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Long-term water sorption and solubility of experimental bioactive composites based on amorphous calcium phosphate and bioactive glass

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The aim of this study was to evaluate water sorption and solubility of two series of experimental composites containing amorphous calcium phosphate (ACP) or bioactive glass (BG). Water sorption and solubility were measured for up to 287 days. The surface precipitation of calcium phosphates was evaluated by scanning electron microscopy. The ACP-series showed higher water sorption (223–568 $\mu g/mm^3$) than the BG-series (40–232 $\mu g/mm^3$). In contrast, the ACP-series had generally lower solubility (37–106 $\mu g/mm^3$) than the BG-series (1–506 $\mu g/mm^3$). The constant specimen mass for the ACP-series was attained after 14 days of water immersion, while the mass decrease due to long-term solubility in the BG-series lasted beyond 287 days. Calcium phosphates precipitated in composites with the BG filler loading of 10 wt% or more, as well as in all of the ACP-containing composites. The experimental composite series showed water sorption and solubility considerably higher than commercial materials.

Keywords: Amorphous calcium phosphate, Bioactive glass, Remineralizing composite, Bioactive composite, ISO 4049

INTRODUCTION

Experimental bioactive composites offer the potential to prevent secondary caries, which has been recognized as one of the major causes for failure of composite restorations[1]. Remineralizing composites based on amorphous calcium phosphate (ACP) are characterized by the release of calcium and phosphate ions[2], with the capability for dental hard tissues remineralization demonstrated *in vitro*, *in situ* and *in vivo*[3-5]. Their bioactivity originates from the high solubility of ACP, especially in an acidic environment, which produces calcium and phosphate solutions supersaturated with respect to hydroxyapatite and allows its precipitation[6,7]. Experimental composites with ACP fillers in the micrometer range have shown mechanical properties inferior to those of commercial composites[2,6], but decreasing the ACP particle size to the nanometer range can yield mechanical properties similar to those of commercial composites[8].

Another group of experimental composites based on bioactive glass (BG) is characterized by the surface precipitation of hydroxyapatite when exposed to water[9,10]. This process is initiated by the dissolution of reactive glass particles which creates a hydrated silica layer on their surface, enabling the heterogeneous nucleation of hydroxyapatite[10]. There are multiple potential benefits of the hydroxyapatite precipitation: sealing of the marginal gap[11], improving the durability of the bonded interface by inhibiting the hybrid layer degradation by collagenolytic enzymes[12] and desensitizing the dentin by the occlusion of dentinal tubules[13].

The filler in bioactive composites is a mixture of bioactive (releasing) and reinforcing (inert) particles. Bioactive fillers are water-soluble and enable the ion release, while reinforcing fillers are stable and contribute to mechanical properties of a cured composite. The bioactive filler impairs mechanical properties, as it is unsilanized and unable to reinforce the composite structure by bonding to the methacrylate network[6]. In bioactive composites, the resinous component is usually more hydrophilic than in conventional commercial composites, in order to allow water diffusion that is sufficient for the ion release[14]. However, high hydrophilicity is an unwanted property because it accelerates the degradation of the composite structure and consequently impairs mechanical properties[15].

This study investigated two series of experimental bioactive composites, which were formulated with two different photo-curable resin systems and two types of bioactive fillers (ACP and BG), following previous studies[9,16-20]. The ACP-series was based on a hydrophilic Bis-EMA/TEGDMA/HEMA resin and 40 wt% of ACP fillers[16-18]. Such a composition was required for a sufficient ion release and remineralizing capability[2,6]. The BG-series was based on a comparatively less hydrophilic Bis-GMA/TEGDMA resin system and contained a lower amount of BG fillers (varying between

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0–40 wt%), as these compositions demonstrated the capability to precipitate hydroxyapatite in a preliminary study[9]. Thus, both experimental series were bioactive, despite considerable compositional differences which should reflect on water uptake and release of soluble species during water immersion. The aim of this study was to investigate the effect of compositional variations in experimental ACP- and BG-series on long-term water sorption and solubility. The dynamics of mass change during water immersion were used to indicate the duration of the ion release. The null hypotheses were that: (I) there is no difference in the dynamics of mass change over time due to water sorption and solubility, and (II) there is no difference among materials with respect to the amount of absorbed water or release of soluble species.

MATERIALS AND METHODS

Preparation of composite materials

Experimental composite materials were prepared by mixing the resin, filler and photoinitiator system in a dual asymmetric centrifugal mixing system (Speed Mixer TM DAC 150 FVZ, Hauschild, Hamm, Germany). The mixing was performed in lightproof containers at 2,700 rpm, through five one-minute intervals separated by one-minute breaks. After mixing, obtained composite pastes were deaerated in vacuum for 12 h and used within one month after the preparation.

The components of experimental composites denoted as ACP-series and BG-series are given in Tables 1 and 2, respectively. The composition of ACP-series followed previous studies[16-18] and was based on zirconia-hybridized ACP prepared as described in reference[21]. The ACP-series comprised five composites with 40 wt% of ACP and two control composites which contained only reinforcing fillers (Table 1).

The BG-series comprised five experimental composites with varying ratios of bioactive/reinforcing fillers (Table 2). In addition to the two experimental series, a restorative (solid) composite (Tetric EvoCeram, shade A2, LOT: V40834, EXP: 10/13/2020, Ivoclar Vivadent, Schaan, Liechtenstein, abbreviation: TEC) and a flowable composite (Tetric EvoFlow, shade A2, LOT: V36426, EXP: 09/02/2020, Ivoclar Vivadent, abbreviation: TEF) were used as references.

Water sorption and solubility

Water sorption and solubility were determined by a modified ISO 4049 gravimetric method[22]. Composite specimens were prepared at room temperature (22±1°C) in discoid Teflon molds with the diameter of 6 mm and height of 2 mm. The molds were placed on the polyethylene terephthalate (PET) strip, filled with uncured composite and covered with another PET strip. The composite paste was then pressed between two glass

Table 1 Composition of the ACP-series

Material		Filler composition (wt%)		Total filler ratio (wt%)	Resin		Filler load (vol%)
		Zr-ACP	Reinforcing fillers		wt%	Composition	
ACP-containing composites	ACP40	40	0 Ba 0 Si	40	60	67% Bis-EMA 23% TEGDMA 10% HEMA photoinitiator system: 0.2% CQ 0.8% 4E	28
	ACP40-Ba10	40	10 Ba 0 Si	50	50		35
	ACP40-Si10	40	0 Ba 10 Si	50	50		37
	ACP40-Ba5Si5	40	5 Ba 5 Si	50	50		36
	ACP40-Ba9Si1	40	9 Ba 1 Si	50	50		35
non-bioactive controls	Ba40	0	40 Ba 0 Si	40	60		22
	Ba40Si10	0	40 Ba 10 Si	50	50		32

Zirconia-hybridized ACP (Zr-ACP): prepared by wet chemistry according to (19); particle size: 2–5 µm

Barium-fillers (Ba): SiO_2 55.0%, BaO 25.0%, B_2O_3 10.0%, Al_2O_3 10.0%, particle size (d50/d99 [µm]) 0.77/2.28, silanization 6 wt%, product name/manufacturer: GM39923/Schott, Mainz, Germany.

Silica-fillers (Si): $SiO_2 \geq 99.8$, primary particle size: 12 nm, silanization 4–6 wt%, product name/manufacturer: Aerosil DT/Evonik Degussa, Hanau, Germany.

Bis-EMA: ethoxylated bisphenol-A-dimethacrylate, Esstech, Essington, PA, USA; TEGDMA: triethyleneglycol dimethacrylate, Esstech; HEMA: 2-hydroxyethyl methacrylate, Esstech; CQ: camphorquinone, Aldrich, Milwaukee, WI, USA; 4E: ethyl-4-(dimethylamino) benzoate, Aldrich.

Table 2 Composition of the BG-series

Material		Filler composition (wt%)		Total filler ratio (wt%)	Resin		Filler load (vol%)
		Bioactive glass	Reinforcing fillers (Ba:Si=2:1)		wt%	Composition	
non-bioactive control	BG-0	0	70	70	30	60% Bis-GMA 40% TEGDMA	48
BG-containing composites	BG-5	5	65	70	30	photoinitiator system: 0.2% CQ 0.8% 4E	48
	BG-10	10	60	70	30		48
	BG-20	20	50	70	30		51
	BG-40	40	30	70	30		52

Bioactive glass: SiO_2 45%, Na_2O 25%, CaO 25%, P_2O_5 5%, particle size (d50/d99 [μm]): 4.0/13.0, silanization: none, product name/manufacturer: G018-144/Schott.

Barium-fillers (Ba): SiO_2 55.0%, BaO 25.0%, B_2O_3 10.0%, Al_2O_3 10.0%, particle size (d50/d99 [μm]): 1.0/4.0, silanization 3.2 wt%, product name/manufacturer: GM27884/Schott.

Silica-fillers (Si): $SiO_2 \geq 99.8\%$, primary particle size: 12 nm, silanization 4–6 wt%, product name/manufacturer: Aerosil DT/Evonik Degussa.

Bis-GMA: Bisphenol-A-glycidyl methacrylate, Esstech; TEGDMA: triethyleneglycol dimethacrylate, Esstech; CQ: camphorquinone, Aldrich; 4E: ethyl-4- (dimethylamino) benzoate, Aldrich.

slides and curing was performed with a LED curing unit (Bluephase G2, Ivoclar-Vivadent; wavelength range 380–515 nm, irradiance 1,200 mW/cm^2) for 20 s on each side. The edges of cured specimens (*n*=6 per experimental group) were gently ground on P1200 abrasive paper to remove flashes and the specimens were transferred to a desiccator containing freshly dried silica-gel. Specimens were weighed to the accuracy of 0.1 mg with an analytical balance (XS204, Mettler Toledo, Greifensee, Switzerland) every 24 h until the difference between two subsequent measurements dropped below 0.1 mg, *i.e.* until the constant mass was attained. This value was recorded as the initial mass (m_1). Specimen dimensions were measured using a digital caliper so that the diameter was measured at two perpendicular planes and thickness was measured at the center and four equally spaced points along the circumference. From these data, mean values of diameter and thickness were calculated. The specimens were then immersed in individual flasks containing 4 mL of distilled water at 37°C. The flasks had a conical bottom so that only the outer specimen border was in contact with the flask wall, in order to expose the maximum specimen surface to water. After determined time intervals, specimens were removed from the water, blotted using cellulose pads until free from visible moisture, waved in the air for 15 s and weighed to obtain $m_2(t)$, where t denotes the time interval after the start of the immersion. For the ACP-series, specimens were weighed 1, 3, 7, 14, 28 and 60 days after the start of immersion, while the specimens from BG-series were weighed in the same time periods and additionally every 14 days up to 196 days and thereafter every 30 days up to 287 days. This was done because in a preliminary study the ACP-series attained equilibrium already after 14 days, while some of the BG-containing composites showed a prolonged solubility[20]. The distilled water was replaced every 7 days in order to avoid pH changes. After the immersion period was over, specimens were transferred to the desiccator and weighed every 24 h until a constant mass (variations below 0.1 mg) was attained, to record the final mass (m_3). The total desorption period was 28 days.

The mass change at each time point (t) during water immersion was calculated as $\Delta m=(m_2(t)-m_1)/m_1$. Water sorption and solubility in μg/mm^3 were calculated according to the following equations: water sorption= $(m_2(eq)-m_3)/V$; and solubility=$(m_1-m_3)/V$, where $m_2(eq)$ denotes mass at the equilibrium and V denotes specimen volume.

Scanning electron microscopy

To investigate the surface precipitation of calcium phosphates, specimens of the same geometry as those used for the water sorption and solubility study were prepared by the same curing protocol and immersed separately in 10 mL of phosphate buffered saline (PBS) at 37°C for 28 days. Specimens were dried in a desiccator at room temperature (22±1°C) and examined unpolished and unsputtered using a scanning electron microscope (SEM, Quanta FEG 400, FEI, Eindhoven, the Netherlands). SEM images were recorded in low vacuum using a large-field detector with a voltage of 5 kV.

Statistical analysis

Within a given material, mean values of mass change among different time points were compared using repeated measures ANOVA with Bonferroni *post-hoc* test. Due to the heterogeneity of variances, water sorption and solubility among materials were compared using Welch ANOVA followed by Games-Howell *post-hoc* test. Statistical analysis was performed in SPSS 20 (IBM, Armonk, NY, USA), with the level of significance set at 0.05.

RESULTS

Mass changes during immersion

Mass change as the percentage of the initial specimen mass is plotted in Fig. 1. Two distinct patterns can be observed. The first pattern is common for conventional composites and features the increase in specimen mass followed by a plateau that signifies equilibrium. This behavior can be seen in all the composites from the ACP-series, BG-0, BG-5, neat resins and two commercial reference composites. The second pattern was observed in composites BG-10, BG-20, and BG-40 and is characterized by the curve not reaching the equilibrium plateau, but rather starting to descend due to a considerable and long-lasting solubility of bioactive fillers.

The neat resin of the ACP-series showed the highest mass increase (45%), ACP40 plateaued at 32%, while the ACP-based composites with 10% inert fillers attained their equilibriums at 24–26% (Fig. 1a). The control composites Ba40 and Ba40Si10 equilibrated at 10–13% mass increase. Much lower mass gains were observed in the BG-series (Fig. 1b), in which the neat resin equilibrated at 4%, similar to BG-5 (3%). The composites with 10–40 wt% of BG did not equilibrate until the end of the study (287 days). Since there were enough data points to obtain an excellent fit ($R^2>0.99$) to the function $y=a+b*e^{-cx}$, where a, b and c denote fit parameters, the approximate equilibrium mass values were obtained by extrapolation, as illustrated in Fig 2. In the mentioned function, as x values approach infinity, the y values asymptotically approach the parameter "a". Thus the parameter "a" can be regarded as the mass change that would be attained at the equilibrium. The described extrapolation for the materials BG-10, BG-20, and BG-40 gave mass changes of −4, −9 and −13%, respectively (Fig. 2). It should be noted that parameters of the fit function other than "a" were not attributed any concrete physical meaning and that such a function was chosen empirically, due to simplicity and goodness of fit. Otherwise, complex calculations exist for modeling the diffusion of water and soluble species through composite specimens[23] which were outside the scope of the present work.

A simple parameter indicating the dynamics of water sorption and the concurrent release of soluble species is the time at which equilibrium is attained (Table 3). This time was determined as the first time point for which the repeated measures ANOVA showed statistically similar values of specimen mass with all of the subsequent time points. All of the composites from the ACP-series attained equilibrium at 14 days, similar to the flowable commercial reference, TEF. Longer times were needed to reach equilibrium for BG-0 and BG-5 (70 and 56 days, respectively), which were comparable to that of the solid commercial reference TEC (56 days). The composites BG-10, BG-20 and BG-40 continued to lose their mass after 287 days of immersion due to the

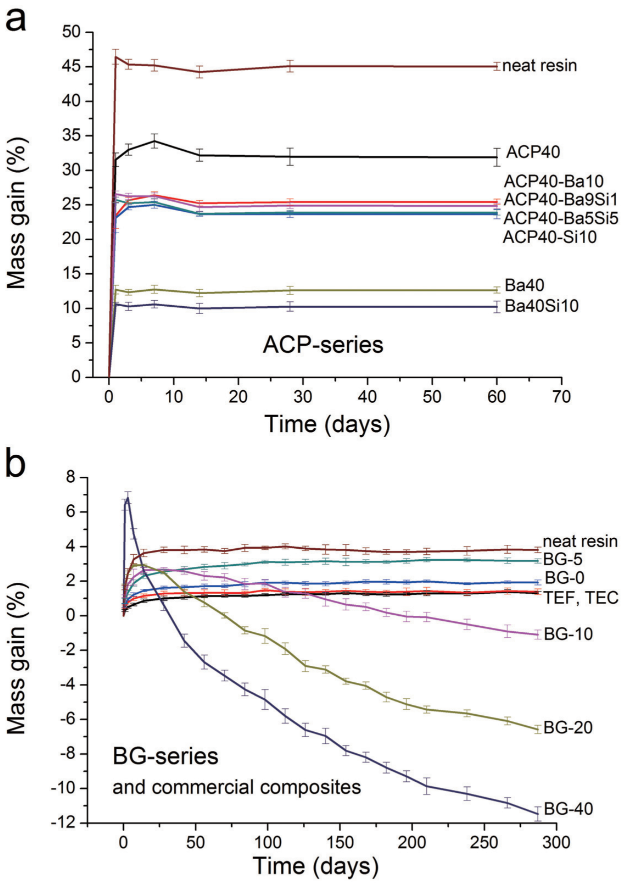

Fig. 1 Mass changes during aqueous immersion of composites filled with amorphous calcium phosphate (ACP-series) for up to 60 days (a) and composites filled with bioactive glass (BG-series) for up to 287 days (b).
Error bars denote ±one standard deviation.

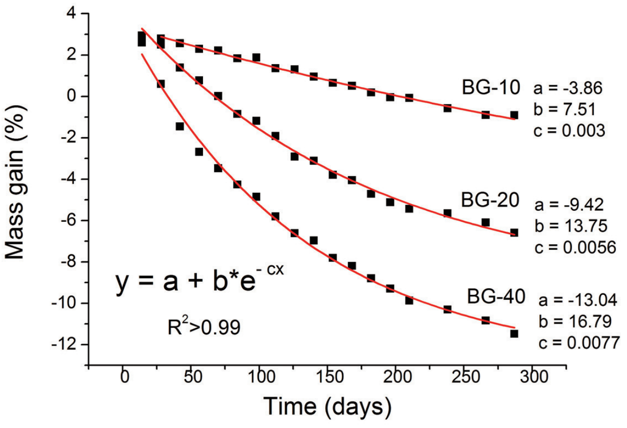

Fig. 2 Fit function and fit parameters used to approximate the mass at equilibrium for the highly soluble materials BG-10, BG-20 and BG-40.

long-term solubility, thus their time of equilibrium was not precisely determined.

Table 3 Time of the equilibrium, defined as the time point beyond which mean specimen mass values were statistically similar

	Material	Time (days)
ACP-series	ACP40	14
	ACP40-Ba10	14
	ACP40-Si10	14
	ACP40-Ba5Si5	14
	ACP40-Ba9Si1	14
	Ba40	14
	Ba40Si10	14
	neat resin	14
BG-series	BG-0	70
	BG-5	56
	BG-10	>287
	BG-20	>287
	BG-40	>287
	neat resin	7
commercial references	TEC	56
	TEF	14

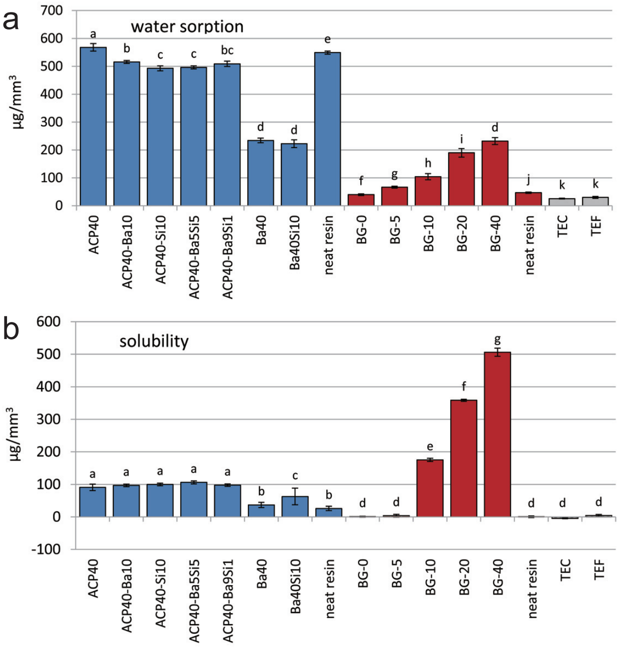

Fig. 3 Water sorption (a) and solubility (b) expressed in µg/mm^3.

Blue, red and grey bars represent ACP-series, BG-series and commercial reference composites, respectively. Error bars denote ±one standard deviation.

Water sorption and solubility

Water sorption and solubility values expressed in µg/mm^3 are shown in Fig. 3. For reference, the upper limit values for dental composites stipulated by the ISO 4049[22)] are 40 µm/mm^3 for water sorption and 7.5 µm/mm^3 for solubility. These values were exceeded in both experimental series, while commercial composites TEC and TEF fulfilled the ISO 4049 requirements. The ACP-series showed 2–10 times higher water sorption than the BG-series. On the other hand, the composites with a high amount of BG (BG-20 and BG-40) showed 2–5 times higher solubility than the ACP-series. The data for neat resins indicate much higher water sorption and solubility for the neat resin of the ACP-series (Bis-EMA/TEGDMA/HEMA) compared to the neat resin of the BG-series (Bis-GMA/TEGDMA). It should also be noted that

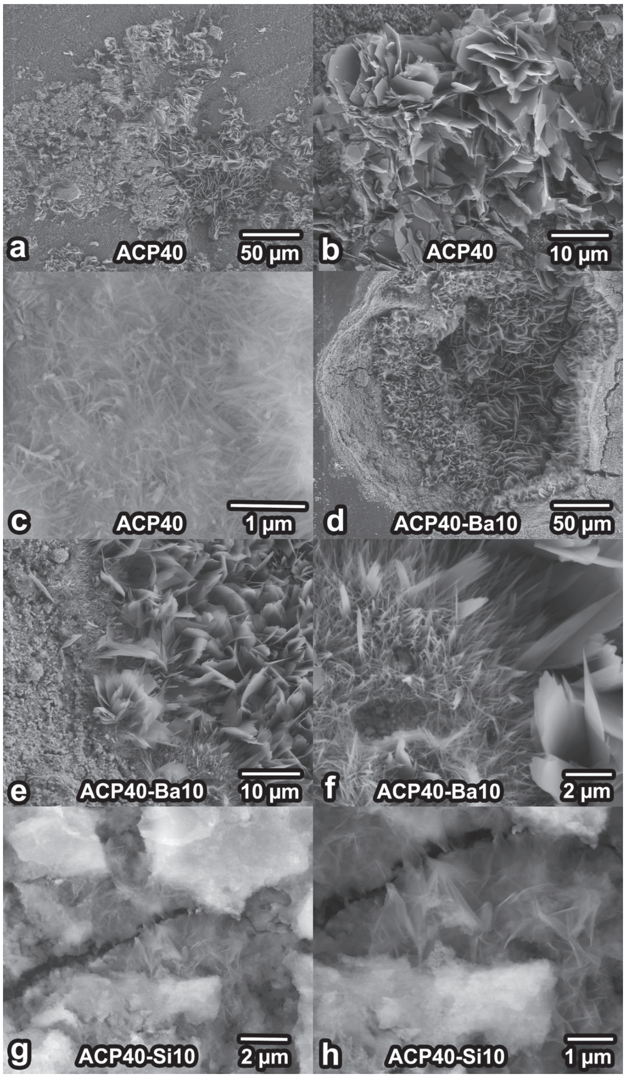

Fig. 4 Micrographs of the representative ACP-containing composites.

Corresponding scale bars are shown within each micrograph.

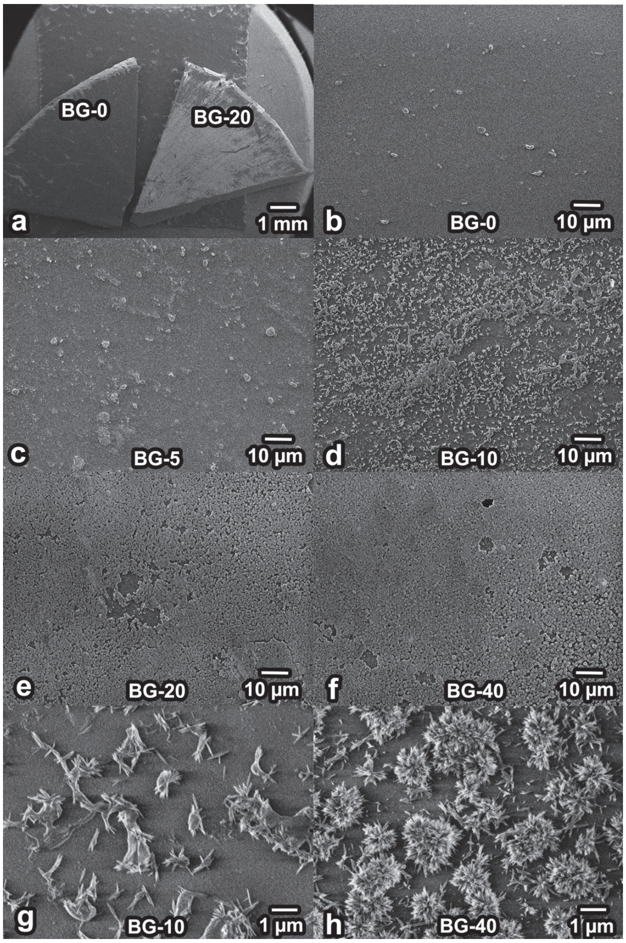

Fig. 5 Micrographs of the BG-series.
Corresponding scale bars are shown within each micrograph.

negative solubility (−4 μg/mm^3) was measured for TEC.

Scanning electron microscopy

Figure 4 shows SEM micrographs of specimen surfaces after aqueous immersion for ACP40, ACP40-Ba10 and ACP40-Si10, as representative for the ACP-containing composites. In ACP40, the surface precipitate comprised larger plate-like crystals (Figs. 4a, b) and finer needle-like crystals (Fig. 4c). Similarly, in ACP40-Ba10, larger plate-like crystals (Figs. 4d, e) were mixed with smaller needle-like crystals (Fig. 4f). In ACP40-Si10, only the needle-like crystals were observed (Figs. 4g, h).

Figure 5 shows SEM micrographs of specimen surfaces after aqueous immersion for the BG-series. Under a low magnification in Fig. 5a, a "clean" surface of the control composite BG-0 is shown in comparison to the hydroxyapatite-covered surface of BG-20. In composites with 0 and 5 wt% of BG fillers, flat and clean composite surfaces with no surface precipitate were observed (Figs. 5b, c), whereas the composites with higher BG amounts precipitated a hydroxyapatite layer which became denser as the BG amount increased (Figs. 5d–f). Figures 5g, h are close-up of the hydroxyapatite crystals in BG-10 and BG-40, respectively. Solitary needle-like crystals were observed in BG-10, which were in the case of BG-40 agglomerated in globular structures. Micrographs of the other non-bioactive composites resembled those of BG-0 and were thus not presented individually.

DISCUSSION

This study investigated the water sorption and solubility of two experimental series of remineralizing composites using the modified ISO 4049 gravimetric method. Gravimetry is the simplest method of quantifying the water uptake and release of soluble components from a cured composite during water immersion[22]. Despite being a rather insensitive and rough method[24], gravimetry is useful in investigations of highly hydrophilic bioactive composites, as its discriminative power increases with higher water sorption and solubility.

Mass changes during water immersion

Water uptake in a composite specimen is a diffusion-controlled process which lasts until the specimen is fully saturated with water[25]. Concurrently with the water uptake, soluble components are eluted from the composite. In plots of the mass change as a function of immersion time, the loss of mass due to solubility is usually masked by the mass increase due to the water uptake, as the effect of the latter process is much larger[23]. Such a behavior is common for commercial composites[26] and was also observed in this study for the composites of the ACP-series, neat resins, BG-0 and BG-5 (Fig. 1). A different behavior was observed for highly soluble materials: BG-10, BG-20, and BG-40, whose curves reached a peak value and then reversed into a mass decrease, approaching the equilibrium below the initial specimen mass (Fig. 1b). The curves of mass change for these materials were dominated by the effect of solubility rather than water sorption. A similar pattern of the decrease in specimen mass (although less pronounced) was reported previously for a self-adhering composite and was explained by monomer elution due to the high hydrophilicity of the resin system and high resin/filler ratio[26]. In this study, the high solubility observed in BG-10, BG-20 and BG-40 could not be ascribed to the elution of residual monomers as neither their counterparts with lower BG amount (BG-0 and BG-5) nor the corresponding neat resin showed an appreciable mass decrease (Fig. 1b). Thus the mass loss of BG-10, BG-20 and BG-40 was likely to be attributed to the long-term release of soluble species from bioactive fillers.

Significantly different times required to reach the equilibrium were observed between the ACP- and BG-series (Table 3). The fact that the ACP-series attained equilibrium after 14 days indicates that their ion release was limited to a very short period of water immersion. This might be because ACP in an aqueous environment undergoes the conversion into hydroxyapatite very rapidly and the ion release from ACP fillers stops once they are transformed into the inert hydroxyapatite phase[7]. The loss of mass in the ACP-series due to the ion release was undetectable in Fig. 1 because it was masked by the immense water sorption. In contrast, the BG-series demonstrated a more sustained release, illustrated by the materials BG-10, BG-20, and BG-40 showing a considerable loss of mass that continued even after 287 days. This gradual dissolution could be the main benefit of BG over ACP, as it indicates a long-term bioactivity. On the other hand, the prolonged loss of mass indicates gradual deterioration of mechanical properties over time[15]. Considerably different patterns of the mass change during immersion among different composite formulations led to the rejection of the first null-hypothesis.

Water sorption

In conventional composites, water uptake occurs predominantly in the methacrylate network, as filler particles are rendered hydrophobic by the silane coating[27]. Thus the amount of absorbed water is inversely related to the filler amount because the latter decreases the percentage of the water-absorbing resinous component[24]. This does not apply to bioactive materials containing highly hydrophilic fillers which may increase water sorption compared to that of the neat resin, as demonstrated by the data for ACP-40 and all of the BG-containing composites (Fig. 3a). The contribution of bioactive fillers to water sorption of a composite material[28] depends on the relative hydrophilicity of bioactive fillers and neat resins. For example, the resin system of the ACP-series was extremely hydrophilic and the addition of 40 wt% of ACP fillers increased the water sorption for only 3%. In contrast, the addition of 40 wt% of BG fillers to the much less hydrophilic resin system of the BG-series resulted in a five-fold increase in water sorption (Fig. 3a). The addition of hydrophobic reinforcing fillers to experimental formulations expectedly decreased the water sorption: ACP-composites with 10 wt% of reinforcing fillers showed modestly reduced water sorption compared to ACP-40, however, their sorption was still twice higher than that of the control composites Ba40 and Ba40Si10. In the BG-series, changing the ratio of bioactive/reinforcing fillers showed a pronounced effect on water sorption: BG-40 had six times higher water sorption than BG-0, which is probably related to the aforementioned low hydrophilicity of the resin system.

The hydrophilicity of a resin system is determined by the presence of polar functional groups[29]. Functional groups such as hydroxyl (–OH) are capable of forming hydrogen bonds with water molecules, thus attracting and retaining water within the polymer network[30]. In this study, HEMA was the most hydrophilic monomer which contains one –OH group per molecule and a relatively low molecular mass (130 g/mol). Bis-GMA contains two –OH groups per molecule, but due to a considerably higher molecular mass (513 g/mol), the number of –OH groups per unit of mass is lower than in HEMA. TEGDMA molecule lacks the –OH groups but shows affinity to water due to its hydrophilic ether linkages[31]. Bis-EMA is a structural analog of the Bis-GMA molecule without the –OH groups, resulting in a more hydrophobic behavior. The relative hydrophilicity and thus water sorption decreases in the order HEMA>TEGDMA>Bis-GMA>Bis-EMA[29]. The vast difference in water sorption between two experimental series (2–10 times higher in the ACP-series) could be attributed to the extremely hydrophilic HEMA-containing monomer system of the ACP-series compared to the Bis-GMA/TEGDMA monomer system of the BG-series (water sorption of 550 and 47 µg/mm^3, respectively). The composition of resin system for the ACP-series was justified by previous studies which showed that it allowed optimal ion release[2,6]. However, an extreme water sorption in the ACP-series is unfavorable for mechanical properties and durability[15]. Moreover, the immense water uptake causes swelling of the restoration, which exerts unwanted expansion forces to cavity walls[32]. In this regard, the BG-series may be expected to show better results in a clinical setting due to its more acceptable water sorption, which was in the range of commercial glass-ionomer cements (50–250 µg/mm^3)[33,34].

In addition to the diffusion through polymeric matrix, water sorption can take place by flowing along the filler/matrix interface[35]. In conventional composites with silanized glass fillers, this occurs as the composite ages and siloxane bonds are hydrolyzed[36], while in experimental bioactive composites with a high amount of hydrophilic unsilanized filler (up to 40 wt%), the interfacial path of diffusion exists from the beginning of immersion[37,38]. As the water sorption advances, micro-cracks are formed at the filler/matrix interface, facilitating further water sorption and degradation of the composite structure[39]. This phenomenon probably contributed to the very high water sorption observed in both experimental series. The contribution of particle size to the interfacial surface area was comparable for ACP and BG fillers, with particle diameters of 2–5 and 4 µm, respectively[16], Table 2.

A study of experimental ACP-based composites which were compositionally similar to the ACP-series investigated in the present study reported the mass increase due to water sorption in the range of 1–5%[40]. A large discrepancy with our findings (Fig. 1) could be explained by methodological differences in evaluating water sorption; the mentioned study measured the water sorption after exposing the composite specimens to an air atmosphere of 75% relative humidity, while in the present study the specimens were immersed in distilled water. The equilibrium was presumably attained at higher amounts of absorbed water in the case of the full immersion compared to the humid air exposure, thus explaining higher water sorption values obtained in the present study.

Solubility

The solubility of conventional composites measured by the ISO 4049 method reflects the release of various soluble components of resin composites. Virtually any composite ingredient (fillers, monomers, photoinitiator system, additives) can be eluted[36,41], but most of the eluted species in conventional composites pertain to unreacted monomers and soluble additives, while filler particles have a minor contribution to the overall solubility[41]. However, in bioactive composites evaluated in this study, most of the soluble species originated from bioactive fillers. This fact is evidenced by much higher solubility values of composites containing ACP or BG fillers than those of the corresponding control materials and neat resins (Fig. 3b): the ACP-containing composites had 2–3 times higher solubility than the control materials (Ba40 and Ba40SI10), while the BG-containing composites had up to 500 times higher solubility than the control (BG-0).

Solubility values of control composites and neat resins reflect the release of residual monomers and thus can be judged by the ISO 4049 requirements. The solubility of the neat resin and control materials of the ACP-series exceeded the upper limit stipulated by the ISO 4049, indicating potential toxicity issues for the highly hydrophilic resin system. In contrast, the neat resin and control material of the BG-series showed much lower solubility, which indicates acceptable biocompatibility of the Bis-GMA/TEGDMA resin system. This remains an issue which requires further investigations.

The significantly higher solubility for the neat resin of the ACP-series than for the neat resin of the BG-series is attributable to the presence of highly diffusive HEMA in the former. It should be noted that such a high solubility occurred despite the high degree of conversion of ACP-composites and comonomers of the same composition[18,42,43]. The ACP-containing composites had considerably higher solubility than the corresponding neat resin, which was not significantly influenced by the admixture of reinforcing fillers. This is in agreement with a previous study which showed that addition of 10 wt% of reinforcing fillers to the ACP-containing composites did not impair their ion release[2,6]. Much higher solubility was observed in composites with 10 wt% or more of BG fillers. In these composites, increasing the amount of BG increased the solubility by two mechanisms: (I) by enhancing the water diffusion through the composite, as mentioned earlier; and (II) by increasing the total amount of reactive glass available for dissolution. Interestingly, the materials BG-0 and BG-5 showed statistically similar solubility, while BG-10 had solubility two orders of magnitude higher. It could be speculated that a certain minimum amount of BG filler exists (between 5 and 10 wt%), which is required for a sufficient water diffusion that would allow the release of soluble species. Due to considerable differences in water sorption and solubility among materials, the second null-hypothesis was rejected.

The calcium and phosphate ion release for the ACP-composites of the same composition as in the current study was demonstrated previously[2,6]. Obtained results were used to calculate the ion activity product with respect to hydroxyapatite, which proved to be more than sufficient to form hydroxyapatite crystals. Although the ion release from the experimental bioactive composites was not evaluated in this study, their bioactivity was demonstrated through the capability to form calcium phosphate precipitate on the surface of specimens immersed in PBS. The precipitate was formed in all of the ACP-containing composites due to their high amount of ACP (Fig. 4), while in the BG-series at least 10 wt% of BG fillers was required in order to form the precipitates (Fig. 5). In the ACP-series, two different crystal morphologies were identified: in ACP40 and ACP40-Ba10, larger plate-like crystals were mixed with smaller needle-like crystals, while in the silica-containing ACP-composites only the needle-like crystals were formed. The plate-like crystals resemble the morphology of dicalcium phosphate dihydrate (brushite), while the smaller needle-like crystals represent hydroxyapatite[2]. The predominance of the needle-like crystals in the silica-containing ACP-composites and the BG-composites with more than 10 wt% of BG can be explained by silica acting as a heterogeneous nucleation spot for hydroxyapatite[2,9].

Previous studies investigated the degree of conversion of the ACP-series[16,18] and BG-series[19]. The curing conditions in the present study were considered to be sufficient for the composites to reach their maximum attainable degree of conversion, which is reported in the aforementioned studies. The ACP-containing composites showed a high maximum degree of conversion (about 80%) with only slight variations due to different reinforcing fillers[18]. In contrast, the maximum degree of conversion in the BG-series declined systematically with higher BG amount: composites with 0, 5, 10, 20 and 40 wt% of BG showed conversions of 81, 80, 78, 72 and 62%, respectively[19]. Thus the higher water sorption and solubility in composites with the higher BG amount could be partly attributed to their lower degree of conversion.

Since hydroxyapatite formation occurred in both the ACP- and BG-containing composites, it is possible that some of the absorbed water was not extracted during the desiccator storage because it remained bound within hydroxyapatite, on the surface of filler particles[31] or within the methacrylate network[26]. The phenomenon of non-extractable water remaining bound within the composite material was previously described as "negative solubility"[44,45] and was observed in this study for the commercial composite TEC. It is thus possible that true solubility values of experimental composites may be even higher than those observed, as the remaining water might have partially masked the loss of mass.

The issue of high water sorption and solubility in bioactive composites

The designing of experimental bioactive composites usually involves trade-offs among opposing properties. A certain level of hydrophilicity is a prerequisite for bioactivity, which is attained at the expense of accelerated degradation of mechanical properties[39] and dimensional instability[24]. While all of the contemporary composites inevitably suffer from the deterioration of mechanical properties when exposed to water[15], this process is expected to be more pronounced in bioactive composites, which have lower initial mechanical properties[2,6].

A potential positive effect of water sorption is a volumetric expansion which could reduce tension on the tooth/restoration interface produced by polymerization shrinkage. Depending on the amount of water sorption, the polymerization shrinkage stress can be partially or fully relieved[46], or even surpassed to produce a net expansion[32]. In commercial composites, the water sorption is usually insufficient and too slow to efficiently counteract the detrimental effects of polymerization shrinkage[15]. However, water sorption in bioactive composites might be finely-tuned to help mitigate the consequences of polymerization shrinkage. The hygroscopic expansion in bioactive composites may work together with the ion release and hydroxyapatite precipitation to aid in the prevention of secondary caries.

CONCLUSIONS

Two experimental composite series with compositions based on previous studies showed water sorption and solubility considerably higher than commercial materials, largely exceeding the threshold values stipulated by the ISO 4049. ACP-based composites showed higher water sorption, but lower solubility than BG-based composites. Monitoring of mass changes up to 287 days revealed that the ACP-composites provided short-term release of soluble species compared to the much longer release observed for the BG-composites. In further studies of experimental bioactive composites, water sorption and solubility should be considered and carefully balanced in order to attain optimal bioactivity while minimizing the unwanted effect on other material properties.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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