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Formation of composites comprised of calcium deficient HAp and cross-linked gelatin

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Abstract Cross-linked gelatin/calcium deficient hydroxyapatite (CDHAp) composites were prepared at or near physiologic temperature. α-tricalcium phosphate (α-TCP) or a mixture of tetracalcium phosphate and dicalcium phosphate were used as CDHAp precursors. Glutaraldehyde was used to cross-link the gelatin fibers. CDHAp formation reached completion in the presence of cross-linked gelatin fibers. Effects of cross-linking concentrations, proportions of gelatin fiber, molecular weight of gelatin and the temperature of the hydration reaction on the formation of CDHAp were studied. Cross-linked gelatin acts as a nucleating agent for CDHAp formation. The pH variations during CDHAp formation are lower at the onset of the reactions in the presence of cross-linked gelatin fibers. Although cross-linked gelatin fibers enhance CDHAp formation their composites have low mechanical strengths. Swelling of gelatin appears to be a major factor that limits the strengths of the CDHAp/cross-linked gelatin composites.

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1 Introduction

Gelatin is produced from the thermal denaturation or physical and chemical degradation of the collagen [1–4]. Denaturation of collagen involves the breaking of the triple-helix structure of collagen into random coils to produce gelatin [5]. Gelatin dissolves in water to form colloidal sols at higher temperature and congeals into gels at room temperature, which mimic thermoplastic polymers [6]. At room temperature some segments of the gelatin molecules are physically associated, forming a "physical crosslink" [7]. Chemical cross-linking of the gelatin can produce insoluble biodegradable and biocompatible products [8]. Consequently, the concept of renaturation is of interest when developing materials with mechanical properties that can be tailored for biomedical applications [7].

Gelatin is non-antigenic at physiological conditions [8]. However, the main limitation of gelatin for its use as a tissue substitute is its rapid dissolution in aqueous environments, leading to fast degradation of grafts [9]. Degradation of gelatin also has a strong influence on the mechanical property of a gelatin-containing graft, as it decreases mechanical properties.

Cross-linking of gelatin is a potential means of improving thermal and mechanical stability [10, 11]. Different methods of cross-linking, including physical and chemical methods, have been previously studied. Physical methods include drying, heating or exposure to gamma or ultraviolet radiation. For example Terao et al. [12] studied the cross-linking of gelatin using gamma irradiation. However, it is difficult to control the extent of cross-linking using this technique [13]. Chemical techniques using different cross-linking agents have also been reported. Yao et al. [14] studied the cross-linking of the gelatin using formaldehyde. Draye et al [15] prepared hydrogel films by

cross-linking gelatin with dextran dialdehyde. Among the chemical cross-linking agents, glutaraldehyde is by far the most widely used [16–19]. Glutaraldehyde has the ability to stabilize collagenous materials [20]. Damink et al. [21] studied the chemistry of the cross-linking of collagen using glutaraldehyde. Ahmed et al. [22] studied the cross-linking of collagen for peripheral nerve repair. Matsuda et al. [23] studied the bioadhesion of gelatin films cross-linked with glutaraldehyde to natural tissues. Bigi et al. [24] investigated the mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde cross-linking. Apostolov et al. [25] compared the swelling behavior between native and cross-linked gelatin. According to all these studies the properties of gelatin were improved using cross-linking treatments.

Gelatin-based materials can be used in applications for repair of both soft and hard tissues. Gelatin powder has been used as a template for the preparation of macroporous brushite cement from a mixture of β-tricalcium phosphate (β-TCP) and monocalcium phosphate monohydrate (MCPM) [26]. The influence of biological macromolecules, such as gelatin, on the formation and transformation of calcium phosphate precipitates has been investigated by Brecevic et al. [27], where the interaction between gelatin and calcium phosphate was characterized by particle electrophoretic mobility, pH and turbidity measurements. Composites composed of gelatin and calcium phosphates have been studied as artificial bone replacement materials [28]. Bigi et al. [29] prepared and characterized hydroxyapatite-gelatin films to produce biomaterials suitable for fulfilling specific mechanical functions. Chang et al. [30] used the biomimetic technique to form organized hydroxyapatite-gelatin nanocomposites in vitro. The hydroxyapatite nanocrystals were precipitated from an aqueous solution of gelatin at pH 8 and at 38°C. Bonding occurred as a result of the interaction between the carboxyl group of the gelatin and calcium ions of the hydroxyapatite. TenHuisen et al. [31] studied the reaction between calcium phosphate reactants and gelatin at 38°C. The heat liberated during HAp formation in presence of gelatin was followed using isothermal calorimetry technique.

The influence of gelatin on the setting properties of α-TCP has attracted interest. Biological effects and cytotoxicities of composites combining tricalcium phosphate and glutaraldehyde cross-linked gelatin for orthopedic application were reported by Yao et al. [32]. Recently, Bigi et al. investigated the effect of gelatin on the setting time, compressive strength, phase evolution and microstructure of calcium phosphate cement [33, 34]. It was found that the presence of gelatin accelerated the setting reaction, and improved the mechanical properties of the cement. The improved mechanical properties of the composites were related to their reduced total porosity and more compact structure.

The objective of the present work was to prepare CDHAp/cross-linked gelatin composites employing hydration reactions of different CDHAp precursors. The influence of the proportion of cross-links in the gelatin matrix on the rate of CDHAp formation was evaluated. The effects of temperature and the extent of cross-linking and proportion of gelatin fibers in the composites on the rate of CDHAp formation were studied. Variations in pH, and mechanical properties of CDHAp/cross-linked gelatin composites were determined.

2 Materials

2.1 Mineral phases

Calcium deficient hydroxyapatite, CDHAp, was prepared by two reactions. The first route entailed the hydration of α-TCP and the second one was the hydration of a physical mixture of TetCP/DCPA (tetracalcium phosphate/dicalcium phosphate anhydrous) powders in deionized water. Syntheses involved pyroprocessing following by the use of mortar and pestle to break up the large particles, following by ball milling under heptane to avoid surface hydrolysis that could occur with milling in air. After the heptane was removed, attrition milling was carried to attain the final particle sizes.

α-TCP was prepared by a solid-state reaction between reagent grade calcium carbonate ($CaCO_3$ Osram Sylvania, PA) and thermally synthesized calcium pyrophosphate. Calcium carbonate was milled with calcium pyrophosphate at room temperature for 16 h in heptane. The slurry was filtered and air-dried overnight. The dried mixture of calcium carbonate and calcium phosphate was sintered at 1,180°C for 1.5 h. After sintering, the α-TCP was directly air quenched to avoid the formation of the β-polymorph. The α-TCP was ground by using mortar and pestle and milled in a ball mill for 16 h in heptane at room temperature. The α-TCP slurry was filtered and dried under vacuum at room temperature overnight, re-milled in an attrition mill for 8 h to get an average particle size of 3–4 μm. The formation of the phase-pure fine product of α-TCP was confirmed by XRD. CDHAp formation was accomplished by the in situ hydrolysis of α-TCP in water at the required temperature according to the following reaction:

$$3\,\alpha\text{-}Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9HPO_4(PO_4)_5OH$$

Powder X-ray analysis was used to confirm the formation of the CDHAp.

The second route for the preparation of CDHAp was by the hydration of a combination of thermally synthesized tetracalcium phosphate, TetCP, and dicalcium phosphate,

DCPA. These were proportioned to produce a calcium deficient hydroxyapatite that has Ca/P molar ratio of 1.5. TetCP was prepared by solid-state reactions of particulate $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$ (MCPM) (FMC, NY). Initially the calcium carbonate was mixed and milled with MCPM for 24 h in heptane at room temperature. The slurry was filtered, dried in an oven at 100°C for ~16 h. The dry mixture was sintered at 1,310°C for 2 h. Phase purity of TetCP was confirmed by X-ray diffraction analysis (Scintag, Inc., Sunnyvale, CA) at a scan rate of 4°/min., by comparing with standard JCPDS card # 25-1137 for TetCP. After two cycles of ball milling and attrition milling, TetCP was mixed with MCPM at a molar ratio of 1.5 and re-milled in heptane for 24 h. The resultant slurry wasfiltered, dried and stored under vacuum. CDHAp formation was formed according to the following reaction:

$$6\,CaHPO_4 + 3\,Ca_4(PO_4)_2O \xrightarrow[H_2O]{} 2\,Ca_9HPO_4(PO_4)_5OH$$

2.1.1 Gelatin

Gelatin (Type A, 75 and 225 Bloom) (Aldrich-Sigma, Inc) was dissolved in distilled water at 45^oC for 1h. The Bloom number is a measure of the stiffness of gelatin as determined by the depth of penetration; with stiffer gelatin having a higher the Bloom number. The solution was spun at room temperature to prepare gelatin fibers. The spinning was carried out using a centrifugal fiber spinner. Fiber was spun at room temperature onto a stainless steel surface. The fibers were left for 1 h on the surface, and then detached. This process produced loose mats of fibers with diameters ranging from about 20–80 μm.

One half a gram samples of gelatin fibers were cross linked in 100 ml of solutions containing 0.1, 0.5, 1.0 or 2.5 wt% of glutaraldehyde (GA) [(Aldrich-Sigma, Inc)] using phosphate buffer saline (pH 7.4) for 24 h at room temperature. After GA treatment, the fibers were rinsed with distilled water for 0.5 h, washed with sodium chloride (1 M) solutions for 0.5 h. The fibers were then immersed in glycine (Aldrich-Sigma, Inc) for 0.5 h to convert any residual aldehyde groups to carboxyl groups. The fibers were rinsed with distilled water for 30 min and air-dried at room temperature overnight. Except where noted 225 bloom gelatin was used.

3 Methods

3.1 Composite synthesis

Cross-linked gelatin fibers were mixed with CDHAp precursors. Three proportions of cross-linked gelatin fibers, 5, 10 and 20 wt. %, were used in producing composites. Pre-composites comprised of cross-linked gelatin and the inorganic precursors to HAp were inter-ground in a mortar and pestle. These were then hydrolyzed at 37, 45 and 56°C.

3.2 Isothermal calorimetry

The rates of the formation of CDHAp from the hydration of α-TCP or TetCP/DCP in the presence and absence of gelatin fibers were determined by isothermal calorimetry. In the isothermal calorimetric technique, the pre-composite of gelatin and inorganic precursors were weighed into an Au plated cupper calorimeter cup. The calorimeter cup was sealed with parafilm, and a placed into the closed calorimeter chamber. A syringe containing a mass of de-ionized water equivalent of the weight of the precomposite was placed in the upper house of the calorimeter. The calorimeter cup and syringe were allowed to equilibrate for 0.5 h. After the thermal equilibrium was established, the reaction was initiated by injecting the de-ionized water into the calorimeter cup. Isothermal reaction was accomplished by connecting the calorimeter chamber to a water bath. The thermopiles surrounding the calorimeter cup respond to the heat produced by the hydration of the inorganic precursor and convert the thermal output to a voltage. Variations in voltage were acquired as the reactions proceeded using a data acquisition card (PCMCIA, model NI4350, National instruments, Inc, TX). The data were collected using LAB VIEW software. The output data were plotted as rate of reaction in milli watts verses time. The accumulated heats (total heats of reaction) were calculated by integrating the rate data. The accumulated heat curves are presented as KJ/mole of calcium deficient hydroxyapatite formed. The formation of HAp in all composites was confirmed by XRD. The weight ratio of inorganic precursor-to-deionized water when CDHAp formed in the absence of gelatin was 3 to 1; composite formation in the presence of gelatin required a ratio of 1 to 1.

3.3 Solution chemistry

The pH changes of the hydration of the inorganic precursors in presence and absence of gelatin fibers were monitored using a glass electrode connected to a pH meter (940 Orion, Thermal electron corporation, MA). The pH measurements were carried out at constant temperature. The data were collected by computer using Orion software. In a typical pH experiment, 1 g of inorganic precursor or pre-composite was stirred in 200 ml of de-ionized water in presence of bubbling nitrogen gas to minimize exposure to atmospheric CO_2. A datum point was collected either every 30 or 60 s depending on the temperature. After terminating

the experiments, the solutions were filtered and rinsed with acetone to stop any further reaction. The solids present were air-dried for 2 h and the formation of calcium deficient hydroxyapatite was confirmed using X-ray diffraction.

3.4 Composite characterization

In order to follow the progress of the CDHAp formation, composite specimens of CDHAp formed in the presence of cross-linked gelatin by hydration were subjected to powder X-ray diffraction. A scintag automated diffractometer was used to analyze the composite with a scan rate 4°/min, from 10° to 40° 2θ.

The fracture surface morphologies of the CDHAp formed in the presence of cross-linked gelatin were examined and investigated via scanning electron microscope (Hitachi S-3000H SEM). Prior to examination, the samples were dried in an oven at 50°C for 10 min, coated with gold for 1 min.

3.5 Mechanical property characterization

Determinations of mechanical properties of the CDHAp formed in the presence of cross-linked gelatin were performed at room temperature. Selected samples of CDHAp composites were subjected to diametrical and compressive tests. Prior to testing, the CDHAp precursors in the presence of cross-linked and treated gelatin were mixed into a paste in a mortar at room temperature. The ratio of powder-to-liquid was 1-to-1. The pastes were carefully placed in Teflon molds and allowed to set to harden in an incubator at 37°C and 100% relative humidity for 48 h.

Tensile tests of the composites were performed using a diametrical compression test (Brazilian test) [35, 36]. The dimensions of the composites samples prepared for tensile strength were 6.25 mm in diameter and 6.25 mm in height. The composite sample dimensions for compressive testing were 6.25 mm in diameter by in 12.5 mm height. The specimens were tested using an automated Instron machine with 1KN load cell and the cross-head speed was set at 0.3 mm/min. The tensile strength was calculated according to Eq. 1

$$(\sigma_t) = 2P/\pi DT \quad (1)$$

where σ_t the tensile strength at failure, P is the load required for failure, D is the diameter of the specimen and T is the thickness of the specimen. And, the compressive strength was calculated according to Eq. 2.

$$(\sigma_p) = P/\pi r^2 \quad (2)$$

where σ_p is the compressive strength at failure, P is the load required for failure and πr^2 is the cross sectional area of the sample.

4 Results and discussion

4.1 Isothermal calorimetric studies

4.1.1 Effect of the cross-linking density

Figure 1 plots the heat evolution curves for the hydration of α-TCP in the presence and absence of 225 bloom gelatin fibers that had been subjected to different glutaraldehyde concentrations at physiologic temperature. The composite proportions were 20 wt% cross-linked gelatin fibers and 80 wt% CDHAp precursors. The gelatin was cross-linked using 0.1, 0.5, 1 or 2.5 wt%, GA solution. During the first 10 h of the reaction, the same behavior was exhibited, irrespective of the extent of cross-linking. After 2.5 h the total values of heat evolution of calcium deficient hydroxyapatite formed in presence of cross-linked gelatin fibers was double of that of CDHAp formed alone. Generally after about 10 h of reaction, all curves began to level off and attained a value close to the total heat of hydration of α-TCP. Figure 1 also shows that the total heat evolution curve of the composites containing a 2.5 wt% cross-linked density of glutaraldehyde of gelatin fibers attains a lower value than that of CDHAp formed in the absence of gelatin. These data illustrate that the presence of cross-linked gelatin fibers enhance CDHAp formation regardless of the extent of the cross-linking.

The influence of cross-linked gelatin on the nucleation of CDHAp is shown in Fig. 2. This figure shows the rates of heat evolution at 37°C during the hydrolysis of α-TCP in the presence of different cross-linking densities of gelatin fibers, when proportion of cross-linked gelatin fibers in the com-

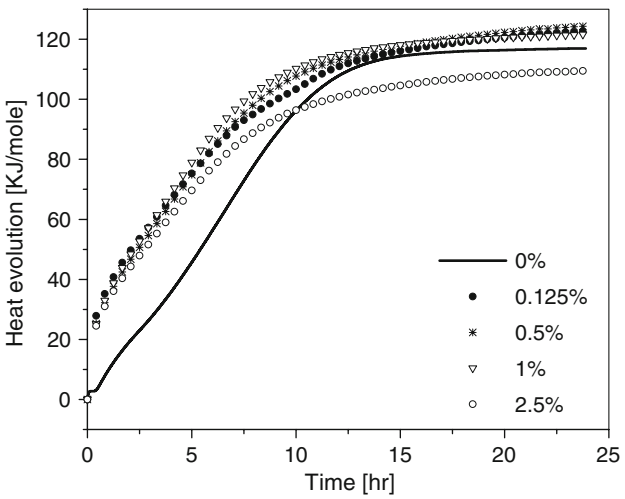

Fig. 1 Heat evolution curves during the formation of CDHAp at 37°C from pre-composites containing 80% α-TCP and 20% cross-linked gelatin as a function of the glutaraldehyde concentration used when cross-linking the gelatin

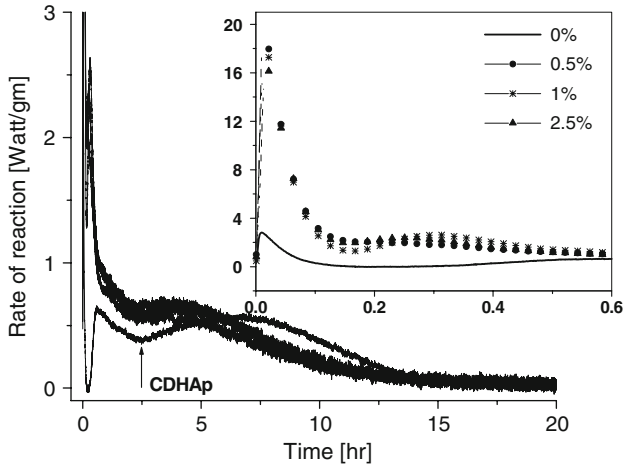

Fig. 2 Rate of heat evolution curves for the formation of CDHAp at 37°C from pre-composites containing 80% α-TCP and 20% cross-linked gelatin as a function of the glutaraldehyde concentration used when cross-linking the gelatin. Insert shows heat evolution during the first 0.6 h of reaction; the expanded y-axis reveals the intensities of the mixing peaks

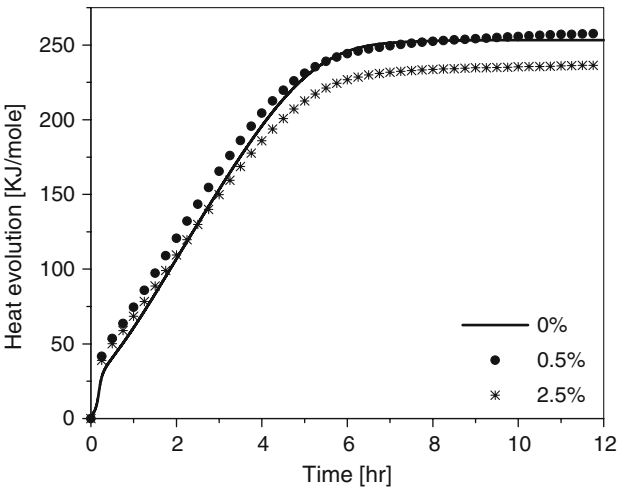

Fig. 3 Heat evolution curves during the formation of CDHAp at 37°C from pre-composites containing 80% TetCP/DCP and 20% cross-linked gelatin as a function of the glutaraldehyde concentration used when cross-linking the gelatin

posite is 20 wt%. The insert to the figure shows the heat evolution characteristics during the first 0.6 h of reaction and reveals the extents of the mixing peaks. Regardless of the extent of cross linking, the nucleation peak of the CDHAp formed by the hydration of α-TCP reached a maximum value at shorter times than that of CDHAp formed in the absence of cross-linked gelatin fibers. The nucleation peak of CDHAp formed in the presence of cross-linked gelatin reached a maximum value after 20 min while that of CDHAp formed in the absence of cross-linked gelatin required more than 30 min to reach its maximum. These data indicate that cross-linked gelatin fibers facilitate nucleation of CDHAp formed at physiologic temperature.

Extent of cross-linking of gelatin fibers had much weaker effect on the hydration of TetCP/DCP to produce CDHAp as shown in Fig. 3. Cross-linked gelatin only slightly accelerates the formation of CDHAp. The total heat of CDHAp formed in the presence of the gelatin crosslinked at high density of glutraledhyde is modestly lower than that of CDHAp formed alone.

4.1.2 Effect of proportion of gelatin fiber

Figure 4 illustrates the total heat evolution curves as a function of time for the hydration of α-TCP in the presence of different proportions of cross-linked gelatin fibers. In this experiment, fibers were cross-linked using 0.5 wt% a glutaraldehyde at physiologic temperature. The presence of cross-linked gelatin fibers enhanced the formation of CDHAp during the first 10 h of reaction. The higher the proportion of the cross-linked gelatin fiber, the higher the rate of the formation CDHAp during this period.

The total heat evolution curves for CDHAp formed during the hydration of TetCP/DCP in the presence 0, 5, 10, and 20% of cross-linked gelatin (0.5% glutaraldehyde) at 37°C were very similar. This suggests that any interactions involving the gelatin do not significantly influence total heat evolution at 37°C.

4.1.3 Effect of gelatin molecular weight

Two molecular weights of gelatin, 75 bloom and 225 bloom, were studied. The influence of the molecular weight of cross-linked gelatin on the rate of the hydration reaction of α-TCP at 37°C in composites containing 20 wt% gelatin is shown in

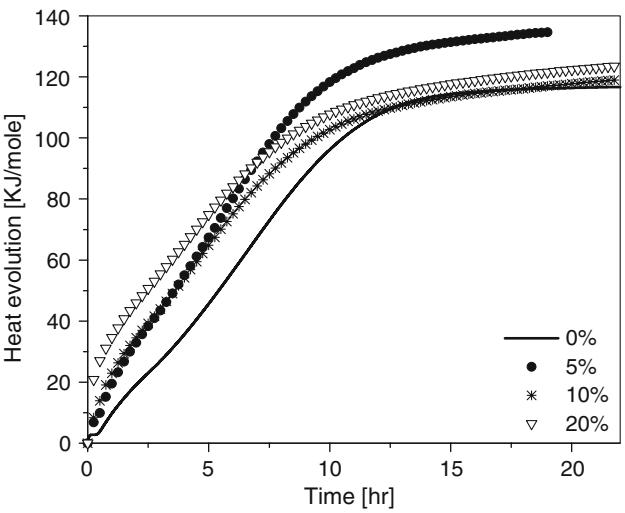

Fig. 4 Heat evolution curves during the formation of CDHAp at 37°C from α-TCP and pre-composites containing various proportions of α-TCP and gelatin cross-linked using a 0.5 wt% glutaraldehyde)

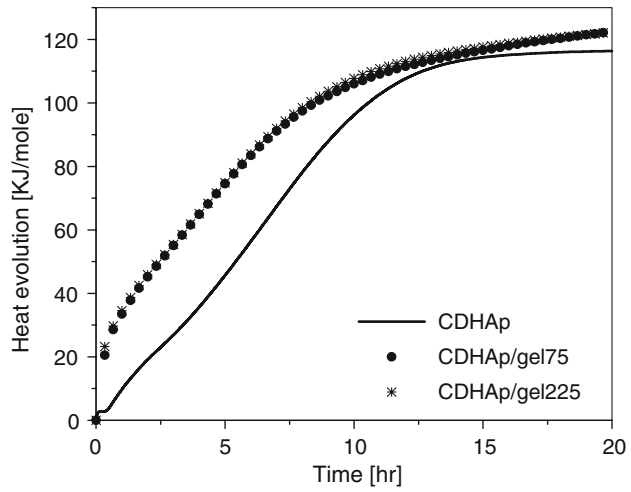

Fig. 5 Heat evolution curves during CDHAp formation from the hydration of α-TCP at 37°C as a function of the molecular weight of cross-linked gelatin fibers (cross-linked using 0.5 wt% glutaraldehyde; 80 wt% TCP and 20 wt% gelatin)

Fig. 5. No differences in the values of the total heat evolved during the formation of CDHAp were observed between the molecular weights of gelatin. Thus, the molecular weight of gelatin has no significant effect on the kinetics of the hydration reaction of α-TCP at physiologic temperature. Similarly, the molecular weight also has little influence on the kinetics of the hydration reaction of TetCP/DCP to produce CDHAp at physiologic temperature.

4.1.4 Effect of Temperature

Figure 6 shows the influence of temperature on the hydration reaction of α-TCP in the presence of 20 wt% of 225 bloom gelatin cross-linked at a glutaraldehyde density of 0.5 wt%. The hydration reactions were studied at 37, 45 and 56°C. The rates of CDHAp formation in the presence of cross-linked gelatin increased with temperature. However, the total heat evolved after the complete of reaction at 56°C was modestly lower than that of CDHAp formed in the absence of gelatin. X-ray diffraction analysis revealed complete formation of CDHAp and the total heat of formation of CDHAp does not show a meaningful dependence on reaction temperature over this range. Thus, these data show that the cross-linked gelatin induces the formation of CDHAp regardless of temperature. The lower total heat evolution at the higher temperature appears to be associated with denaturation. The denaturation temperature of the gelatin used in this study was determined by DSC to be 52°C. Thus, the related absorption of latent heat as gelatin denatures appears to have an impact on the total heat evolved during the composite formation.

4.1.5 Solution chemistry

The variations in pH at 37 and 45°C during the hydration of α-TCP in the presence of 20 wt% of gelatin cross-linked using 0.5 wt% glutaraldehyde are shown in Fig. 7. This figure compares the pH variations during hydration of α-TCP in the presence and absence of cross-linked gelatin. The trends in pH variations are the same at both temperatures. At 37°C, the pH change during CDHAp formation in the presence and absence of cross-linked gelatin occurred in two steps. At 45°C the pH variations during its formation in the presence and absence of cross-linked gelatin occurred in three steps. At 37 and 45°C, the durations of initial steady state pH values during the hydration

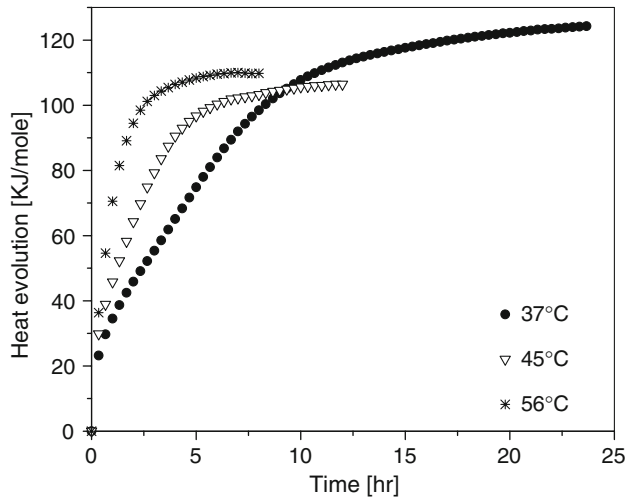

Fig. 6 Heat evolution curves during CDHAp formation at 37, 45 or 56°C from pre-composites containing α-TCP and cross-linked gelatin (cross-linked using 0.5 wt% glutaraldehyde; 80 wt% TCP and 20 wt% gelatin)

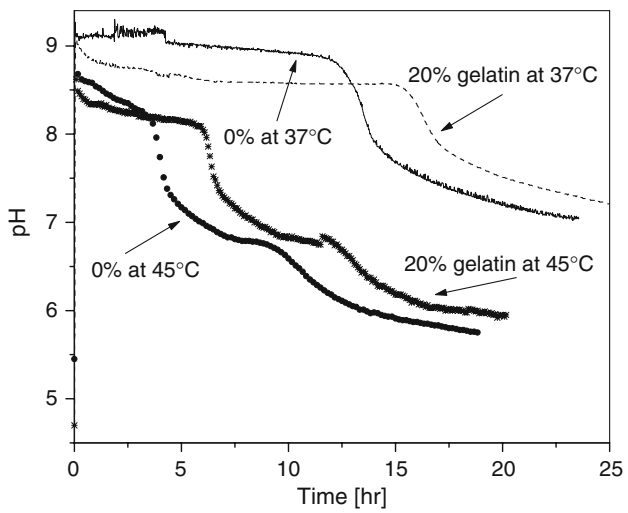

Fig. 7 pH variations as a function of time during the hydration of α-TCP and α-TCP in the presence of 20 wt% of cross-linked gelatin (cross-linked using 0.5 wt% glutaraldehyde) at 37 or 45°C

of α-TCP in the presence of cross-linked gelatin persisted for 12 and 3 h, respectively.

This figure also shows that there is a correlation between the pH variations and the rates of hydration reaction. The increase in the rate of hydration reaction of α-TCP leads to more a rapid pH variation. The pH values during CDHAp formation at 45°C approach 7 by the end of second step. Similar behavior was observed when CDHAp formed at 37°C. However, the pH eventually attained at 45°C was lower.

Figure 7 also shows that the cross-linked gelatin extends the times of onset of the pH variations of CDHAp formation regardless of temperature. After the pH values start to decrease, the variations in the presence of cross-linked gelatin are smaller than those in the absence of cross-linked gelatin. This suggests that the presence of cross-linked gelatin facilitates the preferential dissolution of α-TCP and affects a more rapid transformation to CDHAp. At 37°C, after about 15 h of reaction, the pH in the presence of cross-linked gelatin shows a rapid decrease rapidly until it reaches a value close to 7. These results explain why the rates of conversion of α-TCP as established by isothermal calorimetric data were higher in the presence of cross-linked gelatin fibers. At 45°C the influence of cross-linked gelatin on pH during the hydration of α-TCP shows similar behavior. In all instances the pH values reside in a range unlikely to be damaging to surrounding tissues.

4.1.6 Mechanical property measurements

The elasticity of gelatin fibers is understood to decrease due to the introduction of covalent cross links involving the glutaraldehyde [37, 38]. The influence of cross-linked gelatin on strength was evaluated by measuring the diametrical and compressive strength of the CDHAp composites prepared by the hydration reaction at 37°C. The weight ratio of the pre-composite solids to deionized water was 1:1. The extent of cross-linking did not preclude swelling when mixing with water and the swelling behavior was found to increase the proportion of deionized water needed to the prepare the pastes. The paste containing cross-linked gelatin was allowed to react for 7 days. The CDHAp samples formed in the absence of gelatin were allowed to react for 48 h at 37°C in 100% relative humidity as this duration was sufficient for complete reaction.

Figure 8 shows the results of the diametrical strength of CDHAp formed from the hydration of α-TCP and TetCP/DCP in the presence and absence of cross-linked gelatin at physiologic temperature. The incorporation of the cross-linked gelatin greatly decreases the diametrical strength of CDHAp-gelatin composites. The CDHAp containing 20 wt% of cross-linked gelatin exhibits diametrical strength of 1.2 ± 0.1 MPa, when formed from TCP and about 0.6 ± 0.1 MPa when formed from TetCP/DCP.

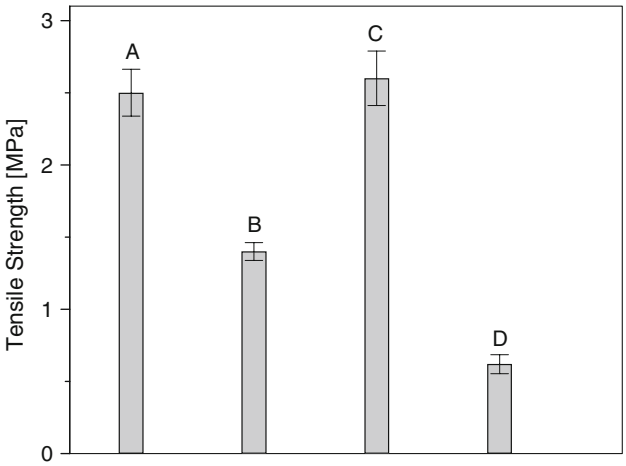

Fig. 8 Diametrical strengths of CDHAp formed from α-TCP (A); α-TCP + 20% crosslinked gelatin (B); formed from TetCP/DCP (C); TetCP/DCP + 20% crosslinked gelatin (D)

CDHAp formed in the absence of cross linked gelatin exhibits a diametrical strength of about 2.5 ± 0.2 MPa, regardless of the precursor used.

Two important factors cause the decrease in the diametrical strengths of CDHAp cross-linked gelatin composites. The first is the degree of porosity in the composites. The ratio between the inorganic precursor and deionized water when CDHAp formed in the absence of cross-linked gelatin was 3 to 1. However, to form composites in the presence of gelatin required a ratio of 1 to 1. The additional amount of water involved in the preparation of the composite containing cross-linked gelatin increased the degree of porosity, which in turn reduced the diametrical strengths. The second factor is that the CDHAp crystallites do not adhere to the cross-linked gelatin.

The fracture morphology of CDHAp formed from the hydration of α-TCP in the presence of cross-linked gelatin, as observed by SEM was characterized by the presence of agglomerates of platelet-shaped crystallites, porosity, and featureless areas. No evidence of gelatin fiber pull-out or of interaction HAp crystallites with the gelatin fibers was observed. This is indicative of poor adhesion between CDHAp and gelatin and would lead to lower composite tensile strengths.

Figure 9 shows the compressive strengths of CDHAp formed by the hydration of α-TCP and TetCP/DCP in the presence and absence of 20 wt% of cross-linked gelatin fibers at physiologic temperature. The presence of cross-linked gelatin also decreases the compressive strengths of the composites.

Increasing the glutaraldehyde concentration was found to induce an increase in the strengths of CDHAp-gelatin composites. The increase in the strengths occurs because the higher concentration of glutaraldehyde

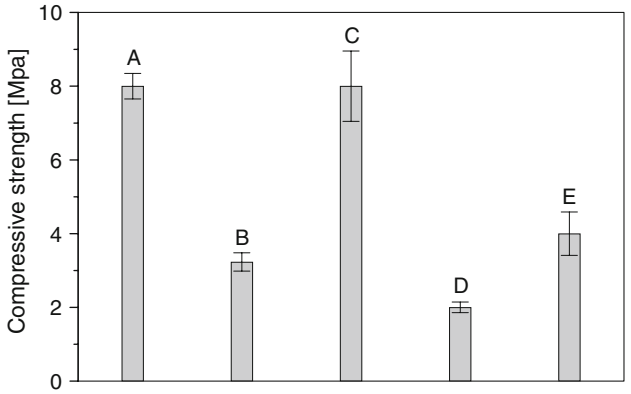

Fig. 9 Compressive strengths of CDHAp composites formed from TCP or TetCP/DCP as follows: (A) TCP, (B) TCP + 20% cross-linked gelatin, (C) TetCP/DCP, (D) TetCP/DCP + 20% gelatin cross-linked using 1.0 wt% glutaraldehyde and (E) TetCP + DCP + 20% gelatin cross-linked using 5.0 wt% glutaraldehyde

provokes a marked reduction in the extensibility of the gelatin fibers.

5 Conclusions

The presence of GA cross linked gelatin accelerated the rate of CDHAp formation regardless of the reaction temperature. The extent of acceleration correlated with the proportion of gelatin present thus indicating the ability of cross linked gelatin to aid in the nucleation of CDHAp. However, neither its presence nor the extent of cross-linking affected the total amounts of heat evolved as α-TCP or TetCP/DCP converted to CDHAp, thus indicating the absence of a mechanistic change. The molecular weight of gelatin had no significant effect on the kinetics of CDHAp formation.

Analyses of variations in solution chemistry during CDHAp formation revealed that pH variations were smaller in the presence of cross-linked gelatin fibers. The pH trends in pH variations, however, were in the presence and absence of cross-linked gelatin fibers, again indicating the absence of a change in the mechanisms of CDHAp formation.

The presence of cross-linked gelatin fibers in the CDHAp produced from either α-TCP or TetCP/DCP resulted in decreases the diametrical and compressive strengths of the composites when compared to the mechanical properties realized in the absence of gelatin. The presence of gelatin required higher proportions of water in order to adequately mix the constituents thereby producing composites with higher porosities. Elevated porosity and the poor adhesion with CDHAp crystallites reduced the diametrical and compressive strengths of the CDHAp/ cross-linked gelatin composites.

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