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Effect of the Monomer and Filler Systems on the Remineralizing Potential of Bioactive Dental Composites Based on Amorphous Calcium Phosphate

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ABSTRACT

Amorphous calcium phosphate (ACP), because of its high solubility and its rapid transformation to hydroxyapatite (HAP) in aqueous environments, has been utilized as the filler phase in the preparation of bioactive polymer-based composites that have remineralization potential. The goal of this study was to evaluate the effects of chemical structure and compositional variations of seven photo-polymerizable matrix monomer systems on the long-term release of calcium and phosphate ions, i.e., their remineralizing potential, when used with three types of ACP filler systems: unhybridized ACP (u-ACP), silica-hybridized ACP (Si-ACP), and zirconia-hybridized ACP (Zr-ACP) composites. The monomer systems evaluated were: 2,2-bis[p-(2′-hydroxy-3′-methacryloxypropoxy)-phenyl]-propane (Bis-GMA)/triethyleneglycol dimethacrylate (TEGDMA) [BT resin], Bis-GMA/TEGDMA/2-hydroxyethyl methacrylate (HEMA) [BTH resin], Bis-GMA/TEGDMA/HEMA/zirconyl methacrylate (ZrMA) [BTHZ resin], TEGDMA/pyromellitic glycerol dimethacrylate (PMGDMA) [TP resin], a urethane dimethacrylate, [U], and two HEMA-modified U resins with the mass fraction of 6.6% and 13.2% HEMA, ([U66H] and [U132H], respectively). All the visible light polymerizable composites consisted of 60% resin and 40% ACP filler on a mass fraction basis. It was shown that ion release from the immersed composites was affected by both the chemical structure and composition of the monomer system as well as the type of filler system. Whereas BT, U and U66H resins formulated with u-ACP gave low to moderate levels of ion release, the resins with elevated amounts of HEMA (BTH, BTHZ and U132H) gave high and sustainable release of mineral ions. The remineralizing capacity of BT, U, U66H and U132H composites generally increased when hybridized fillers were employed instead of u-ACP. Utilization of hybrid ACPs had, however, no effect on ion release from BTHZ composites. The TP composites initially gave high ion release, but it was not sustainable because of significant Ca-binding by the matrix due to the high content of carboxylic acid groups derived from the PMGDMA comonomer. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: amorphous calcium phosphate; composites; resin matrix; hybrid fillers; remineralization

INTRODUCTION

Due to their close association with the formation,

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TABLE 1. Calcium Phosphates of Biological Significance

Name	*Acronym*	*Compositional Formula*
Amorphous calcium phosphate	**ACP**	$Ca_3(PO_4)_2 \cdot 3H_2O$*
Dicalcium phosphate dihydrate	**DCPD**	$CaHPO_4 \cdot 2H_2O$
Octacalcium phosphate	**OCP**	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$
Tricalcium phosphate	**TCP****	$Ca_3(PO_4)_2$
Tetracalcium phosphate	**TTCP**	$Ca_4(PO_4)_2O$
Hydroxyapatite	**HAP**	$Ca_{10}(PO_4)_6(OH)_2$

* Approximate compositional formula[3,4].
** α-, and β- forms.

progression and arrest of enamel and dentin caries, calcium phosphates (CaPs) have been utilized in preventive and restorative dental materials [1]. CaPs of biological significance are listed in Table 1. Crystalline HAP is considered to be the final, stable product in the precipitation of calcium and phosphate ions from neutral or basic solutions.

Amorphous calcium phosphate (ACP) is unique among CaPs in that it lacks the long-range, periodic atomic scale order of crystalline materials. Although the unique structure of ACP has been a subject of considerable interest, of greater relevance is understanding the dynamics of conversion of ACP to HAP by spontaneous precipitation as well as understanding the factors that control the instability of ACP in aqueous solutions. The preparation and properties of ACP, its kinetic and thermodynamic relationship to crystalline CaPs, its structural nature and putative presence and role *in vivo* has been recently reviewed [2].

The role of ACP in HAP formation and especially its possible role in biological calcification, more than anything else, raises the importance of ACP above the level of mere laboratory curiosities and places ACP in the mainstream of calcium phosphate chemistry [2–5]. The relatively high solubility of ACP and its conversion to HAP in aqueous environments might pose limitations in applications where structural, mechanical and chemical stability is desired. However, these same properties make ACP suitable as a potential mineralizing agent. When compounded with appropriate resins, the bioactivity of ACP fillers may be particularly useful in enhancing the performance of composites, sealants and adhesives by providing a sustained milieu of Ca^{2+} and PO_4 ions for contiguous tooth enamel and/or dentin. Thus the extended, time-releasing characteristics of polymeric ACP based materials may be especially advantageous in sealant and adhesive applications designed to prevent demineralization (e. g., in orthodontically treated teeth).

Recent research in this laboratory has led to the development of several unique, experimental bioactive composites based on polymer matrix phases derived from the ambient polymerization of dental acrylic monomers containing ACP as the filler phase [6, 7]. Pure ACP proved to be an unsuitable filler because of its rapid internal conversion to HAP upon exposure to water. However, when the ACP was stabilized by ions that retard its conversion (primarily pyrophosphate ($P_2O_7^{4}$), and to the lesser extent Mg^{2+}), it was possible to take advantage of the relatively high solubility of ACP and obtain substantial release of Ca^{2+} and PO_4 ions from such composites. Moreover, the levels attained in solution from these releases were sustainable, and were shown to promote recovery of mineral-deficient tooth structures in *in vitro* situations [8]. It has also been shown [9] that only a small portion of theoretically available mineral ions is actually being released into external solutions. Compared to conventional silanized glass-filled dental composites, such bioactive composites are relatively weak, mainly because ACP, even when silanized [9], does not act as a reinforcing filler for dental polymers in a manner similar to that of commonly used silanized glass fillers. In order to make ACP more interactive with polymeric matrices and more amenable to treatment by silane coupling agents, tetraethoxysilane (TEOS) and $ZrOCl_2$ have been evaluated as potential hybridizing agents for ACP with encouraging results [10]. Although the nature of these hybridized ACPs has yet to be fully established preliminary results are encouraging with regard to their use as composite fillers [10].

The objective of this study was to assess the effects of the chemical structure and compositional variations of seven resin matrices: (a) 2,2-bis[p-(2′hydroxy-3′-methacryloxypropoxy)phenyl]-propane (Bis-GMA) and triethyleneglycol dimethacrylate (TEGDMA) [BT resin]; b) Bis-GMA, TEGDMA and 2-hydroxyethyl methacrylate (HEMA) [BTH resin]; c) Bis-GMA, TEGDMA, HEMA and zirconyl methacrylate (ZrM) [BTHZ resin]; d) TEGDMA and pyromellitic glycerol dimethacrylate [11] (PMGDMA) [TP resin]; e) 1,6-bis(methacryloxy-2-ethoxycarbonylamino)-2,4,4-trimethylhexane (UDMA) [U resin]; f) and g) UDMA plus HEMA [U66H and U132H resins with different levels of HEMA (mass fraction of 6.6 % and 13.2 %, respectively)], on the release of Ca^{2+} and PO_4 ions from composites utilizing these resin matrices and

TABLE 2. Acronyms, names and sources of the resin components

Acronym	*Name*	*Manufacturer*
Bis-GMA	2,2-Bis[*p*-(2′-hydroxy-3′-methacryloxypropoxy)-phenyl]propane	Freeman Chemical Corp., Port Washington, WI
TEGDMA	Triethyleneglycol dimethacrylate	Esstech, Essington, PA
HEMA	2-Hydroxyethyl methacrylate	Esstech, Essington, PA
PMGDMA	Pyromellitic glycerol dimethacrylate	
UDMA	1,6-Bis(methacryloxy-2-ethoxycarbonylamino)-2,4,4-trimethylhexane	Esstech, Essington, PA
ZrMA	Zirconyl methacrylate	Rohm Tech. Inc., Malden, MA
CQ	Camphorquinone	Aldrich Chem. Co., Milwaukee, WI
EDMAB	Ethyl-4-*N,N*-dimethylaminobenzoate	Aldrich Chem. Co., Milwaukee, WI
IRGACURE®369	2-Benzyl-2-(dimethylamino)-1-(4-(4-morpholinyl)-phenyl)-1-butanone	Ciba, Hawthorne, NY
DAROCUR®4265	2-Hydroxy-2-methyl-1-phenyl-1-propanone plus diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide	Ciba, Hawthorne, NY

three types of ACP fillers, namely unhybridized ACP, silica- and zirconia-hybridized ACP.

EXPERIMENTAL

Resin formulations

The matrix resins were formulated from the commercially available dental monomers listed in Table 2. The components of the two photoinitiator systems used for visible-light photopolymerization also are given in Table 2. Resins designated as BT, BTH, BTHZ, U, U66H and U132H (Table 3) were photoactivated by inclusion of camphorquinone (CQ) and ethyl-4-N,N-dimethylaminobenzoate (EDMAB) as the photo-oxidant and photo-reductant, respectively. For the TP resin, however, a second photoinitiator system consisting of a mixture of 2-benzyl-2-(dimethylamino)-1-(4-(4-morpholinyl)phenyl)-1-butanone (IRGACURE®369), 2-hydroxy-2-methyl-1-phenyl-1-propanone plus diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (DAROCUR®4265) and CQ was selected to enhance photopolymerization and storage stability. The chemical structures of the monomers and photoinitiating systems are given in Fig. 1 and Fig. 2., respectively.

Synthesis and characterization of ACP fillers

ACPs fillers were synthesized employing the protocol proposed by Eanes *et al.* [12]. P_2O_7-stabilized ACP was precipitated instantaneously in a closed system (under CO_2-free N_2 in order to minimize CO_2 adsorption by the precipitate) at 22°C upon rapidly mixing equal volumes of a 800 mmol/L $Ca(NO_3)_2$ solution and a 536 mmol/L Na_2HPO_4 solution that contained a molar fraction of 2 % of $Na_4P_2O_7$. The reaction pH was maintained between 10.5 and 11.0. This ACP is designated unhybridized ACP (u-ACP). TEOS-and $ZrOCl_2$-hybridized ACPs (Si-ACP and Zr-ACP, respectively) were prepared by simultaneously adding appropriate volumes of either a previously prepared TEOS solution (mass fraction of 10 % TEOS, 10 % ethanol, 10 % tartaric acid and 70 % water) or 0.25 mol/L $ZrOCl_2$ solution and $Ca(NO_3)_2$ solution to the Na_2HPO_4 solution. The TEOS solution was designed to prevent premature gelation of TEOS. Amounts of the two solutions of hybridizing agent

TABLE 3. Composition of Photoactivated Resins (mass fraction, %)

Component/Resin	*BT*	*BTH*	*BTHZ*	*TP*	*U*	*U66H*	*U132H*
Bis-GMA	49.50	35.50	35.50	—	—	—	—
TEGDMA	49.50	35.50	35.50	48.65	—	—	—
HEMA	—	28.00	27.20	—	—	6.60	13.20
PMGDMA	—	—	—	48.65	—	—	—
UDMA	—	—	—	—	99.00	92.40	85.80
ZrMA	—	—	0.80	—	—	—	—
CQ	0.20	0.20	0.20	0.40	0.20	0.20	0.20
EDMAB	0.80	0.80	0.80	—	0.80	0.80	0.80
IRGACURE®369	—	—	—	0.80	—	—	—
DAROCUR®4265	—	—	—	1.50	—	—	—

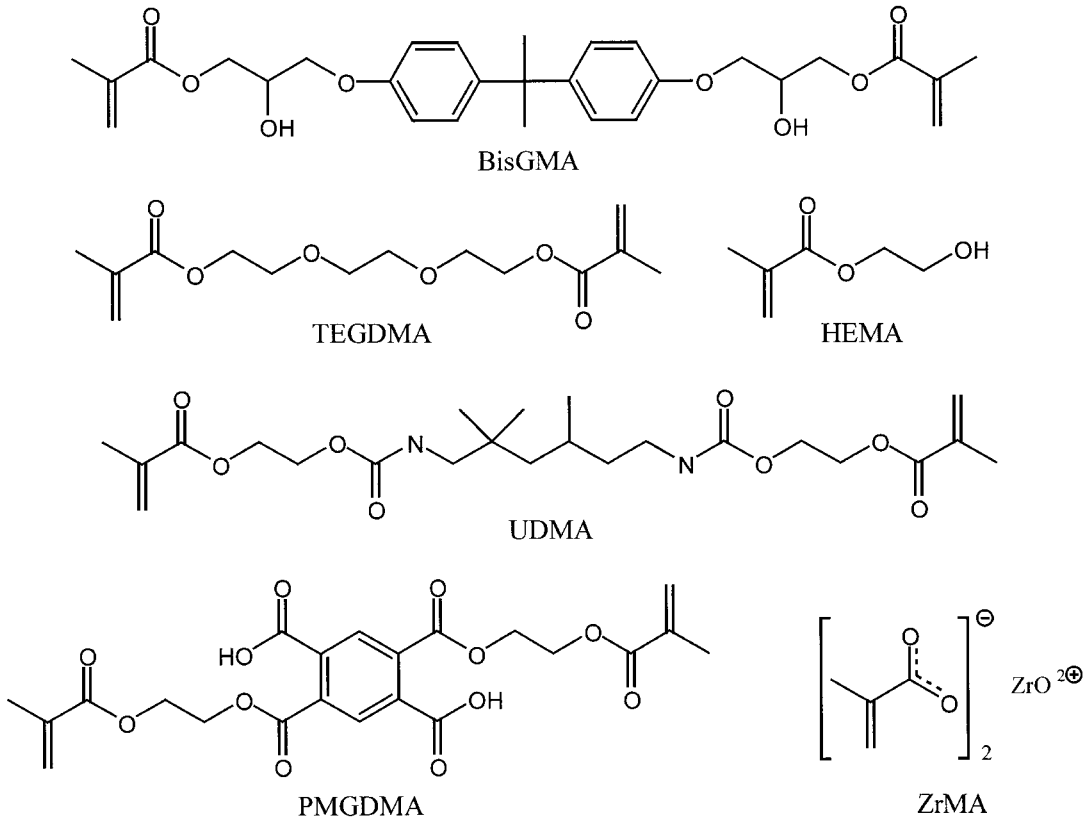

FIGURE 1. Chemical structure of the monomers used to formulate the resins.

were adjusted to achieve molar ratios of $ZrOCl_2$:-Na_2HPO_4 and TEOS: Na_2HPO_4 equal to 0.1. The reaction pH was maintained between 9.0 to 9.3 and 8.6 to 9.0 for the preparation of Si-ACP and Zr-ACP, respectively. The suspensions were filtered, and the solid phase washed with ice-cold ammoniated water and lyophilized.

The amorphous state of lyophilized solids was verified by powder X-ray diffractometry (XRD) and Fourier-transform infrared spectroscopy (FTIR).

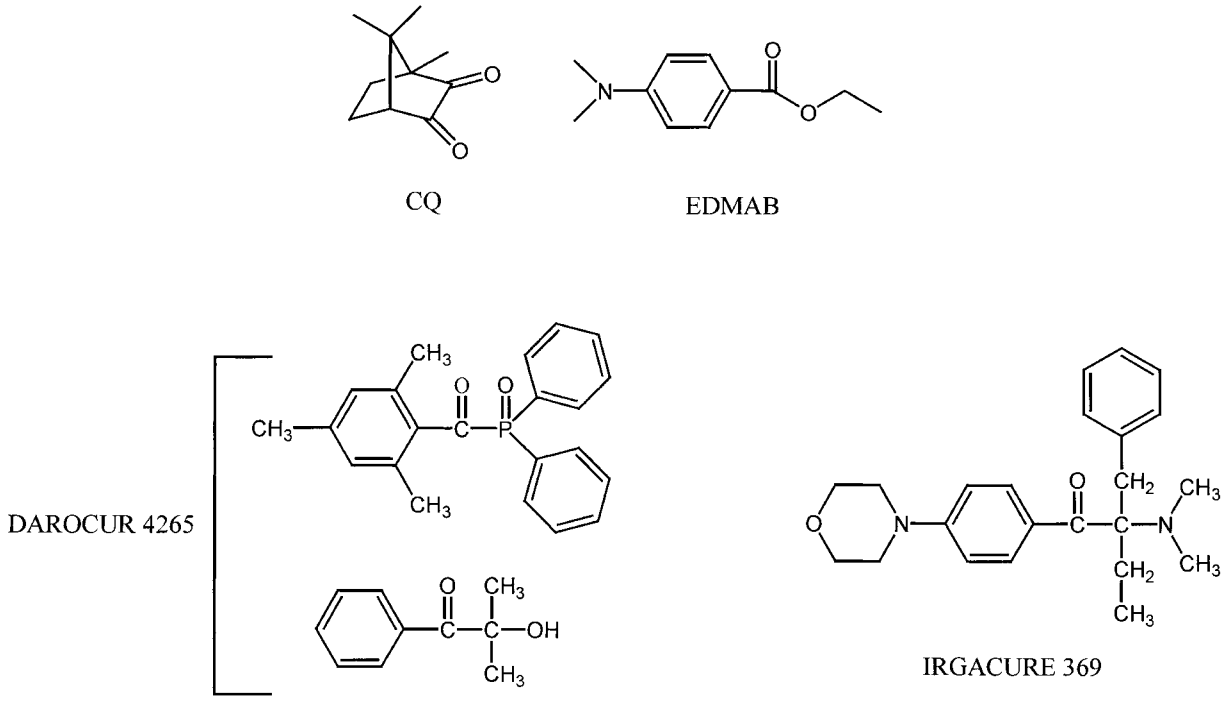

FIGURE 2. Chemical structure of the components of the photoinitiating systems.

TABLE 4. ACP Fillers Used for Preparation of Composite Disk Specimens

Filler	*ACP-stabilizing ion**	*Hybridizing agent@*	*Ca/PO_4 molar ratio*	*SSA (m^2/g)*
u-ACP	Pyrophosphate	None	1.50 ± 0.09 (7)#	19 ± 1 (3)
Si-ACP	Pyrophosphate	TEOS	1.59 ± 0.06 (7)	39 ± 2 (3)
Zr-ACP	Pyrophosphate	$ZrOCl_2$	1.91 ± 0.09 (7)	37 ± 4 (3)

* Initial concentration: mole fraction of 0.02 based on Na_2HPO_4.
@ Initial concentration: mole fraction of 0.10 based on $Ca(NO_3)_2$.
# Results are expressed as mean value ± standard uncertainty. () indicates number of runs in each experimental group.

The XRD profiles of the powdered samples were recorded in the 4° to 60° 2θ range with CuKα radiation ($\lambda = 1.54$ Å) using a Rigaku X-ray diffractometer (Rigaku/USA Inc., Danvers, MA, USA) operating at 40 kV and 40 mA. The samples were step-scanned in intervals of 0.010° 2θ at a scanning speed of 1.000°/min. The same scanning conditions also were applied to the composite disk specimens that were examined for the internal ACP into HAP conversion upon exposure to aqueous environments.

The FTIR spectra (4000 cm^{-1} to 400 cm^{-1}) of the fillers in KBr pellets were recorded using a Nicolet Magna-IR FTIR System 550 spectrophotometer (Nicolet Instrument Corporation, Madison, WI, USA) purged with dry CO_2-free air. Specimen pellets were made of approximately 2 mg of the solid and 400 mg of KBr. Specimens were examined for the traces of Ca-pyrophosphate, HAP and/or other crystalline CaPs by comparison with FTIR spectra of ACP containing the admixed CaPs of interest.

The specific surface area (SSA) of the solids (expressed as the area divided by mass (m^2/g) was determined by the triple point method [13] using the Quantasorb sorption system (Quantachrome Corp., Greenvale, NY, USA). This method uses nitrogen as the adsorbate gas and helium as the inert nonadsorbable carrier. The process of adsorption and desorption is monitored by measuring the change in the thermal conductivity of the gas mixture. The adsorption peak results from the decrease in nitrogen concentration caused by its adsorption on the powder surface. Three replicate measurements were performed for each sample.

The Ca/PO_4 ratio of the solids after dissolution in HCl was calculated from solution Ca^{2+} and PO_4 values. Concentration of Ca^{2+} was determined by atomic absorption spectroscopy (AAS) with a Perkin Elmer Model 603 spectrophotometer (Perkin Elmer, Norwalk, CT, USA) using an air-acetylene flame and the 422.7 nm line. Standard Ca^{2+} solutions were prepared from weighed amounts (±mass fraction 0.1%) of NIST Standard Reference Material $CaCO_3$ (dried at 250°C for 2 h). Both standard Ca solutions and the unknown samples contained 1000 μg/g $LaCl_3$. PO_4 was determined as a blue molybdato complex (in acidified ammonium molybdate solution containing ascorbic acid and a small amount of antimony [14] using a UV/VIS Carey Model 219 spectrophotometer (Varian Analytical Instruments, Palo Alto, CA, USA) at a wavelength of 882 nm. The PO_4 standards were prepared from weighed amounts (±mass fraction 0.1%). of KH_2PO_4 (dried at 105°C for 2 h). Absorbances of both standards and unknowns were determined at essentially the same aging time (approximately 1 h) by observing color development in the sample cell versus an H_2O blank.

Preparation and characterization of composite disk specimens

Composite pastes made up of various resins (Table 3; mass fraction 60 %) and ACP fillers (Table 4; mass fraction 40 %) were formulated by hand spatulation. The homogenized pastes were kept under vacuum (2.7 kPa) overnight to eliminate the air entrained during mixing. The pastes were then molded in disks (15.8 mm to 19.8 mm in diameter and 1.55 mm to 1.81 mm in height) by filling the circular openings of flat Teflon molds, covering each side of the mold with a Mylar film plus a glass slide, and then clamping the assembly together with spring clips. The disks were photopolymerized by irradiating each face of the mold assembly for 120 s with visible light (Triad 2000, Dentsply International, York, PA, USA). After postcuring at 37°C in air for 24 h, the intact disks were directly examined by XRD.

Mineral ion release from the composites

The dissolution/transformation behavior of the composite specimens was examined at 37°C in HEPES-buffered (pH = 7.40) 240 mOsm/kg saline solutions. Each individual disk specimen was suspended by means of a stainless steel wire frame in 100 mL of continuously stirred test solution. Ion release kinetics was followed by taking aliquots at appropriate time intervals, filtering (Millex GS filter assemblies; Millipore, Bedford, MA, USA) and measuring the Ca^{2+} and PO_4 concentrations. Upon completion of the immersion tests (350 h or longer), the disks were removed, dried, and again examined by XRD. Three or more separate runs

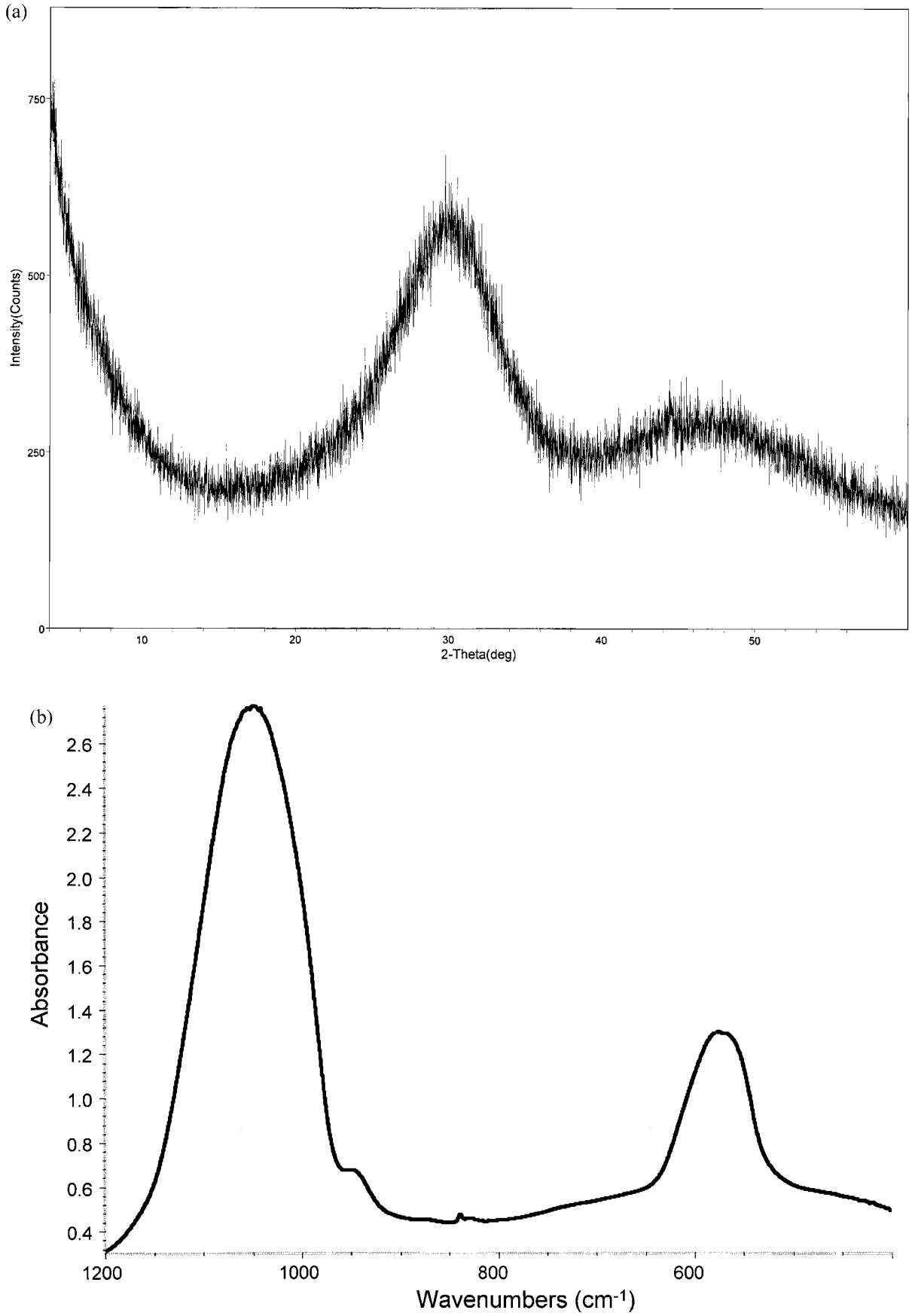

FIGURE 3. Typical XRD pattern (a) and FTIR absorbance spectra (b) of the ACP fillers used in this study.

TABLE 5. Average Values ± Standard Uncertainty of the Maximum Concentration of the Mineral Ions Released from Composites After >336 h of Immersion in Buffered Saline Solutions and the Corresponding Thermodynamic Stability, ΔG° (calculated according to eqn. [1])

Filler	*Resin*	*No. of runs*	*Ca^{2+} (mmol/L)*	*PO_4 (mmol/L)*	*ΔG° (kJ/mol)*
u-ACP		10	0.39 ± 0.03	0.24 ± 0.03	−(3.88 ± 0.09)*
Si-ACP	**BT**	7	0.90 ± 0.12	0.64 ± 0.09	−(4.79 ± 0.27)
Zr-ACP		—	—	—	—
u-ACP		3	1.10 ± 0.01	0.57 ± 0.01	−(5.20 ± 0.04)
Si-ACP	**BTH**	—	—	—	—
Zr-ACP		—	—	—	—
u-ACP		16	0.93 ± 0.11	0.59 ± 0.06	−(4.88 ± 0.22)
Si-ACP	**BTHZ**	17	0.98 ± 0.11	0.64 ± 0.05	−(5.00 ± 0.20)
Zr-ACP		24	0.99 ± 0.08	0.63 ± 0.06	−(5.02 ± 0.18)
u-ACP		6	0.50 ± 0.03	0.95 ± 0.05	−(4.42 ± 0.11)
Si-ACP	**TP**	8	0.94 ± 0.05	0.27 ± 0.01	−(4.28 ± 0.10)
Zr-ACP		8	1.16 ± 0.09	0.20 ± 0.02	−(4.33 ± 0.13)
u-ACP		6	0.42 ± 0.02	0.90 ± 0.05	−(4.17 ± 0.11)
Si-ACP	**U**	9	1.59 ± 0.18	1.05 ± 0.05	−(6.01 ± 0.18)
Zr-ACP		9	1.24 ± 0.12	0.87 ± 0.06	−(5.53 ± 0.19)
u-ACP		6	0.35 ± 0.02	0.91 ± 0.03	−(3.95 ± 0.11)
Si-ACP	**U66H**	9	1.50 ± 0.13	1.09 ± 0.04	−(5.96 ± 0.18)
Zr-ACP		9	1.73 ± 0.10	1.06 ± 0.04	−(6.12 ± 0.10)
u-ACP		6	0.80 ± 0.08	0.78 ± 0.04	−(4.90 ± 0.17)
Si-ACP	**U132H**	9	2.25 ± 0.20	1.31 ± 0.06	−(6.62 ± 0.19)
Zr-ACP		9	1.59 ± 0.13	0.94 ± 0.05	−(5.92 ± 0.14)

* Negative ΔG° value indicates solution supersaturated with respect to stoichiometric HAP.

were performed for each experimental group. Kinetic ion-release data were corrected for variations in the total area of disk surface exposed to the immersion solution. Exposed disk areas ranged from 450 mm^2 to 690 mm^2, with the majority of the values clustered around 500 mm^2. Therefore, the filtrate Ca^{2+} and PO_4 values were normalized to 500 mm^2 using the simple relation for a given surface area, A: normalized value = (measured value) × (500/A).

The thermodynamic stability, ΔG^o, of immersion solutions containing the maximum concentrations of Ca^{2+} and PO_4 ions released from the composite disks was calculated with respect to stoichiometric HAP using the Gibbs free-energy expression:

$$\Delta G^o = -2.303(RT/n)\ln(IAP/K_{sp}) \qquad [1]$$

where IAP is the ion activity product for HAP, K_{sp} is the corresponding thermodynamic solubility product, R is the ideal gas constant, T is the absolute temperature, and n is the number of ions in the IAP (n = 18). IAP and ΔG^o were calculated by the solution equilibrium calculation program EQUIL (MicroMath Scientific Software, Salt, Lake City, UT, USA).

Statistical analysis

Experimental data were analyzed by multi-factorial ANOVA (α = 0.05). To determine significant differences between specific groups, all pairwise multiple comparison procedures were performed.

RESULTS AND DISCUSSION

No observable differences in structural features of u-ACP and the hybridized ACPs (Si-ACP and Zr-ACP) were found in their XRD patterns or FTIR spectra. A typical XRD pattern and FTIR spectrum of the ACPs used in this study are given in Fig. 3. a, b. The XRD pattern has the very broad peak features characteristic of amorphous materials that lack three dimensional repeating order. The FTIR spectra showed only the two wide bands that are typical for noncrystalline calcium phosphate: a) v_1 and v_3 phosphate stretching in the region 1200 cm^{-1} to 900 cm^{-1}, and b) v_4 phosphate bending in the region 630 cm^{-1} to 500 cm^{-1}.

The Ca/PO_4 molar ratio of u-ACP and Si-ACP showed no significant differences (Table 4), but showed a significant increase for Zr-ACP fillers. Notably, the SSAs of both hybridized fillers were, on average, twice that of u-ACP (one-way ANOVA: $p < 0.0001$; two-tail test: $p < 0.019$). However, the differences in SSA between Si-ACP and Zr-ACP were insignificant ($p > 0.10$).

The levels of released mineral ions from u-ACP composites were significantly affected by the resin composition (Table 5). The solution concentrations

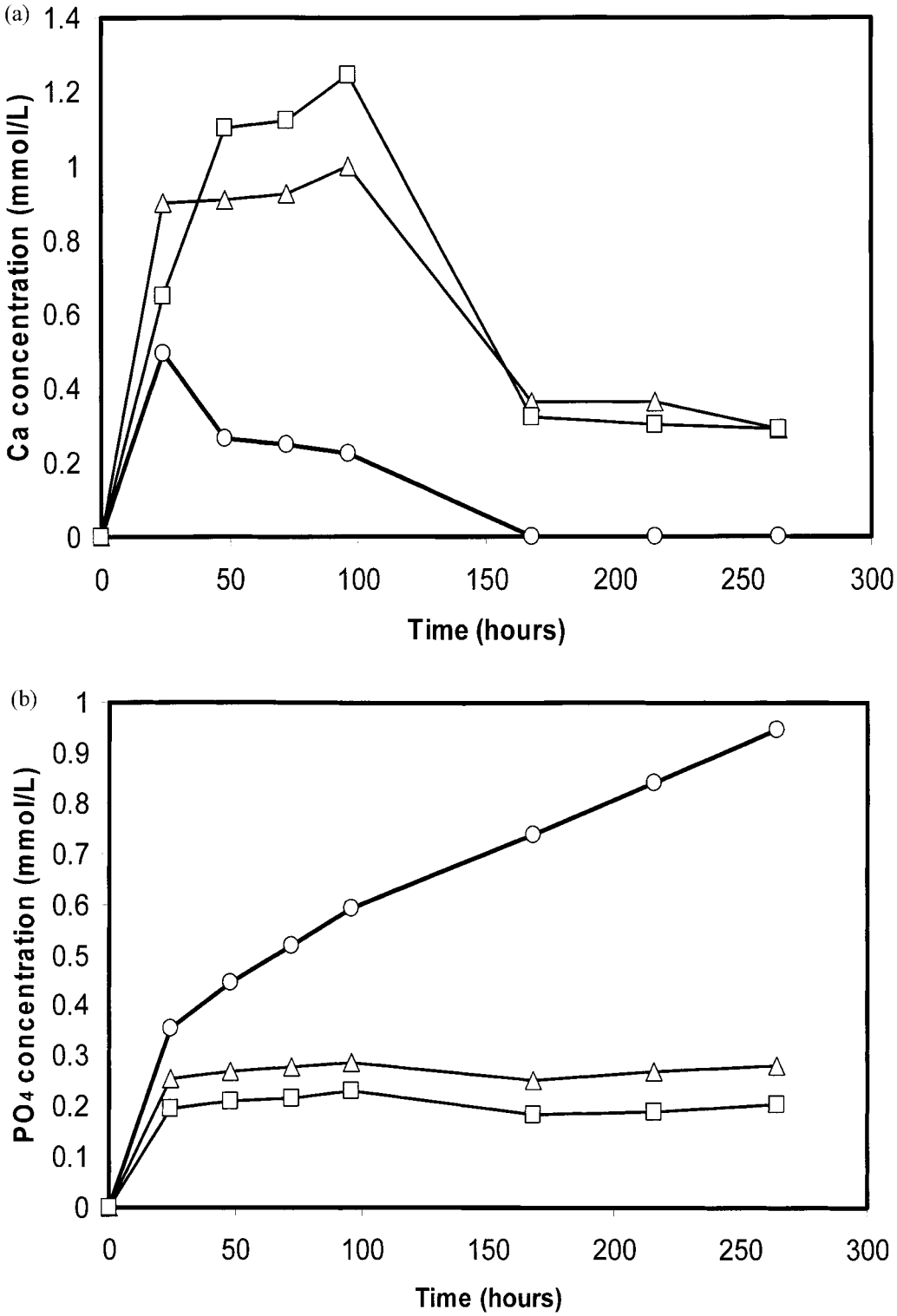

FIGURE 4. Concentration of Ca^{2+} (a) and PO_4 (b) ions released from the composite disks upon their immersion in buffered saline solution: Pyro-ACP (○), Si-ACP (△) and Zr-ACP (□). Standard deviations of indicated values were: ≤0.028 mmol/L (Ca^{2+}) and ≤0.015 mmol/L (PO_4) for Pyro-ACP, ≤0.030 mmol/L (Ca^{2+}) and ≤0.014 mmol/L (PO_4) for Si-ACP, and ≤0.045 mmol/L (Ca^{2+}) and ≤0.010 mmol/L (PO_4) for Zr-ACP.

of both calcium and phosphate ions more than doubled when HEMA alone (BTH resin), or HEMA and ZrMA (BTHZ resin) were added as comonomers to the BT resin (Bis-GMA and TEGDMA in a 1:1 mass ratio). Identical effects were observed when Si-ACP was used as filler in BT composites. On the other hand, the introduction of hybridized fillers in BTHZ resins had no effect on their ion-releasing abilities.

The solution profiles of Ca^{2+} and PO_4 ions

released from composite disks made of u-ACP, Si-ACP and Zr-ACP with TP resin upon immersion in buffered saline are illustrated in Fig. 4. Contrary to the results observed with BT, BTH and particularly BTHZ, the high initial levels of Ca^{2+} reached within (74 to 96) h of immersion of TP composites were not sustainable over extended time periods. Significant reduction in Ca-release by the hybridized disks occurred between 100 h and 150 h of immersion resulting in soluble (available) Ca^{2+} concentrations of only (0.30 to 0.35) mmol/L. A similar profile, showing a maximum concentration of Ca^{2+} (0.50 mmol/L) reached within 24 h followed by a steady decrease to undetectable levels by 160 h, was obtained for the u-ACP composites. PO_4 release from both types of hybridized ACP composites leveled off at considerably lower concentrations ((0.20 to 0.27) mmol/L) after only 24 h of immersion. Unlike the Ca^{2+} release, the PO_4 release continued to steadily rise during the immersion of u-ACP composites.

Sustainable Ca^{2+} releases from u-ACP based UDMA composites containing no HEMA (U resin) or mass fraction of 6.6 % HEMA (U66H resin) were comparable to the release obtained with the BT resins but significantly lower than the release from BTH and BTHZ resins. With the highest level of HEMA (U132H resin) it reached a value close to that observed for u-ACP/BTHZ composites. However, when hybridized ACPs were used as fillers for the UDMA resin composites, Ca^{2+} release was significantly higher than the release from BT, BTH and BTHZ composites. PO_4 release from both u- and hybridized-ACP U, U66H and U132H composites was generally higher than the release from identically formulated BT, BTH, BTHZ and/or TP composites. The only comparable value was the release of PO_4 from u-ACP/TP composites.

When the solution data were recalculated in terms of the ionic activity product (IAP) and supersaturation with the respect to stoichiometric HAP, i.e., ΔG^o, the following order of decreasing remineralizing potential has been established: Si-ACP/U132H >[Zr-ACP/U66H, Si-ACP/U, Si-ACP/U66H, Zr-ACP/132H] >Zr-ACP/U >[u-ACP/BTH, Zr-ACP/BTHZ, Si-ACP/BTHZ, u-ACP/U132H, u-ACP/BTHZ, Si-ACP/BT] >[u-ACP/TP, Si-ACP/TP, Zr-ACP/TP, u-ACP/U] >[u-ACP/U66H, u-ACP/BT]. Composites listed within the brackets showed statistically insignificant differences in ΔG^o ($p > 0.05$, Tukey multiple comparison tests).

As demonstrated by XRD and FTIR analysis, hybridized ACP fillers synthesized in the presence of a mole fraction of 10% hybridizing agent (either TEOS or $ZrOCl_2$) based on $Ca(NO_3)_2$ exhibited no detectable difference in their predominantly amorphous structure. However, Ca^{2+} and PO_4 contents of these solids revealed some compositional differences. The reason for the higher Ca/PO_4 ratio in Zr-ACP compared to Si-ACP and u-ACP is not apparent but may arise from the loss of PO_4 via formation of some soluble zirconium phosphate salts that are removed during the washing of Zr-ACP. Findings that the SSAs of hybridized ACP fillers were considerably higher than the SSA of u-ACP may be explained by the altered mode of ACP particle agglomeration in the presence of TEOS and $ZrOCl_2$. We have previously reported [14] that this observed higher SSA may account for increased mechanical strength of hybrid ACP-based BTHZ composites. The constancy of the SSA and the mass fraction (%) of Si (2.74 to 3.63) [15] in TEOS-hybridized ACPs suggest that the silica is not incorporated into the interior of the filler particles but instead, is bound on the surface of the particles. Excess TEOS is probably removed during filtering and washing of the freshly precipitated hybridized solids.

The kinetics of ion release from ACP-filled composites is determined to a large extent by the permeability of the polymer system to water, the nature of the polymer matrix network structure (as determined by such factors as degree of cure; crosslink density; the type, abundance and location of hydrophilic functional groups; hydrogen bonding interactions; etc.), internal pH, and the kinetics of internal conversion of ACP into HAP. The conversion of ACP into HAP can be expressed by the following equation [3]:

$$8Ca_3(PO_4)_2 3H_2O + Ca^{2+} + 4OH^- + H_2O \rightarrow 5Ca_5(PO_4)_3OH + HPO_4^- \quad (2)$$

Despite the buildup of HAP within the composite, Ca^{2+} and PO_4 levels in the immersion solution steadily rose to high levels of supersaturation. Corresponding XRD patterns [6–9, 16] clearly show the slow development of HAP inside the composite. Apparently, the stabilization of ACP by $P_2O_7^{4-}$ ions sufficiently slowed the rate of conversion to HAP so that over the extended periods of immersion the remaining ACP in the disks never was reduced to a level where its dissolution could no longer maintain solution saturation. In addition, the finding that HAP crystals rapidly reduced mineral ion concentrations by inducing precipitation when directly introduced into the immersion solution [7] suggests that these concentrations also could only have been sustained if the conversion to HAP within the disks was a series of a localized events, each tightly confined by the resin binder to the space originally occupied by the converted ACP. This confinement would greatly restrict growth as well as prevent the HAP from coming into direct contact with the immersion solution and acting as seeds for spontaneous precipitation. The solution stability of BT, BTH, BTHZ, U, U66H and U132H composites further suggests that any exposed ACP (largely free of polymer) rapidly dissolved during the initial immersion of the disks; this resulted in a resin-rich surface that was not conducive to nucleating HAP directly from the Ca^{2+}/PO_4 solution. It had been previously found that high ion concentrations in the immersion solutions could be achieved with the dissolution of as little as 1.3 mass % of the initial ACP filler

content in the BT composite [16] and no more that 6.2 to 6.7 mass % of the initial amount of ACP filler in the BTH composite [10]. Moreover, conversion to HAP was even slower when TEOS- or $ZrOCl_2$-hybridized ACPs were used[10]. Additional evidence of the retarding effect of these hybridizers on the conversion of ACP to HAP was obtained from a series of dissolution experiments with hybrid ACP fillers alone in buffered saline. Ca/PO_4 ratios for Si- and Zr-ACP fillers were found to be much higher than those of u-ACP indicating slower formation of HAP in the presence of TEOS and $ZrOCl_2$. Furthermore, reimmersion of the Zr-ACP based composite disks in fresh saline after 400 h of soaking released higher Ca^{2+} and PO_4 levels than did similarly treated u-ACP composite disks.

We have previously reported [3, 4] that the $P_2O_7^{4-}$-stabilized ACP (u-ACP) was the most effective filler for establishing elevated Ca^{2+} and PO_4 ion concentrations upon soaking BT, BTH and/or BTHZ polymeric composite disks in buffered saline solutions. For this reason, $P_2O_7^{4-}$-stabilized ACP was proposed as the most suitable of the ACP fillers for use in dental applications where protection against mineral loss and/or restoration of mineral would be desirable [4, 5]. Immersion of composites made of Si- or Zr-ACP filler (used at optimal levels of mass fraction of 40 %) with the BTHZ resin, also resulted in elevated mineral ion releases that should be conducive to promoting remineralization. However, similarly treated composites made with TP resin, which has adhesive potential for tooth structure due to the carboxylic monomer PMGDMA [11], failed to maintain a favorable remineralization potential due to the reincorporation by the composite of released Ca^{2+} along with a reduction in PO_4 release. Observed Ca^{2+} consumption in TP composites can possibly be explained by the strong affinity of the carboxylic groups of PMGDMA to bind ionically Ca^{2+}. Ca-PMGDMA binding was indeed confirmed[15] in a series of experiments where disks made of unfilled TP resin were exposed to buffered saline solutions that initially contained 1.5 mmol/L Ca^{2+}. The average observed drop in Ca^{2+} concentration of (0.63 ± 0.01) mmol/L correlates well with the Ca^{2+} decreases from reincorporation seen in both types of hybrid ACP/TP composites (0.56 ± 0.04) mmol/L and (0.75 ± 0.06) mmol/L for Si- and Zr-ACP, respectively). Reincorporation via ion binding by carboxylic acid groups in the matrix also explains why no measurable Ca^{2+} was detected in the immersing solutions of u-ACP/TP composites at time intervals >150 h.

From this study it would appear that a strategy for promoting high, sustained ion release requires utilization of a hybrid ACP filler with resin matrices that contain a hydrophilic monomer such as HEMA. As a base resin for these bioactive composites UDMA appears to have advantages over Bis-GMA/TEGDMA and highly carboxylated monomer systems such as PMGDMA/TEGDMA.

CONCLUSIONS

Both the chemical structure and composition of the monomer system used to form the matrix phase of ACP composites can affect their ion release profiles. Generally high ion release was observed with UDMA/HEMA resin matrix systems. TP composites, on the other hand, gave high initial ion release but overall showed the lowest remineralizing abilities due to ion-binding by carboxylic groups of the resin matrix. In addition, the type of ACP filler (hybridized versus unhybridized) also can influence the ion release behavior of polymeric ACP composites. Significantly higher ion release was observed with Si-ACP /U132H composites compared to other types of ACP fillers. A further advantage of these hybrid ACP fillers appears to be their ability to slow down the intra-composite conversion of ACP to HAP.

DISCLAIMER

Certain commercial materials and equipment are identified in this work for adequate definition of the experimental procedures. In no instance does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or that the material and the equipment identified is necessarily the best available for the purpose.

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