JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 7 (1996) 673–682

Low temperature synthesis of a self-assembling composite: hydroxyapatite–poly[bis(sodium carboxylatophenoxy)phosphazene]

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The present study was undertaken to investigate the low temperature formation of a hydroxyapatite–polyphosphazene polymer composite likely to be biocompatible. The temperature range studied (25 to 60°C) was selected to bracket physiological temperatures. The composite precursors consisted of $CaHPO_4 \cdot 2H_2O$, $Ca_4(PO_4)_2O$, and poly[bis(sodium carboxylatophenoxy)phosphazene]. The results indicate that a synergistic relationship exists in the formation of a polyphosphazene network and hydroxyapatite (HAp) matrix phase during composite synthesis. Calcium from the HAp precursors participates in the formation of a Ca crosslinked polymeric network which influences the rate of HAp formation and its morphology. The mechanistic paths taken during composite formation were followed by determining variations in the concentration of species in solution (at physiological temperature), rates of heat evolution, and microstructural development. These analyses indicate that the polymer controls the kinetics of hydroxyapatite formation and the composite microstructure. Low reaction temperatures and a high proportion of polymer facilitate the formation of a highly interconnected composite. The presence of the polyphosphazene allows a metastable calcium phosphate solution to persist for extended periods prior to the formation of hydroxyapatite. The degree of supersaturation and the length of the induction period increase with an increase in polyphosphazene content. The temperature dependence of these induction periods obeyed an Arrhenius relationship.

1. Introduction

There is growing interest in the development of low temperature routes to the synthesis of composites that have properties superior to those of their individual constituents. Composites that form at near-net shape under ambient conditions are of particular interest in biomedical disciplines such as orthopedics, orthodontics, and maxillofacial and cranial reconstruction. Typical materials such as ceramic and metallic preform plugs, filler particulates such as hydroxyapatite and bioglass, and cements such as poly(methyl methacrylate) (PMMA) and glass ionomers are presently being used or tested *in vivo*. With the exception of glass ionomer cements which bond to native tissue, these materials are either mechanically attached through the use of PMMA or are placed within the defect or excavation site without attachment. This results in a relatively weak interface between the prosthetic material and the native tissue.

More recently a calcium phosphate cement has been developed that forms under physiological conditions and has the ability to generate both mechanical and chemical bonds to the surrounding native tissue [1–4]. Reaction occurs between the basic calcium phosphate, tetracalcium phosphate, $Ca_4(PO_4)_2O$ (TetCP), and an acidic calcium phosphate such as monetite, $CaHPO_4$ (DCP); brushite, $CaHPO_4 \cdot 2H_2O$ (DCPD); or monocalcium phosphate monohydrate, $Ca(H_2PO_4)_2 \cdot H_2O$ (MCPM). This cement is initially a formable putty which later hardens into a monolithic form composed of hydroxyapatite (HAp). HAp does not have a fixed composition, and Ca/P molar ratios of HAp formed at low temperature have been reported to range between about 1.33 and 1.67 [5, 6]. In the present study HAp having a Ca/P molar ratio of approximately 1.5 was formed by reaction between TetCP and DCPD.

While the rheological properties of calcium phosphate cements are amenable to a large number of *in vivo* applications, their mechanical properties are limited by brittleness and porosity. Polymeric materials such as gelatin [7], collagen [8], polyacrylic acid

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(PAA) [9], and initiator activated resins [10] have been incorporated into these cements in an attempt to improve their fracture properties and have shown variable results. In the present study we have investigated the effects on the kinetics of HAp formation, the variations in solution chemistry during composite formation, and the composite microstructure resulting from the incorporation of poly[bis(sodium carboxylatophenoxy)phosphazene]. This polymer was selected because of its biocompatibility, solubility in aqueous media, and ability to form a crosslinked hydrogel with multivalent cations (see Fig. 1) [11–14].

Our objective was to establish routes to the formation of a three-dimensionally connected reinforcing polymeric network interdigitated by hydroxyapatite. The rate of composite formation must occur within a time period that is clinically relevant for composite formation to be utilized in *in vivo* applications. Thus, the kinetics of composite formation were determined by measuring the rate of heat evolution by isothermal calorimetry. Because the processes of composite formation cannot be so aggressive as to damage adjacent tissue, the variations in solution chemistry were determined. The data obtained from calorimetry and those obtained by analyses of the solutions were used to establish the mechanistic paths of composite formation. These data, along with microstructural observations, were also used to establish the extent of a synergistic relationship between the formation of an extended polymer structure and the formation of HAp. Establishing this relationship allows control of application-dependent properties, such as *in vivo* resorbability and mechanical properties.

2. Experimental procedure

2.1. HAp precursor synthesis

TetCP was synthesized by a high temperature solid state reaction between reagent-grade DCP (Fisher Scientific) and precipitated calcium carbonate (Fisher Scientific). These particulate reactants were mixed in equimolar quantities, milled to obtain a homogeneous mixture, and fired at 1400 °C for 2 h to produce TetCP which was then milled to a mean particle size of 2–3 μm. DCPD (Aldrich) and TetCP were mixed in a 2-to-1 molar ratio. These react in water to form calcium-deficient HAp as follows (see Reaction 1):

$$3CaHPO_4 \cdot 2H_2O + 1.5Ca_4(PO_4)_2O \rightarrow Ca_9(HPO_4)(PO_4)_5OH \quad \text{(Reaction 1)}$$

Phase purities of HAp precursors and the extents of their reactions during composite formation were determined using X-ray diffraction analysis. Diffraction data were collected using an automated Scintag powder diffractometer with a CuK_α radiation source and a nickel filter. Diffraction patterns were collected using a scan rate of 2° 2θ and a step size of 0.02° 2θ.

2.2. Polymer synthesis

Hexachlorocyclotriphosphazene (Ethyl) was purified by recrystallization from heptane and sublimation at 50 °C (0.05 mm Hg). Poly(dichlorophosphazene) was prepared from hexachlorocyclotriphosphazene as described previously [15]. Tetrahydrofuran (Omnisolv) was dried over sodium benzophenone ketyl and was distilled under nitrogen before use. Sodium hydride (60% dispersed in mineral oil) and potassium *tert*-butoxide (Aldrich) were used as received. Propyl 4-hydroxybenzoate (Aldrich) was purified by recrystallization from methylene chloride.

The sodium salt of propyl 4-hydroxybenzoate was prepared by the slow addition of the ester (77.7 g, 4310 mmol) in THF (500 ml) to sodium hydride (13.8 g, 60% dispersion) in THF (800 ml). A solution of poly(dichlorophosphazene) (10.0 g, 862 mmol) in THF (800 ml) was added slowly to the salt solution at reflux. The refluxing was continued for 72 h. The resultant poly[bis(aryloxy)phosphazene] was purified by precipitation into deionized water (three times), hexane (twice), and ethanol (once) A solution of potassium *tert*-butoxide deprotection agent (58.0 g, 517 mmol) in THF (750 ml) was cooled to 0 °C with an ice bath, water (3.0 ml) was then added slowly. A solution of the purified poly[bis(aryloxy)phosphazene] (10.0 g, 24.8 mmol) in THF (750 ml) was added slowly over a 15 min period. After 5 additional minutes at 0 °C, the solution was allowed to warm to room temperature and was stirred for 48 h. This polymer was purified by dialysis against deionized water (3 days) and then precipitated by acidification with hydrochloric acid to form the caboxylic acid polymer which was collected by filtration. The carboxylic acid polymer was treated with sodium hydroxide to prepare the sodium salt derivative (poly[bis(sodium carboxylatophenoxy)-phosphazene] see Fig. 1), purified by dialysis against deionized water to remove excess sodium hydroxide, and dried at 115 °C.

2.3. Reaction kinetics

Kinetics of composite formation were determined by isothermal calorimetry. The methods used have been described in detail elsewhere [16]. Isothermal calorimetry was performed to determine the rates of HAp formation in deionized water and in polyphosphazene solutions at (DCPD +TetCP)-to-polymer weight ratios of 20-to-1, 10-to-1, and 5-to-1. A summary of the precursor weights and reaction temperatures is shown in Table I. These proportions were chosen to produce composites that contained approximately 60% porosity. In the absence of polymer, the HAp precursors and the water were separately equilibrated to the selected temperature of reaction before mixing. When thermal equilibration was achieved, the water was injected onto the HAp precursor powder. Therefore, the evolution of heat from the time of mixing was determined. This was not possible when polymer was present. In these experiments the polymer was dissolved in deionized water (see Table I) and mixed with the HAp precursor powder for 1.5 min in a sealed stainless steel 10 ml Sorvall container designed for high shear mixing and tissue fragmentation. The solutions were kept cool to inhibit premature reaction by immersing the mixing container in an ethanol–ice bath. In preparation for isothermal

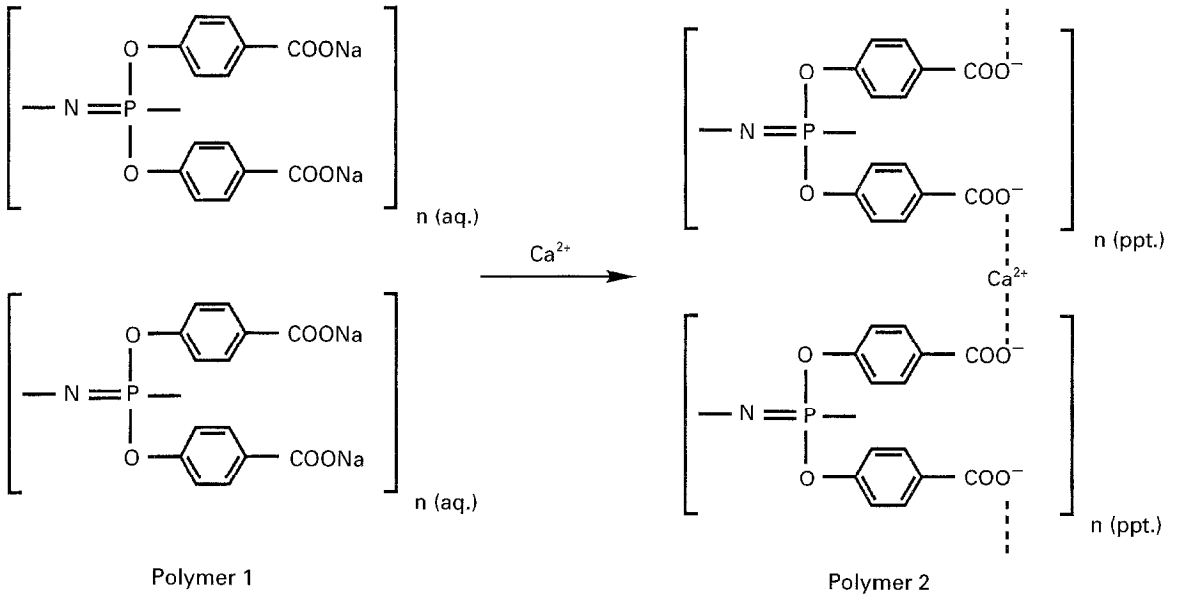

Figure 1 Schematic representation of salt bridge of poly[bis(sodium carboxylatophenoxy)phosphazene] by calcium which results in its precipitation.

TABLE I Precursor weights and reaction temperatures for isothermal calorimetry study

HAp precursor-to-polyphosphazene weight ratio	Weight of D.I. H_2O (g)	Weight of polyphosphazene (g)	Weight of HAp precursor (g)	Reaction temperatures (°C)
Pure mineral	3.00	0.000	3.000	25.0, 30.0, 33.5, 37.4, 45.0, 50.0, 60.0
20-to-1	1.51	0.150	3.000	30.0, 37.4, 45.0, 50.0, 60.0
10-to-1	1.70	0.300	3.000	30.0, 33.5, 37.4, 45.0, 50.0, 55.0, 60.0
5-to-1	1.90	0.600	3.000	30.0, 37.4, 45.0, 50.0, 50.0

calorimetry the slurry was poured into a copper calorimeter cup and was weighed. Samples containing polymer required approximately 20 min to reach thermal equilibrium. This did not affect the analysis of the kinetic data because the reaction mixtures containing polymer did not undergo appreciable reaction during the period of equilibration due to an induction period.

Once thermal equilibration had been achieved, heat evolution was determined by measuring the voltage output as a function of time from thermopiles surrounding the calorimeter cup. A datum point was acquired every 30 s and was stored on a microprocessor. Using a thermoelectric coefficient determined by calibration, these voltages were converted to heats of reactions. Total heat evolution was determined by integrating the rate data using the trapezoidal rule, and plots of heat evolved are presented in kJ/mole of HAp formed.

2.4. Solution chemistry

Analysis of the variations in the solution during composite formation were carried out by measurement of pH and analysis of filtered solutions for Na, Ca, and P by DC plasma emission spectroscopy. Determination of the effects of the presence of the polyphosphazene on pH and on the Na, P and Ca concentrations were performed at a liquid-to-solids (DCPD +TetCP) weight ratio of 5-to-1 and at 37.4 °C. Variations in solution chemistry were determined in the absence of polymer and when the HAp precursor-to-polymer weight ratios were 20-to-1 and 5-to-1. Deionized water or polymer solutions were placed into a 400 ml jacketed reaction vessel at 37.4 °C and were thermally equilibrated. HAp precursor powders were added to the stirring solutions. The solutions were purged continuously with argon gas to minimize exposure to carbon dioxide.

The variation in pH was determined every 20 s using a calibrated combination glass electrode interfaced to an Orion 920 pH meter. Aliquots were extracted at selected times and were filtered through either 0.22 μm Millipore GV filters (polymer absent) or through 2 μm Whatman multigrade GMF 150 graded density microfibre glass filters (polymer present). The latter were used because the polymer solution would not pass through submicron membrane filters. Filtered aliquots were acidified using hydrochloric acid to prevent the precipitation of Ca-, P-, or Na-containing species and to precipitate the polymer. These solutions were filtered though 0.22 μm PTFE syringe filters (Gelman). Filtrates were analysed for Na, Ca, and P by DC plasma emission spectroscopy. Selected solid samples were quenched in acetone to arrest

further reaction and were subjected to X-ray diffraction analysis.

2.5. Microstructure

Conventional electron microscopy was performed on selected samples to investigate the influences of both the reaction temperature and the presence of the polyphosphazene on HAp morphology and to observe the microstructural features of the composite with respect to the polymer-HAp spatial relationship. Microstructure was observed using the first stage of an ISI-DS 130 dual stage scanning electron microscope. All samples were freeze-dried and coated with gold prior to imaging.

3. Results and discussion

3.1. Composite formation kinetics

If composites are to form *in vivo*, the rates of reaction must be clinically acceptable. The objective of measuring the rates of composite formation was to determine the influences of varying quantities of polymer on the kinetics and to establish the rate-limiting mechanism(s) of the reaction. Heat evolution during reaction at constant temperatures between 25 °C and 60 °C were determined using isothermal calorimetry and were all exothermic. Fig. 2 shows the heat evolution curves at selected reaction temperatures during HAp and HAp-polymer composites formation. These sigmoidal curves are typical of hydrolysis reactions and show the following features: (1) an initial period of slow reaction when the polyphosphazene is present (induction period) and (2) one or two periods of reaction where the rate of heat evolution is relatively constant until the reactants have been largely consumed. The rates of these reactions are highly sensitive to the reaction temperature, which indicates that composite formation is highly thermally activated.

In the absence of the polyphosphazene (Fig. 2a) HAp formation below 45–50 °C involves two mechanistic steps as indicated by the two slopes of the heat

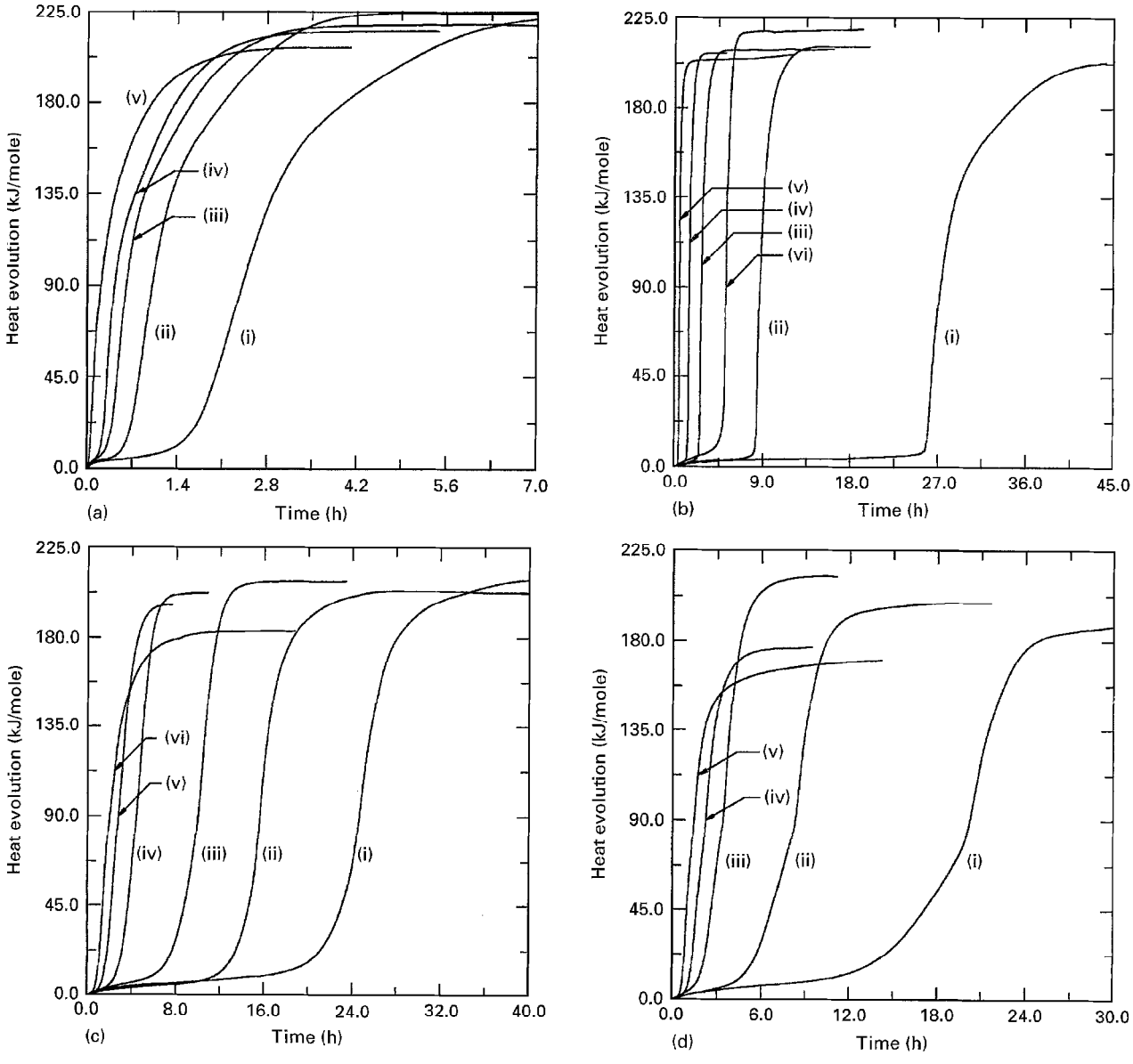

Figure 2 Heat evolution during HAp formation in (a) deionized water [(i) 25 °C, (ii) 30 °C, (iii) 33 °C, (iv) 37.4 °C, (v) 50 °C]; and for HAp/polyphosphazene composites with (DCPD +TetCP)-to-polyphosphazene weight ratios of (b) 20-to-1 [(i) 30 °C, (ii) 37.4 °C, (iii) 45 °C, (iv) 50 °C, (v) 60 °C], (vi) 37.4 °C with a liquid-to-(DCPD +TetCP) weight ratio of 1.9-to-3.0; (c) 10-to-1 [(i) 30 °C, (ii) 33 °C, (iii) 37.4 °C, (iv) 45 °C, (v) 50 °C, (vi) 65 °C]; and (d) 5-to-1 [(i) 30 °C, (ii) 37.4 °C, (iii) 45 °C, (iv) 50 °C, (v) 55 °C].

curves. Above this temperature range the reaction occurs by a single mechanism. This is a result of an increase in the relative dissolution rate of TetCP, which is rate-limiting [17]. X-ray diffraction analyses show that the DCPD is consumed before TetCP when the reaction temperature is 37.4 °C. Therefore, the first mechanistic step is the formation of an apatite with a calcium-to-phosphate molar ratio less than 1.5. The formation of defect apatites with Ca/P molar ratios less than 1.5 have been previously reported [5, 6, 18–20]. Reaction 2, in which DCPD is consumed, is consistent with the formation of calcium-deficient HAp.

$$3CaHPO_4 \cdot 2H_2O + (1.5 - x/4)Ca_4(PO_4)_2O \rightarrow + Ca_{9-x-y}(PO_4)_{6-x}(HPO_4)_x(OH)_{1-x-y} \quad \text{(Reaction 2)}$$
$$0 \leq x \leq 1 \text{ and } y \leq (1-x)/2$$

Reaction 3 shows the second mechanistic step involving the reaction of the apatite formed during Reaction 2 and the remaining TetCP.

$$Ca_{9-x-y}(PO_4)_{6-x}(HPO_4)_x(OH)_{1-x-y} + (x/4)Ca_4(PO_4)_2O \rightarrow Ca_9(PO_4)_5(HPO_4)OH \quad \text{(Reaction 3)}$$

Fig. 2b–d shows the influence of the polymer on the kinetics of HAp formation. The polyphosphazene causes an induction period prior to significant heat evolution. The duration of the induction period decreases as the reaction temperatures increase for all ratios studied. Fig. 3 is an Arrhenius plot indicating that the duration of the induction period can be described in terms of an apparent activation energy (E_{act}) [21]. The E_{act} values obtained are as follows: 115 kJ/mole (20-to-1), 107 kJ/mole (10-to-1), and 103 kJ/mole (5-to-1). The apparent activation energy decreases as the proportion of polymer increases, which indicates that the induction period is more strongly dependent on temperature at lower proportions of polymer. This is due to the presence of relatively less water as the polymer content decreases (see Table I), because the composites were synthesized to form products with approximately 60% porosity.

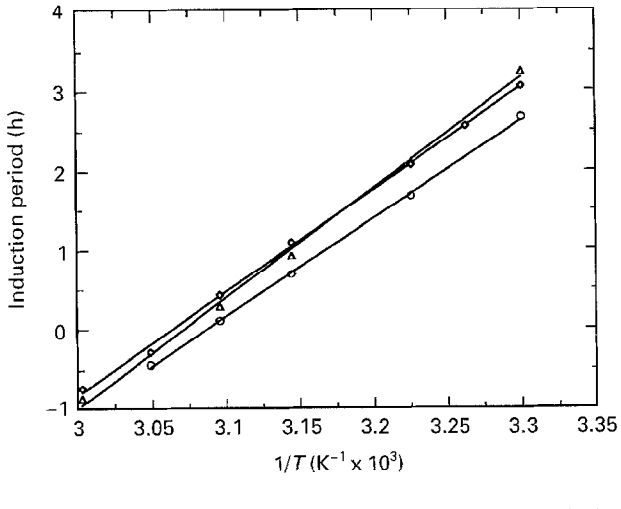

Figure 3 Arrhenius plot of the induction periods for HAp/polyphosphazene composites with (DCPD +TetCP)-to-polyphosphazene weight ratios of 20-to-1 (△), 10-to-1 (◇), and 5-to-1 (○).

When the liquid-to-(TetCP +DCPD) weight ratio is kept constant (resulting in variable porosity), the kinetics decrease with increasing proportions of the polyphosphazene at constant temperature. This is shown by comparing the two calorimetric curves of composites (20-to-1 and 5-to-1) synthesized at 37.4 °C at a liquid-to-(TetCP +DCPD) weight ratio of 1.9-to-3.0 (see Fig. 2b (vi) and Fig. 2d (ii)). The reaction rates decrease with increasing polymer content at constant liquid-to-(TetCP +DCPD) weight ratios. This was observed in the solution chemistry runs and will be discussed later.

The total heat evolved for reactions occurring at and above 50 °C appears to decrease with increasing temperature for the 10-to-1 and 5-to-1 composite systems (see Fig. 2c and 2d). However, complete reaction of identical precursors to the same product phase should result in the same total heat of reaction regardless of the reaction temperature. Such a decrease in the total heat evolved is a result of the inability to measure heat evolution before thermal equilibrium since the precursors were mixed outside the calorimeter chamber. This unavoidable experimental problem limited the maximum temperature at which composite formation could be studied. In spite of these limitations the slope calculations for Arrhenius plots and the observations and trends discussed remain valid.

Composites that contained the two highest proportions of polymer generated two discernable reaction peaks, indicated by the two slopes in the heat curves (Fig. 2c and 2d). The first slope (shorter reaction times) shows an Arrhenius relationship as illustrated in Fig. 4. The apparent activation energies are 50.0 kJ/mole (10-to-1) and 84.9 kJ/mole (5-to-1) which indicates that the temperature dependence of the reaction rate increases with the proportion of polymer. However, this thermal event is not primarily

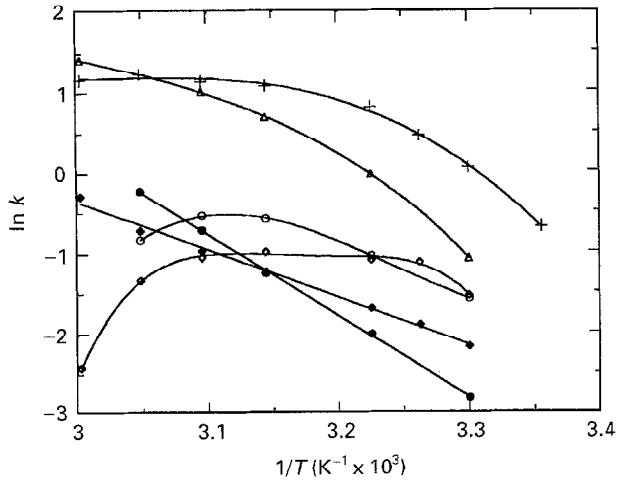

Figure 4 Arrhenius plot for reaction rates of the pure mineral system (+) and for HAp/polyphosphazene composites with (DCPD +TetCP)-to-polyphosphazene weight ratios of 20-to-1 (△), 10-to-1 [first reaction peak (◆) and second reaction peak (◇)], and 5-to-1 [first reaction peak (●) and second reaction peak (○)]. The open symbols represent reaction peaks associated with the major reaction forming HAp; the solid symbols represent reaction peaks associated with the formation of a supersaturated solution and the subsequent precipitation of the HAp crystallite containing polymer network.

associated with the formation of HAp. Rather, as will be shown by the analyses of the solution chemistry, it appears to be related to the slow dissolution of the calcium phosphate reactants, the formation of a solution highly supersaturated with respect to HAp, and the eventual formation of carboxylate-calcium salt bridges and small crystallites of HAp within the polymer network. The details of this mechanism are presently under investigation.

An Arrhenius activation energy could not be calculated for the thermal events associated with the formation of HAp (see Fig. 4). As the temperature increases the rate of HAp formation either becomes constant or eventually decreases. This observation is consistent with earlier studies on HAp formation in the absence of polymer [17, 22]. Because DCPD and HAp have negative thermal coefficients of solubility [23–26], higher temperature promotes the overgrowth of DCPD by HAp [27]. As a consequence the reaction rates are reduced at higher temperature, where this effect begins to influence the rate-limiting step. As discussed earlier, these observations are supported by microstructural development.

The presence of the polyphosphazene introduces an induction period that results in kinetics that are insufficiently rapid for *in vivo* use at physiological temperature. The high thermal activation in this system could be used to overcome part of this problem by initiating the reaction at temperatures higher than 37.4 °C prior to implantation. Another alternative would be to constitute the inorganic precursors to produce a higher Ca/P ratio thereby elevating the Ca in the system. This should promote a more rapid precipitation of the polyphosphazene and thus a faster set of the composite.

3.2. Solution chemistry

Minimal tissue damage from *in vivo* composite formation requires that the pH does not become extremely acidic or basic and that the ionic strength does not reach extremely high values. Therefore, variations in pH, Na, Ca, and P during composite formation were determined. These variations were also correlated with heat evolution data in order to more fully establish the mechanistic paths of composite formation. Fig. 5 shows a plot of pH versus time and illustrates two trends associated with the proportion of polymer. The first is that the pH value throughout the course of reaction is higher when the proportion of the polymer is higher. This is due to the modest basicity of the polyphosphazene. The second trend is the occurrence of an induction period preceeding HAp formation which increases in duration with increasing proportion of the polymer.

Fig. 6 shows the concentrations of Ca, Na, and P in solution as functions of time and polyphosphazene content. For the purpose of analysis, reactions are considered in four stages based on the variations in pH, ion concentrations, and solids present. The first stage does not occur when the polyphosphazene is absent. This stage is characterized by a pH that is nominally constant. The second stage of reaction

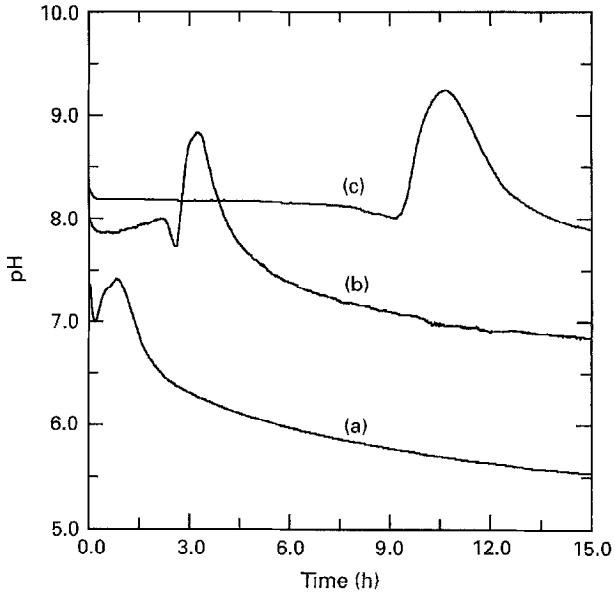

Figure 5 Solution pH as a function of time during HAp formation in (a) deionized water and for HAp/polyphosphazene composites with (DCPD +TetCP)-to-polyphosphazene weight ratios of (b) 20-to-1 and (c) 5-to-1.

involves significant HAp formation. During this second stage there is a decrease in pH (prior to the pH maxima) because TetCP dissolution is rate-limiting. The third stage involves a rise in pH to its maximum value as the previously formed HAp reacts with the remaining TetCP. The fourth and final reaction stage shows a characteristic decrease in pH as the system approaches its final equilibrium state.

Reaction stage 1. The induction period in heat evolution is associated with the first stage of reaction where the pH remains relatively constant. X-ray diffraction indicates the presence of only DCPD and TetCP during this period in both the 5-to-1 and 20-to-1 ratios. The ion concentrations remain nominally constant for the 20-to-1 ratio (Fig. 6b) resulting in near steady-state reaction conditions. Conversely, at the 5-to-1 ratio there are systematic changes in both the calcium and phosphate ion concentrations (Fig. 6c) during the first stage of reaction. The calcium and phosphate concentrations first increase and then decrease. Although X-ray diffraction does not indicate the presence of HAp during this stage, these variations indicate that a calcium phosphate phase is precipitating. Previous studies [3, 7–9, 22, 28] have shown that HAp forms from calcium phosphate precursors as extremely small crystallites. In small proportions, these would be difficult to detect by X-ray diffraction.

The length of the induction period increases with the proportion of polymer present. This trend was also observed in calorimetry performed at a constant liquid-to-(TetCP +DCPD) weight ratio. In solution chemistry the induction periods were approximately 1.75 and 9.0 h for the 20-to-1 and 5-to-1 ratios. Calorimeter runs performed at a calcium phosphate precursor-to-water weight ratio of 1.58-to-1 showed induction periods of approximately 4 and 5 h for the 20-to-1 and 5-to-1 ratio. Therefore, the length of the induction period at near-physiological temperature increases

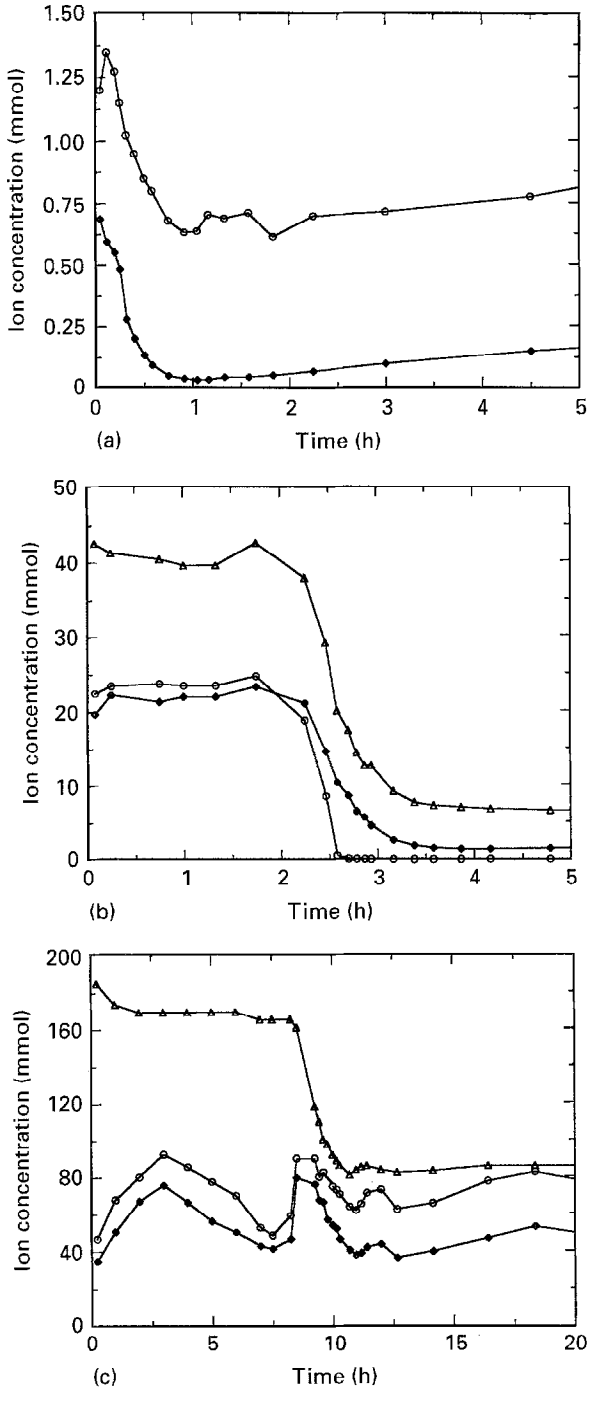

Figure 6 Solution ion concentrations as a function of time during HAp formation (a) deionized water and for HAp/polyphosphazene composite with (DCPD +TetCP)-to-polyphosphazene weight ratios of (b) 20-to-1 and (c) 5-to-1. [calcium (○), phosphate (◇), sodium (△)].

with polymer content, but is sensitive to the relative proportion of water present. As the proportion of water in the system increases, the induction period decreases for the 20-to-1 ratio but increases for the 5-to-1 ratio.

Significant differences in ion concentrations during the first stage are related to the proportion of polyphosphazene present. Calcium and phosphate concentrations and pH increase with the proportion of the polymer. However, the solubility of HAp decreases as the pH increases [23–26]. The elevated concentrations of calcium and phosphate (resulting in solutions highly supersaturated with respect to HAp) and induction period are therefore a result of the presence of the polymer. Thus, while the polyphosphazene increases the supersaturation level, it decreases the rate of HAp crystallization. Carboxylate-containing molecules have been shown to chelate calcium ions in solution, to adsorb on the reactant and product phases, and to retard the reaction to form HAp [9, 28]. The increase in ionic concentrations and the induction period are thus caused by the chelating and adsorbing properties of these functional groups of the polymer. It is probable that the carboxylate groups of the polyphosphazene also coordinate with the calcium ion on the surfaces of the HAp nuclei thus inhibiting their growth.

Reaction stage 2. At the 20-to-1 ratio the concentration of ions in solution before the onset of the second stage remain constant. Complete precipitation of the polymer (as a result of Ca salt bridge formation, see Fig. 1) occurs before the start of the second reaction stage. By the end of the first stage of reaction no further polymer precipitated from the aliquots of solution upon their acidification. Thus it appears that a prerequisite of significant HAp formation is the precipitation of the polyphosphazene by calcium ions. This is consistent with the retardation of HAp formation by the polyphosphazene discussed previously.

Conversely, the onset of the second reaction stage is preceeded by an increase in the calcium and phosphate concentrations after primary nucleation at the 5-to-1 ratio. At this ratio the onset of the second stage is not characterized by the complete precipitation of polyphosphazene. While the transition between the first and second stages is characterized by an observable decrease of soluble polymer, there is still a significant quantity of soluble polymer that exists during the remainder of the reaction. This indicates there is insufficient free Ca in solution to cause the complete precipitation of the polymer and formation of HAp.

During the onset of the second stage all ion concentrations begin to decrease in all three ratios investigated. The three reactions studied exhibit a second stage characterized by a decrease in pH as the HAp precursors begin reacting to form apatite. X-ray diffraction and the decreases in pH indicate that TetCP dissolution is rate-limiting during this second reaction stage. In all experiments the DCPD is consumed prior to TetCP. This has been observed in other studies [29] and results in a product phase with a Ca-to-P molar ratio less than 1.5. The disappearance of DCPD is associated with the pH minimum prior to the peak maximum in pH.

Reaction stage 3. A third stage is characterized by the reaction between the previously formed HAp and the remaining TetCP. Because the DCPD has been exhausted, the hydrolysis of TetCP, results in an increase in pH. This is shown in Fig. 5. The pH continues to increase until the TetCP has been completely consumed, this occurs after 1 h, 3.25 h, and 11 h in the absence of polyphosphazene, at the 20-to-1 ratio, and at the 5-to-1 ratio, respectively. During the third stage the ion concentrations in solution continue to decrease in the three systems investigated (see Fig. 6).

Reaction stage 4. During the fourth and final reaction stage the solid and solution phases approach equilibrium. Fig. 5 shows that the pH continues to decrease even after the precursors have been completely consumed. The ion concentrations are relatively stable but increase slightly. Slow equilibration during this stage is a result of the extremely high surface area of the HAp that formed.

There is an extended time of equilibration during the fourth stage. In both the absence of polymer and at the 5-to-1 ratio, the calcium concentration is greater than the phosphate concentration. This is typically observed in reactions of this type [4, 8, 9, 28]. Conversely, the 20-to-1 ratio displays anomalous behaviour during the third and fourth stages. In that system the phosphate concentration is higher. This indicates that the complete precipitation of the polyphosphazene influences the relative concentrations of calcium and phosphate. At complete reaction the calcium concentration reaches a nominally constant value of approximately 0.1 mmol. The phosphate concentration reaches a minimum of 1.5 mmol and then slowly increases to almost 3 mmol after 5 days. This slow increase in phosphate concentration over time results in a continuous decrease in the pH. As the concentration of the polymer increases, the final phosphate concentration in solution becomes greater. In the presence of carboxylate containing molecules, this increased phosphate concentration has been observed and attributed to the partial substitution of the carboxylate group for phosphate into the apatite structure [9, 28, 30–32]. It is probable that the same phenomenon is occurring in the present study resulting in a polymeric phase that is intimately bound to the HAp.

When soluble polyphosphazene is present after complete reaction of the HAp precursors (5-to-1 ratio), the calcium and phosphate concentrations never decrease to the values associated with the equilibrium solubility of HAp (see Fig. 6c). Even after 140 h the calcium and phosphate levels in solution are approximately 85 mmol and 50 mmmol, respectively, as a result of the chelating ability of the polymer.

In the systems containing polyphosphazene the sodium concentration decreases during the formation of HAp. Sodium substitutes for calcium in the apatite structure [33]. The pH does not shift to extremely high values because the sodium associated with the polyphosphazene and the calcium from the HAp/HAp precursors exchange for one another. While sodium incorporation occurs during the second and third reaction stages, sodium is still present in solution upon complete reaction. Since the pH continues to decrease during this final stage the sodium is not associated with hydroxyls in solution, rather; the solution consists of a phosphate with the generic formula $Na_{(3-x)}H_xPO_4$. Thus, higher phosphate concentrations in the systems containing the polyphosphazene are also present to compensate for the charge of the sodium.

As previously discussed, it will be important to regulate the pH and ionic concentrations during *in vivo* formation of an implanted material in order to minimize local tissue damage. Initial results from solution chemistry suggest that the formation of self-assembling HAp–polyphosphazene composites *in vivo* will not likely induce severe tissue damage. While the presence of the polyphosphazene results in an increase in the pH of the system, the pH values observed should not result in any significant tissue damage in the surrounding area of an implant. In the systems investigated, the sodium concentrations are all below those values typically reported for serum (~145 mmol). While the calcium and phosphate concentrations are approximately an order of magnitude higher in this *in vitro* study than in serum, it is likely that these species are not all completely free ions and some bound to the polyphosphazene.

3.3. Microstructure

The microstructure of an implanted material has been shown to be an important factor with respect to ingrowth of native tissue and/or its resorption rate. The desired properties are dependent on the specific application of the material. The properties of a composite material are also dependent on its final microstructure. The microstructure of HAp and HAp-polyphosphazene composites were thus observed by electron microscopy and correlated with the variations in composition and reaction temperature.

The microstructure of HAp formed in the absence of the polyphosphazene shows a significant change as the temperature increases (Fig. 7). The microstructure

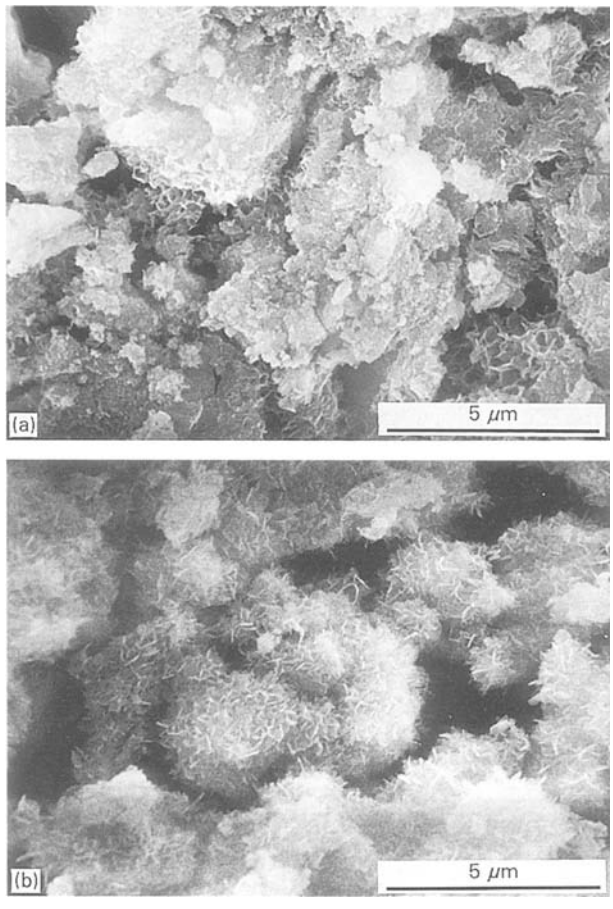

Figure 7 Microstructures of HAp formed in deionized water (a) 30 °C (b) 60 °C.

of HAp formed at 30 °C is characterized by artifacts of the reactant particles interconnected by HAp causing extensive interparticulate connectivity. Conversely, when the same precursors are reacted at 60 °C, HAp appears to form in the volumes originally occupied by the reactant particles. This results in a microstructure composed of discrete particles that are not continuously bonded in all three dimensions (Fig. 7). Thus, at 60 °C a polycrystalline HAp forms that resembles the gross morphological features of the basic reactant precursor, TetCP. This indicates that, at higher reaction temperatures, a critical degree of supersaturation is achieved in close proximity to the TetCP surface. As a consequence, relatively less product phase forms between the reactant precursor particles, and this results in a commensurate decrease in connectivity.

The microstructures of the individual HAp crystallites also change with temperature. HAp formed at 30 °C does not exhibit a distinct microstructure. Rather it crystallizes as polycrystalline spheres, needles, and plates. However, the microstructure of the HAp formed at 60 °C is extremely homogeneous, forming as submicron hexagonal plates. The changes in the microstructure and solubility with temperature are consistent with the lack of a linear Arrhenius relationship describing the temperature dependence of HAp formation.

The presence of the polymer causes significant changes in gross morphology as well as affecting the morphology of the HAp itself (see Fig. 8). In the 5-to-1 composite a three-dimensional network of polymer and HAp can be observed at all reaction temperatures studied (30 °–55 °C). The microstructure is composed of a polymer network which contains individually dispersed HAp needles. The occurrence of this microstructure supports the hypothesis that the polymer network provides HAp nucleation sites. Also present are larger, less well-defined features remniscent of the TetCP particles that are coated with HAp crystallites and polymer. No significant temperature-dependent microstructural variations occurred in this composite indicating that the presence of the polyphosphazene is an important factor in controlling microstructural development. The composite microstructure and past studies on the surface area of HAp formed from DCPD and TetCP [17] suggest that the HAp in these composites should resorb slowly over time. Large pores resulting from entrapped air should induce the ingrowth of native bone.

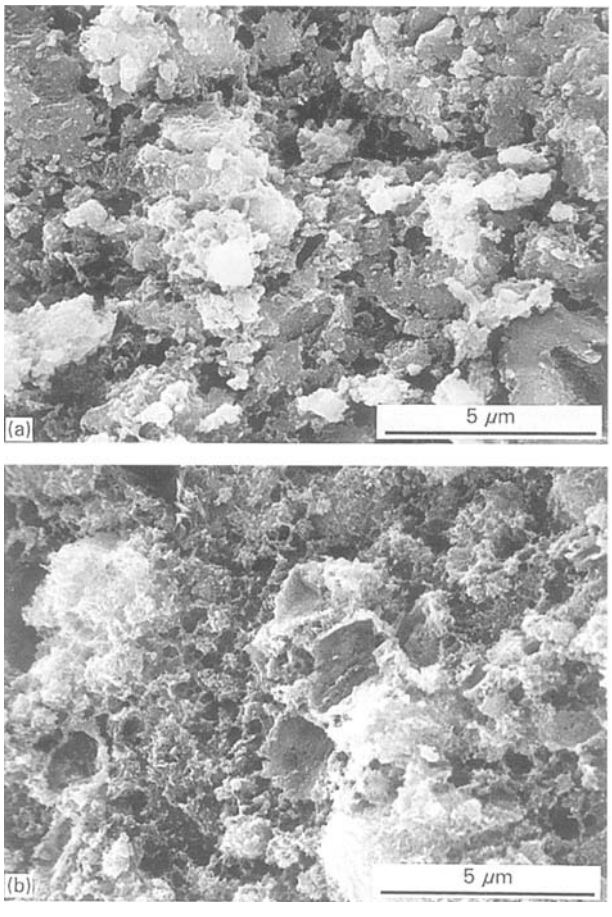

Figure 8 Microstructures of the 5-to-1 HAp/polyphosphazene composite formed at (a) 30 °C (b) 60 °C.

4. Conclusions

Multistep reactions between DCPD, TetCP, and poly[bis(sodium carboxylatophenoxy)phosphazene] occur during the low temperature synthesis of HAp-polyphosphazene composites. Compared to HAp formation in the absence of the polymer, reactions in the presence of the polyphosphazene increased the solution pH due to its slight basicity. It also resulted in an induction period during which the soluble polymer chelates high concentrations of calcium in solution to give a solution that is highly supersaturated with respect to HAp. When polymer is still present in solution after complete reaction, it chelates ions in solution for periods of time in excess of 6 days resulting in the stabilization of a supersaturated solution. At lower polymer contents there is ample calcium to cause complete precipitation of the polyphosphazene. Conversely, at higher proportions the polymer does not completely precipitate from solution due to the lack of sufficient calcium. Microstructure and solution analyses suggest that the polymer also nucleates HAp during this period.

The duration of the induction period could be characterized by an Arrhenius relationship that indicated a stronger temperature dependence with lower polyphosphazene levels. An Arrhenius relationship could not be established for the reaction involving the formation of HAp. As illustrated by microstructural analysis this resulted from changes in the solubility of the HAp and its precursors with temperature.

The present study suggests that a self-assembling composite consisting of HAp and an ionically crosslinkable polyphosphazene may be useful for *in vivo* applications that require filling complex defects. While the kinetics of these reactions are presently insufficiently rapid for *in vivo* formation at physiological temperature, a more rapid rate of setting may be achieved by changing the initial mixing temperature and/or by increasing the Ca/P ratio of the HAp precursors.

Acknowledgements

The authors wish to thank the Penn State Materials Research Institute, NIH Grant DE09421, and Corning Inc. for the support of this work.

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Received 14 February
and accepted 16 October 1995.