
Silica- and zirconia-hybridized amorphous calcium phosphate: Effect on transformation to hydroxyapatite

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Received 14 June 2001; accepted 25 July 2001

Abstract: The goal of this study was to determine the effect that silica and zirconia have on the stability of bioactive amorphous calcium phosphate (ACP) mineral, i.e., in retarding its transformation to hydroxyapatite (HAP). The glass-forming agents, tetraethoxysilane and zirconyl chloride, were introduced individually during the low-temperature preparation of ACP. These hybrid ACPs (Si-ACP and Zr-ACP, respectively) as well as the control, unhybridized ACP (u-ACP), were characterized by X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, specific surface area measurements, and chemical analysis (Ca/PO₄ ratio of the solids) before being dispersed in one of the following four test solutions: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered (pH = 7.40) saline solutions with 0 μg/g fluoride (test solution A1), 1 μg/g fluoride (test solution A2), and 10 μg/g fluoride (test solution A3), or a lactic acid-containing solution (pH = 5.10, adjusted with NaOH; test solution B). Aliquots were taken at predetermined time intervals for solu-

tion Ca and PO₄ analysis. Solids isolated after 30 and 90 min exposure to solution B as well as the final dissolution/transformation products from all four solution experiments were analyzed by Fourier transform infrared spectroscopy and X-ray diffraction. Regardless of the type of experimental solution used, slower conversion to HAP was observed with the hybrid ACPs compared with u-ACP. The retarding effect of the Si or Zr species in the hybridized ACPs is probably due to these ions specifically blocking, by adsorption, potential sites for HAP nucleation and growth. The stability of ACP toward HAP conversion increased in the following order: u-ACP < Si-ACP < Zr-ACP. Hybrid ACP fillers, especially Zr-ACP, could be utilized in applications in which it is desired to enhance performance of composites, sealants, and/or adhesives in preventing demineralization or actively promoting remineralization. © 2001 John Wiley & Sons, Inc.* J Biomed Mater Res 59: 597–604, 2002

Key words: amorphous calcium phosphate; hybridization; silica; zirconia

INTRODUCTION

Calcium phosphates (CaPs) are integrally involved in the formation, progression, and arrest of enamel and dentin caries, and have been utilized in pre-

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 the American Dental Association Health Foundation, or that the material and the equipment identified is necessarily the best available for the purpose.

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Contract grant sponsor: National Institute of Dental and Craniofacial Research; contract grant number: DE13169-02

Contract grant sponsor: American Dental Association Health Foundation

Contract grant sponsor: National Institute of Standards and Technology

ventive and restorative dental materials.¹ CaPs of dental significance are the following: amorphous calcium phosphate (ACP) {approximate compositional formula Ca₃(PO₄)₂ · 3H₂O [2]}, dicalcium phosphate dihydrate (CaHPO₄ · 2H₂O), octacalcium phosphate [Ca₈H₂(PO₄)₆ · 5H₂O], tricalcium phosphate [Ca₃(PO₄)₂], and hydroxyapatite (HAP) [idealized as Ca₁₀(PO₄)₆(OH)₂]. Crystalline HAP is considered to be the final, stable product in the precipitation of calcium and phosphate ions from neutral or basic solutions. However, over the broad range of solution conditions in which precipitation occurs spontaneously, ACP precedes the formation of HAP. ACP is unique among CaPs in that it lacks the long-range, periodic atomic scale order of crystalline materials. Although the uniqueness of ACP has been a subject of considerable interest, of greater relevance to understanding the dynamics of HAP formation by spontaneous precipitation is the instability of ACP in solution. The preparation and properties of ACPs, their kinetic and thermodynamic relationship to crystalline CaPs, their true structural nature

and putative presence and role *in vivo* have been recently reviewed.² The role of ACPs in HAP formation and possible role in biological calcification places them in the mainstream of calcium phosphate chemistry.²⁻⁵

We have recently developed a unique bioactive composite based on a polymer matrix phase derived from ambient polymerization of acrylic monomers and a filler phase consisting of ACP.^{6,7} Pure ACP proved to be an unsuitable filler for this type of composite because of its rapid internal conversion to HAP. However, when the ACP was stabilized by ions that retard its conversion ($P_2O_7^{4-}$ primarily, and to the lesser extent Mg^{2+}), it was possible to take advantage of ACP's relatively high solubility and obtain substantial release of Ca and PO_4 . Moreover, the levels attained in solution from these releases were sustainable, and could promote the recovery of mineral-deficient tooth structures in *in vitro* situations⁸ even though only a small portion of the theoretically available mineral ions is actually released into external solutions.⁷ However, such bioactive composites are relatively weak mechanically because ACP does not act as reinforcing filler in a manner similar to that of silanized glass fillers commonly used in conventional dental composites. To strengthen ACP composites by making ACP more amenable to coupling agents, we have incorporated during ACP synthesis tetraethoxysilane (TEOS) and zirconyl chloride ($ZrOCl_2$) as hybridizing agents with encouraging results.⁹ Preliminary attempts to use conventional silane coupling agents with unhybridized ACP to enhance composite strength have, however, proved unsuccessful.⁷

In this study, the effect of the hybridizing agents TEOS or $ZrOCl_2$ on ACP stability was examined by introducing these agents into the ACP filler during its low-temperature synthesis from aqueous solutions. The stability of the hybrid ACP fillers was assessed by exposing them to different aqueous environments and comparing the results with the dissolution/conversion properties of unhybridized ACP fillers. The results of this study show that slower conversion to HAP occurred with hybridized ACPs compared with unhybridized ACP. This property may make hybridized ACP fillers particularly useful in composites aimed at preventing tooth demineralization and/or actively promoting remineralization of damaged tooth structures because of their potential for enhanced sustained release of calcium and phosphate ions.

MATERIALS AND METHODS

Synthesis and characterization of ACP fillers

ACP fillers were synthesized using the protocol of Eanes et al.¹⁰ Unhybridized, P_2O_7 -stabilized ACP (u-ACP) was

precipitated instantaneously in a closed system (under CO_2 -free N_2 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 between 10.5 and 11.0.

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 a 0.25 mol/L $ZrOCl_2$ solution and the $Ca(NO_3)_2$ solution to the Na_2HPO_4 solution. The TEOS solution was designed to prevent premature gelation of TEOS. The amounts of the two hybridizing agent solutions were adjusted to achieve mole ratios of $ZrOCl_2:Na_2HPO_4$ and $TEOS:Na_2HPO_4$ equal to 0.1. The reaction pH was 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 was washed with ice-cold ammoniated water, freeze-dried, and lyophilized.

The amorphous state of lyophilized solids was verified by powder X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FTIR). The XRD profiles of the powdered samples were recorded in the 4° to 60° 2 θ range with $CuK\alpha$ radiation ($\lambda = 1.54 \text{ \AA}$) using a Rigaku X-ray diffractometer (Rigaku/USA Inc., Danvers, MA) operated at 40 kV and 40 mA. The samples were step-scanned in intervals of 0.010° 2 θ at a scanning speed of 1.000°/min.

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) purged with dry CO_2 -free air. Specimen pellets were made of 1 to 2 mg of the solid and 400 mg of KBr.

Surface morphology of u-ACP and hybrid ACPs fillers was determined by scanning electron microscopy (SEM). Powders were placed onto an SEM stub, sputter-coated with gold, and examined with a JSM-5400 instrument (JEOL Inc., Peabody, MA).

The specific surface area (SSA) of the fillers was determined by the triple point method¹¹ using the Quantasorb sorption system. N_2 was used as the adsorbate gas and He as the inert nonadsorbable carrier. N_2 adsorption and desorption on the powder surface were monitored by measuring the change in the thermal conductivity of the gas mixture. The SSA of the samples (area divided by mass; m^2/g) was determined from three replicate measurements of each sample.

The Ca/PO_4 molar ratio of the fillers after dissolution in HCl was calculated from solution Ca and PO_4 values. Ca concentration was determined by atomic absorption spectroscopy with a Perkin-Elmer No. 603 spectrophotometer (Perkin-Elmer, Norwalk, CT) using an air-acetylene flame and the 422.7-nm wavelength line. Standard Ca solutions were prepared from weighed amounts (mass fraction $\pm 0.1\%$) of NIST Standard Reference $CaCO_3$ (dried at 250°C for 2 h). Both standard Ca solutions and the unknown samples contained 1000 $\mu g/g$ $LaCl_3$. PO_4 was determined as a blue molybdate complex (in acidified ammonium molybdate solution containing ascorbic acid and a small amount of antimony¹² using a Carey Model 219 spectrophotometer (Varian Analytical Instruments, Palo Alto, CA) and a wavelength of 882 nm. The PO_4 standards were prepared from weighed

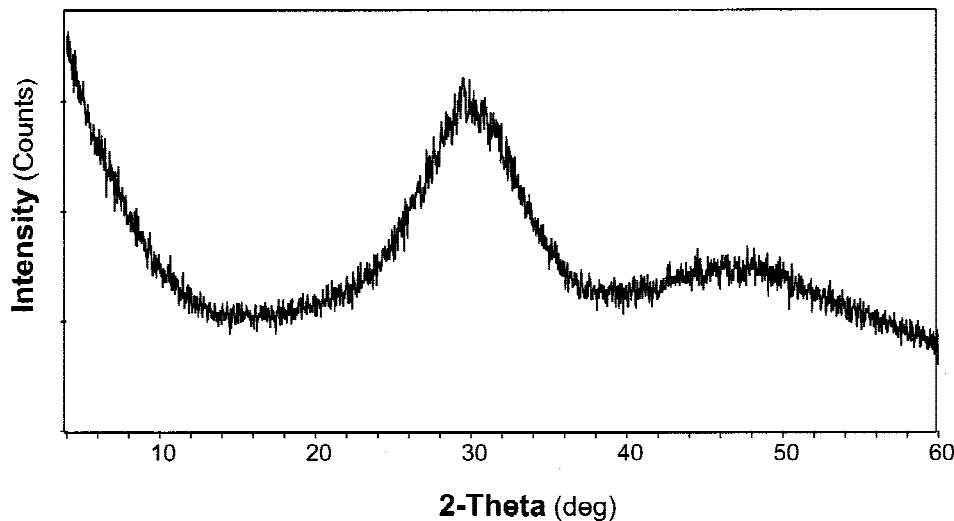


Figure 1. XRD pattern typical of the ACP fillers used in the study.

amounts (mass fraction $\pm 0.1\%$) of KH_2PO_4 (dried at 105°C for 2 h). Absorbances of both standards and unknowns were measured at the same aging time (1 h) by observing color development in the sample cell versus an H_2O blank.

Dissolution kinetic experiments

The dissolution/transformation of powdered ACP fillers was examined at 37°C in four different solutions at two different pH levels: (a) in N-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered (pH = 7.40) 240 mOsm/kg saline solutions containing 0 $\mu\text{g/g}$ F (test solution A1), 1 $\mu\text{g/g}$ F (test solution A2), or 10 $\mu\text{g/g}$ F (test solution A3), and (b) in a 0.1 mol/L lactic acid solution (pH = 5.10, adjusted with NaOH) containing a mass fraction of 1% hydroxymethylcellulose; modified after Tantbirojn et al.¹³ (test solution B). The release of mineral ions was followed upon dispersion of approximately 800 mg of the solid in 100 mL of the test solutions specified above. Aliquots for Ca (atomic absorption spectroscopy) and PO_4 (UV/VIS spectrophotometry) analysis were taken at predetermined time intervals: 0.5, 1, 2, 5, 24, 48, and 72 h.¹⁴ Solids isolated after 0.5 and 1.5 h of exposure to the lactic acid solution as well as all solids isolated after 72 h of exposure to all test solutions were examined by XRD and FTIR.

Incorporation of fluoride into solids exposed to test solutions A2 and A3 for 72 h was assessed by measuring the fluoride content (fluoride ion-selective electrode model 94-09; Orion Research Inc., Boston, MA) upon dissolving the solids in HCl. From an experimental curve relating electrode potential (mV) and fluoride concentration ($\mu\text{g/g}$ or mmol/L), a calibration curve was generated for the range of fluoride concentrations between 0.01 and 1 mmol/L.

Statistical analysis

Experimental data were analyzed by multi-factorial ANOVA ($\alpha = 0.05$). To determine significant differences be-

tween specific groups, all pair-wise multiple comparisons (two sample *t* test assuming unequal variances; confidence interval 95%) were performed.

RESULTS

A typical XRD pattern of the ACP fillers used in this study is given in Figure 1. Broad peak features reveal an amorphous material that lacks repeating three-dimensional order. The FTIR spectrum (Fig. 2) showed two wide bands typical for noncrystalline CaPs: PO_4 stretching in the region 1200 cm^{-1} to 900 cm^{-1} , and PO_4 bending in the region 630 cm^{-1} to 500 cm^{-1} . There were no observable differences in either XRD or FTIR of u-ACP and hybrid fillers. Representative SEM photographs of u-ACP, Si-ACP, and Zr-ACP are provided in Figure 3(a-c). Both the unhybridized and the two hybridized ACPs were highly polydispersed with no distinguishable textural/morphological features. However, SSA analysis revealed significant differ-

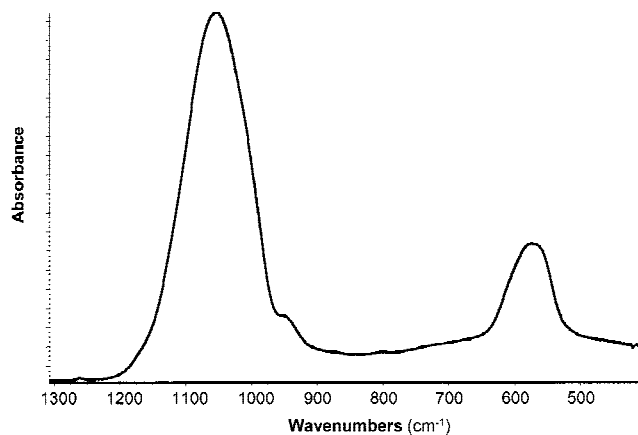


Figure 2. FTIR spectrum typical of the ACP fillers used in the study.

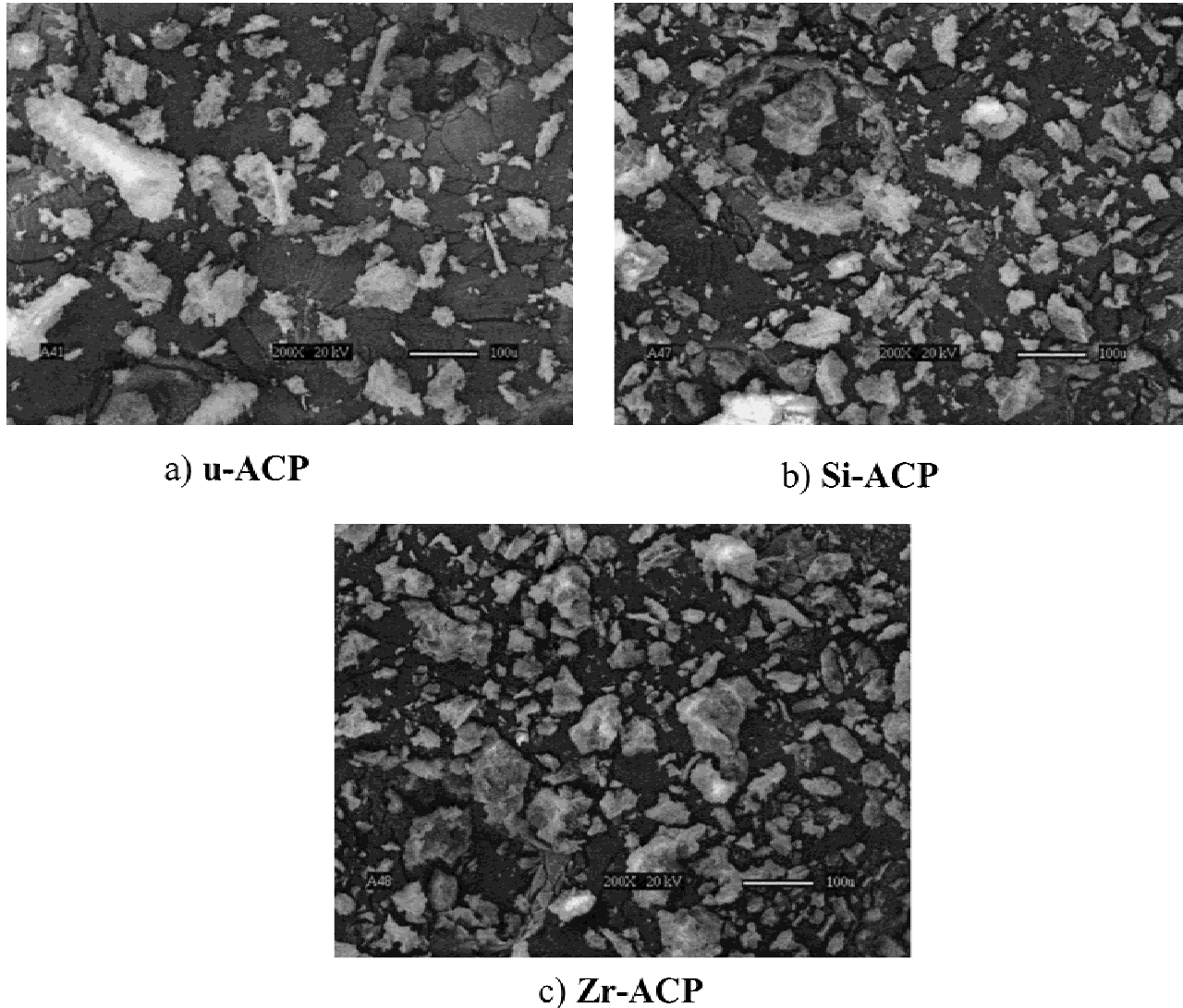


Figure 3. SEM photographs of the fillers utilized in the study: (a) u-ACP, (b) Si-ACP, and (c) Zr-ACP.

ences ($p < 0.05$) between u-ACP, Si-ACP, and Zr-ACP (Table I). The Ca/PO₄ molar ratios of powdered fillers (Table II), however, showed no significant difference between u-ACP and Si-ACP, whereas being very high for the Zr-ACP.

Dissolution of u-ACP, Si-ACP, and Zr-ACP powders dispersed in test solutions A1, A2, A3, or B, showed different kinetic profiles that were dependent on both the type of filler examined and the type of test solution. Kinetic changes in the solution Ca/PO₄ ratio resulting from the dissolution of u-ACP, Si-ACP, and Zr-ACP powders in different test solutions are given in Figure 4(a–c), respectively. Practically no Ca was released from u-ACP dispersed in saline (test solution A1) because of rapid conversion of u-ACP into HAP [Fig. 4(a)]. The conversion of u-ACP was somewhat retarded in F-containing media—measurable Ca levels were registered at 0.5 h and 1 h after dispersing the solid in test solutions A2 and A3, respectively. Si-ACP

and Zr-ACP were, generally, less prone to conversion into HAP as evidenced from Ca/PO₄ profiles obtained in these systems [Figs. 4(b,c)]. The Ca/PO₄ values were initially 2 to 3 times higher in lactic acid-containing media (test solution B) than those in test solutions A1–A3 and remained much higher throughout the course of dissolution process.

XRD patterns of the solids isolated at different time

TABLE I
Specific Surface Area (SSA) of the Fillers Used in the Study

| Filler | Hybridizing Agent | SSA (g/m ²) | |
|--------|--------------------|-------------------------|----------------------|
| | | Mean Value | Standard Uncertainty |
| u-ACP | None | 18.60 | 1.16 |
| Si-ACP | TEOS | 40.37 | 1.94 |
| Zr-ACP | ZrOCl ₂ | 33.34 | 2.44 |

Number of repetitive experiments in each group = 3.

TABLE II
Ca/PO₄ Mole Ratio of the Fillers Used in the Study

| Filler | Hybridizing Agent | Ca/PO ₄ Molar Ratio | |
|--------|--------------------|--------------------------------|----------------------|
| | | Mean Value | Standard Uncertainty |
| u-ACP | None | 1.50 | 0.09 |
| Si-ACP | TEOS | 1.59 | 0.06 |
| Zr-ACP | ZrOCl ₂ | 1.91 | 0.09 |

Number of repetitive experiments in each group = 5.

intervals from test medium B are presented in Figure 5(a–c). Both types of hybridized fillers converted more slowly to HAP than did u-ACP, with the retarding effect being more pronounced with Zr-ACP compared with Si-ACP. FTIR data shown in Figure 6 provide additional support for the above conclusion. The appearance of distinctive PO₄ bands in the ν_1 and ν_3 region (1100 cm⁻¹ and 1033 cm⁻¹) and in ν_4 region (606 cm⁻¹ and 550 cm⁻¹), typical of crystalline HAP, was significantly delayed in the system Zr-ACP/lactic acid. However, fluoride analysis of the solids isolated after 72 h dissolution in test solutions A2 and A3 showed that between 35 to 41% and approximately

28%, respectively, of initial 1 $\mu\text{g/g}$ and 10 $\mu\text{g/g}$ solution fluoride became incorporated into transformed u-ACP and Si-ACP. However, in Zr-ACPs, only about 0.2% of initial solution fluoride (in either A2 or A3) became incorporated into solids during dissolution.

DISCUSSION

ACPs prepared and characterized in this study showed practically no difference in either their long-range (XRD data; Fig. 1) or short-range (local PO₄ environment, FTIR data; Fig. 2) structure, or in their morphologies (SEM data; Fig. 3). However, the apparent absence of observable crystalline features in the ACPs does not rule out the possibility that the ACPs may have had a well-defined local structural unit. Previous studies on the compositional constancy of ACP over a wide range of preparative solution conditions suggest the existence of such a core structure.^{15–19} Betts and Posner^{15,16} proposed that the defining structural unit of ACP is a spatial subset that has a

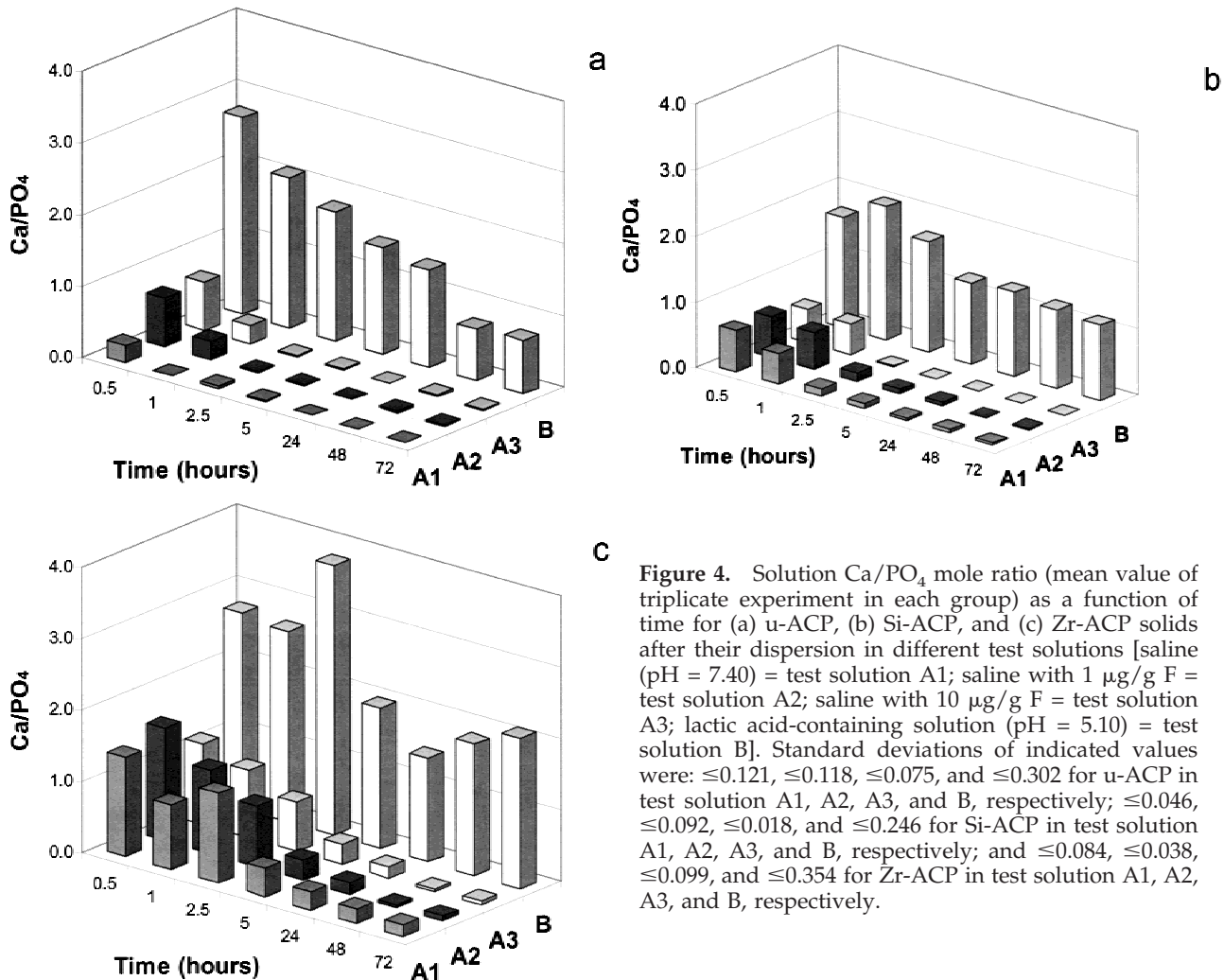


Figure 4. Solution Ca/PO₄ mole ratio (mean value of triplicate experiment in each group) as a function of time for (a) u-ACP, (b) Si-ACP, and (c) Zr-ACP solids after their dispersion in different test solutions [saline (pH = 7.40) = test solution A1; saline with 1 $\mu\text{g/g}$ F = test solution A2; saline with 10 $\mu\text{g/g}$ F = test solution A3; lactic acid-containing solution (pH = 5.10) = test solution B]. Standard deviations of indicated values were: ≤ 0.121 , ≤ 0.118 , ≤ 0.075 , and ≤ 0.302 for u-ACP in test solution A1, A2, A3, and B, respectively; ≤ 0.046 , ≤ 0.092 , ≤ 0.018 , and ≤ 0.246 for Si-ACP in test solution A1, A2, A3, and B, respectively; and ≤ 0.084 , ≤ 0.038 , ≤ 0.099 , and ≤ 0.354 for Zr-ACP in test solution A1, A2, A3, and B, respectively.

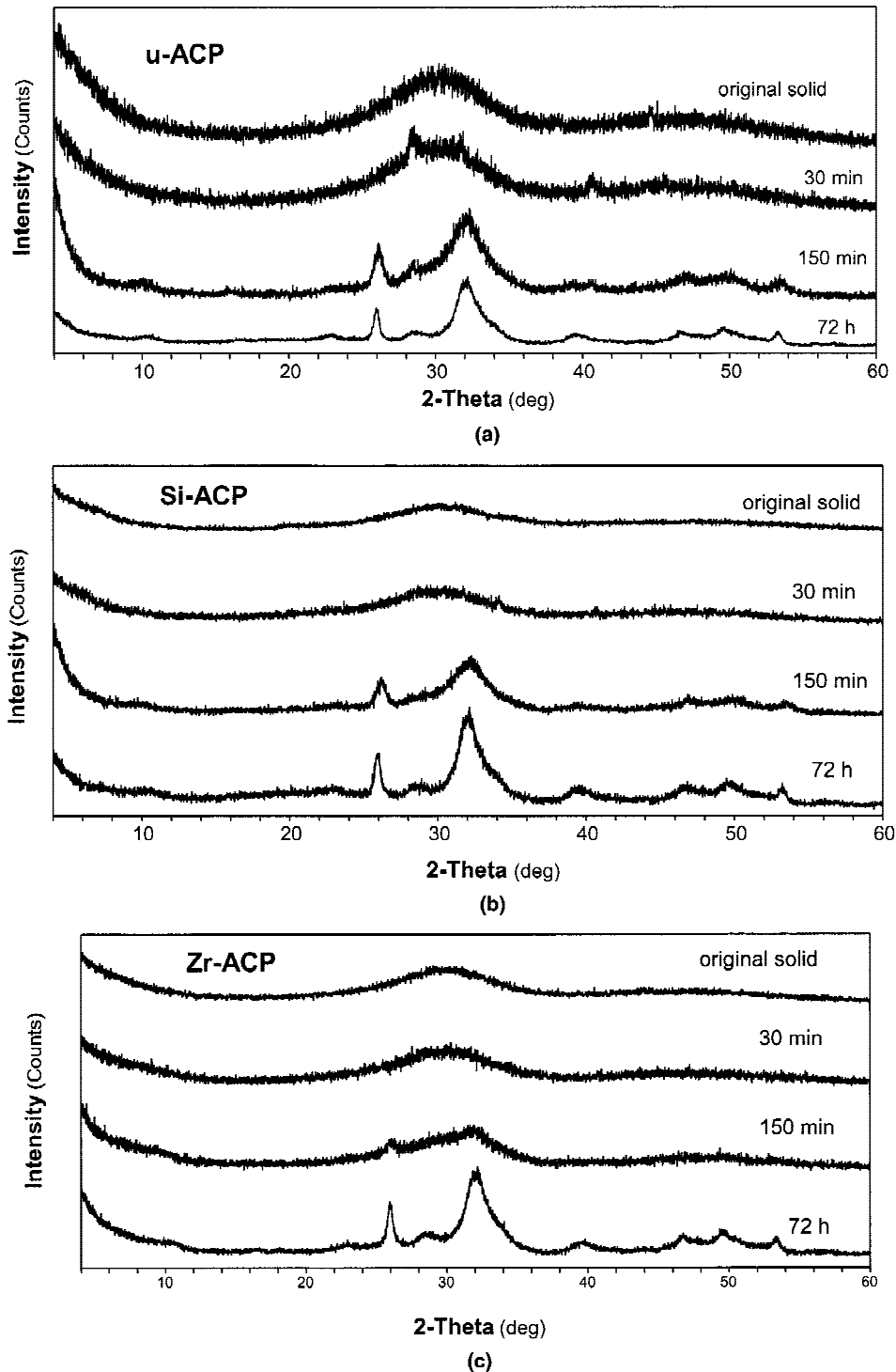


Figure 5. XRD patterns of the solids isolated at different time intervals from lactic acid-containing test solution B: (a) u-ACP, (b) Si-ACP, and (c) Zr-ACP.

$\text{Ca}_3(\text{PO}_4)_2$ composition and these subsets are randomly clustered in ACP with water filling the intervening spaces. Whereas u-ACP and Si-ACP with the mean Ca/PO_4 mole ratio of 1.50 and 1.59, respectively, might satisfy the above model, Zr-ACP with the mean Ca/PO_4 mole ratio of 1.91 requires a different explanation. The co-precipitation of an amorphous, highly

insoluble Zr phosphate salt that occurs parallel to ACP formation might explain the observed high Ca/PO_4 mole ratios of PO_4 -depleted Zr-ACPs. This assumption is based on the fact that, upon dissolving the Zr-ACPs in HCl, a small amount of the solid remained undissolved. When this solid was isolated, dried, and analyzed by XRD and FTIR, it showed structural fea-

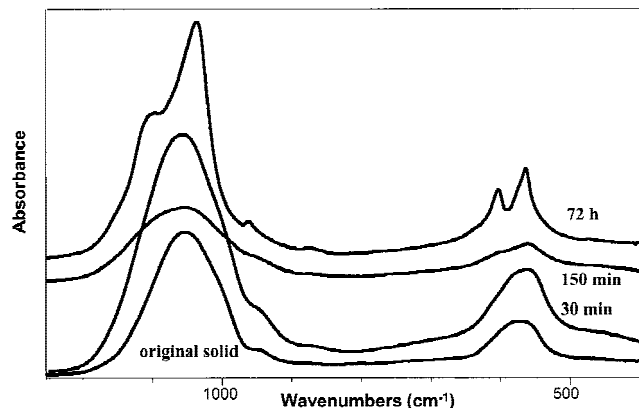


Figure 6. FTIR spectra of Zr-ACP filler upon its exposure to test solution B. Time intervals as indicated.

tures of an amorphous material. The existence of amorphous Zr phosphates of somewhat variable compositions and properties as well as the relationship between the amorphous and crystalline Zr phosphates has been well documented in the literature.^{20,21}

Another interesting finding is the difference in SSAs of the examined ACP solids. The SSAs of both types of hybrid ACPs were considerably higher than the SSA of u-ACP (Table I). The hybridizers may have decreased ACP particle size or degree of agglomeration. This conclusion, however, could not be confirmed by SEM examination. It was, however, found that composites based on hybrid ACP have improved mechanical properties.^{22,23} It is possible that the higher SSA of the fillers in those composites could account for the observed increase in mechanical strength due to more intimate filler/polymer interactions.

Generally, the relatively high solubility of ACP and its ready conversion to HAP in aqueous environments might pose limitations in dental applications in which structural and/or chemical stability are desired. However, these same properties make ACP suitable as a mineralizing agent. As shown in this study, conversion of ACP solids into HAP upon immersion in all the experimental test solutions occurred more slowly when hybridized ACPs were used (as evidenced by solution analysis, XRD, and FTIR; Figs. 4–6). The stability of the examined ACPs, even under the most unfavorable lactic acid conditions decreased in the following order: Zr-ACP > Si-ACP > u-ACP. The mechanism by which Zr, and to a lesser extent Si, retard the conversion of hybrid ACPs into HAP is probably controlled by the adsorption of hybridizing ions at active HAP nucleation/growth sites, thereby affecting the exchange rate of calcium and phosphate ions between the solid phase and the solution.²⁴ The finding that Zr-ACPs, contrary to u-ACP and Si-ACP, practically did not incorporate fluoride during the course of dissolution/transformation remains unexplained.

Slower internal conversion into HAP is a desirable

property of ACP fillers used in composites designed for remineralizing applications, because HAP is not sufficiently soluble to be effective as a remineralizing agent. We have previously reported²⁵ that $P_2O_7^{4-}$ -stabilized ACP was the most effective filler for establishing elevated Ca and PO_4 ion concentrations upon soaking methacrylate-based polymeric composite disks in buffered saline solutions. Results of this study suggest that hybrid ACPs, (Zr-ACP or Si-ACP) should be preferred in applications in which protection against mineral loss and/or restoration of mineral are desirable, because of the enhanced stability of such ACPs. When compounded with appropriate polymeric resins, bioactivity of hybrid ACPs, primarily Zr-ACP, may be particularly useful in enhancing the performance of composites, sealants, and adhesives by preventing tooth demineralization and by actively promoting remineralization. The extended, time-releasing characteristics of these ACP-based materials may be especially advantageous in applications designed to prevent demineralization in orthodontically treated teeth or promote remineralization of white spots and carious lesions.

It has been well documented in the literature that a common loci of mechanical failure in most composites is the filler/polymer interface, especially if the interfacial interaction of the two phases is poor.²⁶ To enhance the integrity of glass-filled composite, the mineral filler surface is commonly treated with coupling agents, such as organofunctional silanes.²⁷ This improves the composite's mechanical properties by promoting the adhesion between mineral substrate and polymer matrix. In this way, the siliceous filler can be bonded to the polymer matrix, and thereby, reinforce the weaker organic phase of the composite and provide a mechanism for stress transfer from the lower modulus matrix to higher modulus filler phase. Introduction of hybrid fillers may be, therefore, beneficial from that point of view as well because they have the potential to promote improved filler/polymer interaction.

CONCLUSIONS

The results of this investigation demonstrate the possibility to enhance the remineralizing potential of bioactive ACP fillers by introducing Zr or Si elements during the low-temperature synthesis of these hybridized fillers. Utilizing hybrid ACP fillers in methacrylate resin composites can enhance both the duration of calcium and phosphate release and also may improve mechanical properties, and thus extend the range of their applications as dental materials.

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