical properties of the polymers containing the trimethacrylate **9** versus the dimethacrylate **8** may be due to the cumulative effects of improved conversion (due to reduced viscosity) and hydrogen bonding. The polymer containing the trimethacrylate **6** had the lowest elastic moduli of all, likely because of a lower overall conversion and the absence of hydroxyl groups. In this temperature region, the elastic moduli of the polymers containing cholic acid derivatives were generally comparable or inferior to those of the two commercial formulations. Above 75 °C,

Table 3. Density of Reacted Methacrylate Groups of Polymers Following Visible-Light Curing and Postcuring

monomer mixture with TEGDMA	methacrylate groups in uncured mixture (mM · g^{-1})	density of reacted methacrylate groups (mM · g^{-1})[a] visible-light cure	postcure
BisGMA (**1**)	5.01	3.61 ± 0.05	4.46 ± 0.05
UDMA (**2**)	5.29	4.18 ± 0.05	4.97 ± 0.05
6	5.47	3.0 ± 0.2	3.8 ± 0.1
8	4.57	3.02 ± 0.09	3.52 ± 0.09
9	5.30	3.18 ± 0.05	4.0 ± 0.2
10	5.93	2.85 ± 0.06	4.51 ± 0.06

[a] The density of reacted methacrylate groups is defined as the product of the concentration of methacrylate groups in the uncured mixture and conversion (from Table 1).

however, the elastic moduli of the commercial formulations were comparable or inferior to those of all polymers containing cholic acid derivatives. In order to better interpret these results, the number of methacrylate groups that have polymerized per gram of polymer (44), for each polymer after visible-light curing and postcuring, has been calculated from the molecular weights of the monomers and the extent of conversion. This parameter, presented in Table 3, has previously been used by Cook et al. for comparing the thermomechanical properties of photopolymerized dimethacrylates (44) and was selected instead of the modulus in the rubbery region (also known for its correlation with the density of mechanically active cross-linking points) owing to the inaccessibility of the latter (i.e., T_g's in excess of 150 °C, the maximum sampled temperature) due to issues of potential thermal degradation, as discussed in the Experimental Section. Comparing this value to the cross-linking density obtained from the rubbery plateau would have permitted greater insight into the structure of the polymer network and may have highlighted further structure–property relationships. With the exception of the postcured polymers containing **10**, all polymers containing cholic acid derived monomers had lower densities of reacted methacrylate groups than the controls. This may partially explain the generally lower, yet comparable, moduli of these polymers with respect to those containing BisGMA or UDMA. It should, nevertheless, be mentioned that the greater thermal stability of the polymers containing the cholic acid derivatives, despite their lower density of reacted methacrylate groups, demonstrates greater rigidity within the polymer network.

Static Mechanical Properties. The flexural moduli and yield strengths of all unfilled polymers and model hybrid composites at room temperature (Table 1) were comparable to values found in the literature (9, 45–47). Different trends were found between the static and dynamic moduli for the polymeric systems, as was already noted in the literature (48). The polymers and composites containing cholic acid derivatives generally had moduli that were comparable to those containing BisGMA and UDMA (Table 1). Generally, among the polymers containing **8**–**10**, the moduli decreased inverse-proportionally to the number of hydroxyl groups. The polymers containing **8** had the highest moduli of all polymers containing cholic acid derivatives and were equivalent to those containing BisGMA and UDMA, which further indicates the importance of hydroxyl groups on the monomer to the value of the modulus. The yield strengths of the other polymers containing cholic acid derivatives were inferior to those containing the commercial models, suggestive of a less flexible network despite the lower density of reacted methacrylate groups.

Different trends were observed for the composites. In particular, the composites containing the trimethacrylate **9** had yield strengths that were equivalent to those containing BisGMA and UDMA. Overall, incorporation of the reinforcing filler material either had no effect or slightly improved the yield strength of the composites versus the unfilled polymers. Differences between the composite formulations with respect to moduli and yield strengths were less pronounced than those for the unfilled polymers, demonstrating the validity of using cholic acid monomers for dental applications.

CONCLUSIONS

This study provides substantial insight into the relationship between the structure of multimethacrylate derivatives of bile acids and the physical properties of polymers and composites made from them. Generally, the bile acid derivatives offered advantages such as reduced polymerization shrinkage and improved high-temperature mechanical properties compared to the model commercial dental materials. At room temperature, these polymers and composites also generally had comparable or slightly lower mechanical properties. These monomers may be further optimized by modifying the nature and length of the group on position 24 (i.e., amides, urethanes, etc.) in order to reduce their viscosity and/or to improve their mechanical performance. The trimethacrylate **9** appears to be one of the most promising candidates based on cholic acid because of the combined advantages of having multiple (three) methacrylate groups as well as a free hydroxyl group. The results presented herein are sufficiently promising to warrant additional structural modifications to increase the susceptibility of the methacrylate groups directly attached to the steroid backbone to polymerize and to modify the rheological properties of the monomers. We intend to pursue further evaluation of such polymers in biological environments and characterize the nature, quantity, and toxicity of leachates from such materials. The latter include biodegradation studies in the presence of salivary esterases given their known influence on the release of leachates from composites (49–51) and the

known modulation of the enzyme activity by bile salts (in particular cholesterol esterase) (52).

EXPERIMENTAL SECTION

Materials. UDMA, TEGDMA, cholic acid, ethylene glycol, triethylamine, (3-methacryloxypropyl)trimethoxysilane (γ-MPS), camphoroquinone (CQ), (*N*,*N*-dimethylamino)ethyl methacrylate (DMAEMA), silicon dioxide (0.5−10 μm, 80% between 1 and 5 μm), pyrogenic silica (0.014 μm), and concentrated HCl were obtained from Sigma-Aldrich (Milwaukee) and used as received. BisGMA was received from Polysciences (Warrington, PA) and purified by column chromatography [100 g of silica per 1 g of BisGMA; ethyl acetate/hexane (1:1, v/v) as the eluent]. Methacryloyl chloride (Aldrich) was distilled immediately prior to use. All solvents were used as received except for dichloromethane, which was dried using a column solvent purification system. Compounds **5**−**10** (Figure 1) were synthesized as described previously (32). All structures were verified by ^{1}H NMR spectroscopy on an Avance 400 Bruker spectrometer (400.26 MHz for protons) in $CDCl_3$.

Filler Silanization. The silicon dioxide (0.5−10 μm, 80% between 1 and 5 μm) and pyrogenic silica were both silanated (separately) by reacting each (200 g) with γ-MPS (20 g) in 600 mL of methanol/water (95:5, v/v) at pH 3.5 (by adding drops of glacial acetic acid) for 1 h at room temperature. The solvent was evaporated in vacuo at room temperature for 1 h and the filler vacuum-dried at 120 °C for 12 h. The presence of methacrylate groups on the filler was confirmed by the presence of the C═CH_2 stretching band (1640 cm^{-1}; characteristic of the methacrylate group) in the photoacoustic Fourier transform infrared (FTIR) spectrum of the powder using a Digilab FTS 6000 spectrometer equipped with a MTEC 300 photoacoustic cell under an atmosphere of helium (Supporting Information). The extent of silanization was ~6 wt % for both fillers as determined by thermogravimetric analysis (20 °C · min^{-1} until 800 °C in air) using a TGA 2950 thermogravimetric analyzer from TA Instruments (Supporting Information).

Preparation of Unfilled Polymers and Composites. Equimolar amounts of each monomer (i.e., bile acid derivative, BisGMA, or UDMA) combined with TEGDMA were weighed in a glass vial and homogenized with dichloromethane, which was then removed in vacuo (moderate vacuum for ~1 h at 4 °C and under gentle agitation with a magnetic bar). The monomer mixture was then weighed in a ceramic mortar and homogenized (using a pestle) in a darkroom with CQ (initiator, 0.6 wt %) and DMAEMA (accelerator, 1.2 wt %). This mixture was spatulated into a stainless steel, bar-shaped mold (2 × 2 × 30 mm) and covered with a microscope slide coverslip to avoid oxygen inhibition (53). The samples were irradiated on one side every 10 mm along their length with an Optilux 401 visible-light gun (400 mW · cm^{-2}, 2 × 60 s at each spot) and then removed from the mold and irradiated in the same fashion on the other side. Postcuring of the polymers was done by placing the bars in a sealed glass vial, which was immersed in an oil bath thermostatted at 120 °C for 24 h. Composites were prepared by initially incorporating the silanated filler into the monomer mixture (3:1, w/w). The filler consisted of 0.5−10 μm SiO_2 and pyrogenic silica (both silanated) (14:1, w/w). These weight ratios are typical for hybrid-type dental composites. After homogenization with the initiator and accelerator, the resulting paste was placed under vacuum (to remove dissolved air), spatulated in the mold, and cured in the same manner as that for the unfilled polymers.

Characterization Techniques. The viscosity of the unfilled monomer mixtures was measured in triplicate on an AR2000 rheometer from TA Instruments at 25 °C using a 40 mm cone-and-plate geometry (55 μm gap) at frequencies between 0.1 and 100 Hz.

Thermograms of the monomers were recorded on a DSC 2910 differential scanning calorimeter (DSC) from TA Instruments at 10 °C · min^{-1} from −50 to +200 °C under a flow of helium. All samples (approximately 7−15 mg) were mixed with 1 wt % benzoyl peroxide (thermal polymerization initiator) and hermetically sealed in aluminum DSC pans. The heat of polymerization of methyl methacrylate served as the standard for calculating the molar heat released by the complete polymerization of a single methacrylate group (100% conversion is assumed). Quantification of conversion by this method is described elsewhere (36). All measurements were performed in triplicate.

NIR spectra were recorded in triplicate on a Cary 500 Scan UV−vis−NIR spectrophotometer between 4500 and 8000 cm^{-1} at a scan rate of 1 cm^{-1} · s. The spectra of the monomer mixtures were recorded in a rectangular quartz vial with a 2-mm optical path length. The spectra of the polymers were recorded by placing two bars side-by-side perpendicular to the incident light. In both cases, the sample was behind an aluminum mask with a 2 × 4 mm hole in it. The thickness of the bars (~2 mm) was used to normalize the spectra and was measured to within 10 μm using a digital micrometer. The degree of conversion is obtained from the difference of the areas of the peak at 6165 cm^{-1} (═CH stretch overtone) before and after polymerization (54).

The elastic shear moduli (G') of the polymers were measured on a 2980 dynamic mechanical analyzer (DMA) from TA Instruments in dual-cantilever geometry. Thermograms were recorded between −50 and +150 °C at a heating rate of 2 °C · min^{-1}, with 10 μm amplitude of deformation and at solicitation frequencies of 1 and 10 Hz. The upper temperature limit of the DMA experiments performed herein was selected based on the measured onset of the thermal decomposition of TEGDMA polymers at, or right below, 200 °C (Supporting Information) and on visible signs of decomposition (i.e., browning of polymers) and irreproducibility of DMA graphs when samples, heated above 150 °C, were reanalyzed for a second time. We therefore concluded that any data gathered above 150 °C had too great a potential to be compromised by this phenomenon in an unpredictable manner, and that numerical data extrapolated from this region would also be compromised.

The flexural moduli of the specimens were measured at room temperature (~22 °C) in three-point bending geometry (20 mm span) at a crosshead speed of 0.75 mm · min^{-1} on an Instron model 4201 mechanical analyzer. This modulus was measured from the slope (at small strain; in the linear region) of their stress−strain curves. The yield strength corresponds to the stress at yield from the stress−strain curves. The dimensions of all of the specimens submitted to mechanical testing were approximately 2 × 2 × 25 mm and were measured to within 10 μm (0.5% maximum error on each individual dimension) using a digital micrometer. All thermograms and stress−strain curves were recorded at least in quintuplicate.

Polymerization Shrinkage. Polymerization shrinkage was measured by axiosymmetric drop analysis (55) using a FTA 200 dynamic contact angle analyzer from First Ten Angstroms. Measuring the polymerization shrinkage by this method is described in detail by Hudson et al. (55). Essentially, this method relies on the proportionality between the volume of a droplet and the area of its cross section, which is conveniently, and accurately, measured using a common contact angle instrument equipped with a digital camera and image analysis software. The monomer mixture containing the initiator (0.6 wt % CQ) and accelerator (1.2 wt % DMAEMA) was prepared in a darkroom. A drop (10 μL) of the latter was placed on a glass microscope slide and placed inside the instrument. The back light of the instrument was fitted with a filter to remove blue light. An image of the cross section of the drop was taken from the side, and then the drop irradiated in situ, without moving

the sample, under a blanket of argon for 2 × 60 s using the light gun described above. Another image was then taken of the polymerized drop. The volume of the droplets before and after curing was calculated (integration around 180°) from the area of the droplet's cross section measured using the image analysis software provided with the instrument. Representative images of a monomer droplet before and after polymerization are shown in Figure 3. Measurements were performed 10 times for each monomer formulation.

Statistical Analysis. All statistical analyses were one-way ANOVA followed by Tukey's post hoc test. Differences between means were considered significant at $p < 0.05$.

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Supporting Information Available: FTIR spectra of filler particles confirming the presence of methacrylate groups, thermogravimetric analyses of silanized fillers to determine the extent of silanization, and thermogravimetric analysis of a TEGDMA homopolymer to determine the onset of thermal decomposition. This material is available free of charge via the Internet at http://pubs.acs.org.

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