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Mechanical and degradation behavior of polymer-calcium sulfate composites

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Abstract Calcium sulfate (CS) is one of the oldest bone graft materials still in use. Its main limitations are poor handling characteristics, poor mechanical properties, and a resorption rate that is too fast for some applications. The present study investigated the effect of viscous polymers, such as carboxymethylcellulose (CMC) and hyaluronan (HY), on the handling characteristics, mechanical properties, and degradation behavior of CS. CMC and HY were added to CS at concentrations from 1–10 wt%. Addition of CMC to CS at more than 4 wt% produced a putty-like material and decreased the density of the composite, while also increasing flexural and compressive strength at higher loadings. Incorporation of CMC produced a concentration-dependent increase in water absorption and degradation rate. At an equivalent loading, HY-containing CS composites showed better compressive strength than CS with CMC. Overall, addition of CMC or HY to CS resulted in composite materials with better handling characteristics and improved mechanical properties after set, however the degradation rate of the augmented materials was increased. These properties suggest that the enhanced CS materials may be useful in certain clinical situations, such as filling non-uniform bone defects and situations that require mechanical integrity of the bone graft substitute during implantation.

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1. Introduction

An estimated 500,000 to 600,000 bone grafting procedures are performed annually in the United States [1]. Currently, synthetic substitutes represent only about 10% of the market, with the majority of grafts being derived from natural bone (e.g., autografts, allografts, and xenografts). Autografts, which are considered the gold standard, still have the commonly acknowledged problems of a need for a second surgery, limited availability, and donor site morbidity. Disadvantages of allografts include limited availability, as well as a lack of viable cells and the possibility (albeit remote) of disease transmission.

Attempts have been made to find a substitute for autografts, and some of the most promising biological results in non-load bearing situations have been seen with resorbable materials, such as calcium sulfate (CS) (e.g., [2–4]). CS is used for dental applications for ridge preservation and augmentation, sinus lifts, and as a barrier membrane as well as for treatment of orthopedic infections, tumors, surgically created voids, joint fusion, and fracture non-unions [5].

Calcium sulfate has a long history of use as a bone graft material and is well known for its osteoconductive properties. Recently, a better understanding of the mechanisms behind its success has been provided by the work of Ricci *et al.*, who showed that CS is replaced by a calcium phosphate lattice as it dissolves and that the release of calcium ions creates a local environment favorable for bone formation [5]. CS has been shown to increase angiogenesis and osteogenesis [6, 7]. Furthermore, CS can serve as a delivery vehicle for both antibiotics [8] and growth factors [9].

Unfortunately, calcium sulfate also has disadvantages. It is difficult to work with in its natural form, it is weak and brittle, and it can have a variable rate of resorption [1, 10].

Table 1 Amount of water needed to produce the most workable form of material

Material	ml/g
CS	0.80
CS + 5%CMC	1.16
CS + 7.5%CMC	1.25
CS + 5%HY	1.25
CS + 10%CMC	1.33

Recent work with CS has focused on using composites to minimize some of these problems [10–12].

The present work seeks to build upon the recent improvements in CS materials. Specifically, we sought to measure various characteristics of CS following the incorporation of carboxymethylcellulose and hyaluronan.

2. Materials and methods

2.1. Sample preparation

Calcium sulfate dihydrate (Sigma, St. Louis, MO) and was heated at 120°C for 72 hrs to produce a hemihydrate [13]. Medium viscosity carboxymethylcellulose and hyaluronan derived from rooster comb were obtained from Sigma.

For sample preparation, either CS alone or CS containing polymer was hand-mixed with deionized water and placed into an appropriate silicone mold. CMC was added at 5, 7.5, and 10 weight%, and HY was added at 5 weight%. Depending on the formulation, water was added to samples at concentrations ranging from 0.25–1.5 ml per g of material. Workability of the material was qualitatively assessed by manually manipulating the mixture between gloved fingers. Amounts of water (Table 1) resulting in the best handling characteristics were used for subsequent testing. The samples were allowed to dry at 40°C for 24 hr and then were removed from the mold. Selected samples were heat-treated at 150°C for 48 hr in an attempt to slow degradation rate by reducing water penetration into the samples. A minimum of eight samples were prepared for each formulation.

2.2. Mechanical testing

Mechanical properties were determined using a custom-built uniaxial testing system that was computer-controlled using LabView (National Instruments, Austin, TX). Samples were loaded until failure. Data were recorded, transferred to a Microsoft Excel spreadsheet, and plotted as force vs. displacement for analysis.

2.2.1. Flexural testing

Flexural testing samples were prepared in rectangular molds having dimensions of 50 mm × 10 mm × 3 mm. Samples were lightly ground on 800 grit diamond paper to give a smooth surface finish, and the average thickness was measured to account for shrinkage of the samples during set. Samples were tested in four-point bending at room temperature in air at a displacement rate of 1 mm/min. The testing jig had dimensions of 4 mm and 28 mm between the inner and outer pins, respectively. Ultimate flexural strength was determined at the point of maximum force before failure using the formula

$$\sigma_{\text{flex}} = \frac{3P_{\max}a}{wh^2},$$

where $P_{\max}$ is the maximum applied force, a is the distance between the inner and outer pins, w is the width of the beam, and h is the height of the beam. Flexural modulus was determined by linear approximation of the initial portion of the load-deflection curve and using the formula.

$$E = \frac{a^2(3L - 4a)}{wh^3}\frac{\Delta P}{\Delta d},$$

where L is the distance between the outer pins and d is deflection.

2.2.2. Compression Testing

For compression testing, cylindrical specimens were cast in molds having 6 mm height and 3 mm diameter. To account for shrinkage, the average diameter and height were measured after drying. The samples were also weighed to determine sample density. Samples then were tested in axial compression at a displacement rate of 1 mm/min. Ultimate compression strength was determined by $\sigma_{\text{comp}} = P_{\max}/\text{A}$, where $P_{\max}$ is the maximum axial load and A is the average cross sectional area. Compressive modulus was determined by linear approximation of the initial linear portion of the load-deformation curve.

2.3. Degradation

Samples having the compression testing geometry also were used for examining degradation. After measuring their initial mass, the samples were incubated at 37°C in 4 ml of PBS, pH 7.4. The PBS was aspirated, and the samples were weighed at 6, 12, 24, and 48 hr and then at two day intervals for 40 days. Samples were then weighed at 4 day intervals until 32 more days had elapsed or the samples had completely degraded. Degradation rate (or the rate of mass loss) was calculated from the initial linearly decreasing portion of the curve that followed absorption of water.

2.4. Statistical analysis

Statistical analysis was performed using INSTAT3 software (GraphPad Software, San Diego, CA). Differences in

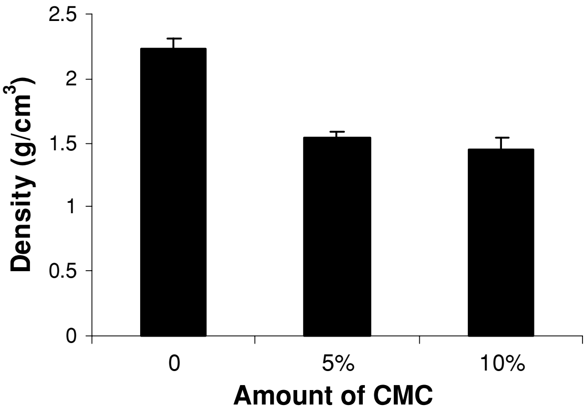

Fig. 1 Density of CS composites as a function of CMC added. Data are mean ± standard deviation ($n = 8$).

mechanical properties between treatments (amount of CMC, HY, or water) were analyzed for statistical significance using one-way ANOVA followed by *post-hoc* comparison using the Tukey test. Differences in maximal water uptake after heat treatment were examined using *t* tests. Degradation rates were compared by determining the significance of differences between slopes of the mass loss curves.

3. Results

3.1. Sample preparation

The handling characteristics of the materials depended on both the amount of polymer added as well as on the amount of water. Above approximately 4 wt% CMC, the material took on putty-like traits. The mixtures became more cohesive and formable as water concentration increased to a certain point, and beyond that the mixture became tacky and difficult to form. The amount of water needed increased with the amount of CMC added (Table 1).

Fig. 1 shows the density of CS composites as a function of CMC loading. There was a significant decrease in the density of the material with the addition of CMC ($p < 0.01$). Adding 5% CMC resulted in a 31% decrease in density, and 10% loading produced a 35% decrease. Although there was an apparent concentration-dependent decrease in density (10% CMC was 5.7% less dense than 5%), the difference was not statistically significant.

3.2. Flexural properties

Fig. 2 shows the flexural strength of CS composites as a function of the amount of CMC added. There was a significant increase in strength for all loadings compared to CS alone ($p < 0.01$). Materials formulated with 5% CMC produced a 99% increase, 7.5% CMC loading produced a 103% increase, and 10% loading produced a 124% increase in flexural strength. Although CS + 7.5% CMC was 2% stronger than CS + 5% CMC, and CS + 10% CMC was 10% stronger than CS + 7.5% CMC, these differences were not statistically significant.

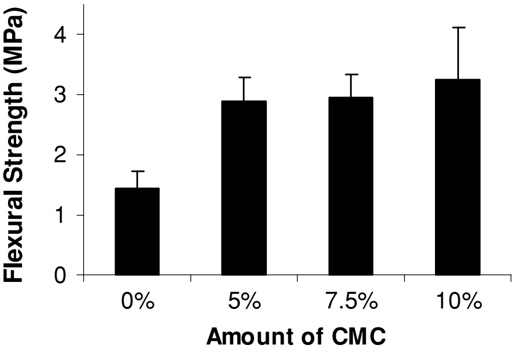

Fig. 2 Flexural strength of CS composites as a function of CMC added. Data are mean ± standard deviation ($n = 8$).

Fig. 3 shows a comparison of the flexural modulus of the same samples. Significant increases over the pure hemihydrate were seen for both the 5 and 10% CMC samples ($p < 0.001$). Loading 5% CMC resulted in a 40% increase, and 10% loading resulted in a 47% increase. There also was a small increase of 5.4% for the 7.5% CMC loading, which was not statistically significant.

Figs 4 and 5 show the flexural strength and modulus for samples loaded with 5% CMC as a function of the

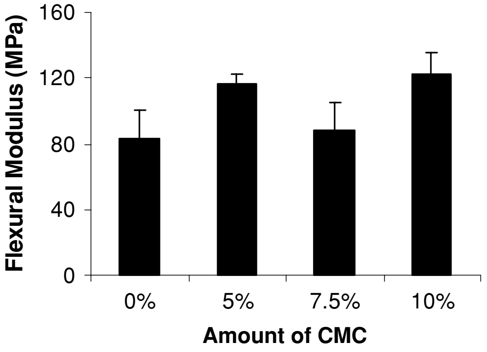

Fig. 3 Flexural modulus of CS composites as a function of CMC added. Data are mean ± standard deviation ($n = 8$–16).

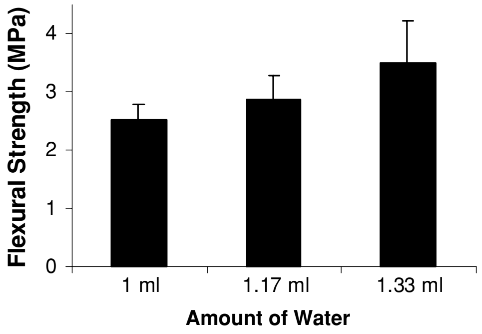

Fig. 4 Flexural strength for CS + 5% CMC as a function of water added. Data are mean ± standard deviation ($n = 8$).

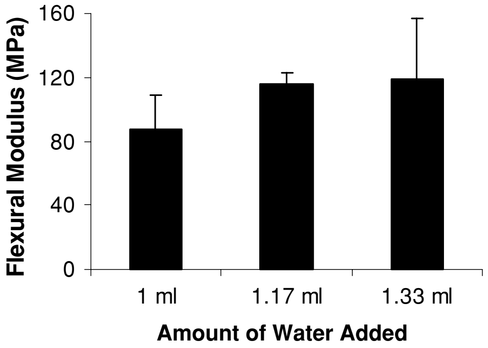

Fig. 5 Flexural modulus for CS + 5% CMC as a function of water added. Data are mean ± standard deviation ($n = 8$).

amount of water added. The water volumes shown are the minimum, optimal (for workability), and maximum values for which a putty-like material could be produced. There was a significant trend of increasing flexural strength with increasing mixing water ($p < 0.001$). Samples prepared with 1.17 ml/g were 14% stronger than samples with 1 ml/g, and samples prepared with 1.33 ml/g were 21% stronger than samples prepared with 1.17 ml/g. The flexural modulus was significantly higher for samples mixed with 1.17 ml/g and 1.33 ml/g over those mixed with 1 ml/g ($p < 0.05$). Samples mixed with 1.17 ml/g had a modulus 33% higher, and the samples mixed with 1.33 ml/g had a modulus that was 35% higher.

3.3. Compressive properties

Fig. 6 shows the compressive strength of the CS materials as a function of CMC added. Compared to unloaded CS, there was a significant increase in strength at the 7.5% and 10% CMC concentrations ($p < 0.001$). The 7.5% CMC samples had 88% higher compressive strength, and the 10% CMC samples had 85% greater strength. In contrast, there was a statistically significant 35% decrease in strength at the 5% loading ($p < 0.01$).

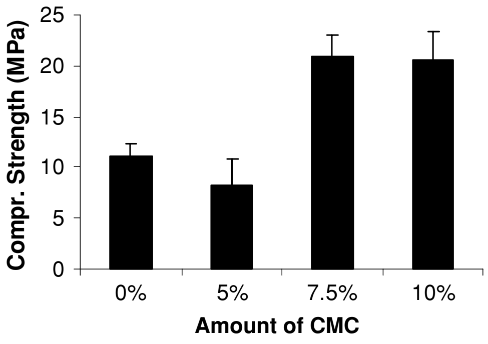

Fig. 6 Compression strength of CS composites as a function of CMC added. Data are mean ± standard deviation (n = 8–16).

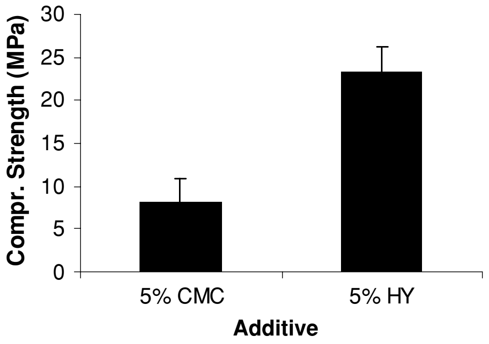

Fig. 7 Compression strength of CS comsposites as a function of additive. Data are mean ± standard deviation ($n = 8$).

Fig. 7 shows the compressive strength at equal loadings of HY and CMC. There was a significant difference in strength ($p < 0.001$). At 5% concentration, HY produced samples that were 110% stronger that plain CS and 185% stronger than samples loaded with 5% CMC. Strength of the HY-containing composites was comparable to that of the 7.5% and 10% CMC materials.

3.4. Degradation

Fig. 8 shows sample degradation with respect to CMC loading. There are two notable observations in this figure. First is the loading-dependence of the water uptake into the materials. All samples began at 100% mass, but this quickly increased as the samples absorbed water. The 0, 5, 7.5, and 10% CMC samples plateaued at 175%, 215%, 290%, and 359% of their initial mass, respectively. The second observation is the loading-dependence of degradation rate. Degradation was nearly linear for all samples, with the slope becoming more negative as the amount of CMC added increased. Using linear regression, the slopes were −1.6%/day, −3.0%/day, −5.8%/day, and −12%/day for 0, 5, 7.5, and 10% CMC loaded samples, respectively.

Fig. 9 shows degradation of 5% and 10% CMC samples dried at 40°C for 24 hr compared with similar samples dried

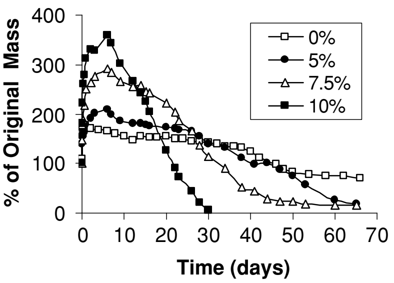

Fig. 8 Degradation of CS composites as a function of CMC added. Data are the mean of eight replicates. Error bars are not shown because they obscure viewing of the curves. They ranged from 3–8% of the mean at early times and from 12–27% at later times.

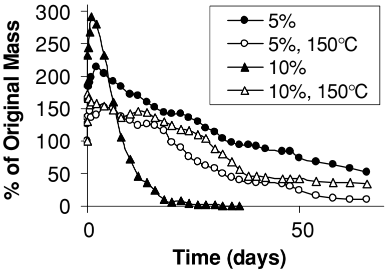

Fig. 9 Degradation of CS composites as a function of heat treatment. Data are the mean of eight replicates. Error bars are not shown because they obscure viewing of the curves. They ranged from 5–15% of the mean at early times and from 13–35% at later times.

at 150°C for an additional 48 hr. There was a marked decrease in water absorption with heat treatment. Untreated 5% CMC samples plateaued at 215% of their initial mass, and heat-treated samples plateaued at only 151%. Untreated 10% CMC samples plateaued at 281% of their initial mass, but heat-treated samples plateaued at 172% of their mass. Heat treatment also slowed degradation of the 10% samples (−2.2% treated vs. −12%/day untreated), and it was slowed slightly for the 5% samples (−2.3%/day treated vs −3.0%/day untreated). Heat-treated samples also were prepared for mechanical testing, but increased brittleness produced samples that were unfit to test.

Fig. 10 shows another direct comparison of 5% loadings of CMC and hyaluronan. CS containing HY absorbed less water than did samples with an equivalent loading of CMC. Whereas HY-loaded samples plateaued at 157% of their initial mass, CMC-loaded samples plateaued at 215%. Degradation also was slowed as the linearly approximated slope for the 5% HY was −1.7%/day, and the slope for the 5% CMC was −3.0%/day.

4. Discussion

4.1. Calcium sulfate

Calcium sulfate has the longest history of any synthetic bone graft material, dating back to 1892 [5]. Being skeptical about the positive reports regarding use of CS, Peltier investigated the material and showed its usefulness for filling a variety of bone defects [2, 14]. Recent work has not only confirmed the ostoeconductivity of CS, but some information regarding the mechanisms of its effects has been obtained [4, 5, 15, 16].

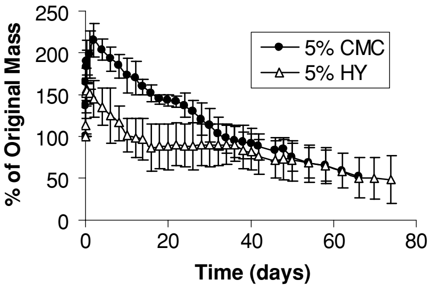

Fig. 10 Degradation of CS composites as a function of additive. Data are mean ± standard deviation ($n = 8$).

Despite its advantages, CS also has deficiencies. It is a brittle and weak material [5]. Also, the cement or plaster form available for dental applications can be difficult and clumsy to apply [10]. Depending on the source, CS can have a variable rate of resorption that makes it unsuitable for some applications [15].

Recent work with CS has focused on using composites to minimize some of these problems. Addition of CMC or hydroxypropylmethylcellulose solves some of the handling problems while maintaining the osteoconductive capacity of CS [10, 12, 17]. The augmented CS serves as a binder for bone grafts (demineralized bone matrix or cancellous bone chips). Wright Medical Technology's MIIG products are injectable CS formulations. Dissolution of CS can be slowed by coating with poly(L-lactic acid) [11].

The strength of calcium sulfate materials is generally given only in terms of compressive strength because it is prohibitively brittle for tensile applications. Mechanical properties also depend on the type of CS; type I dental plasters have the lowest strength, around 5 MPa, and nonimplantable dental stone materials having the greatest strength, exceeding 50 MPa [13]. Other factors that determine properties include additives, initial water to powder ratio, and drying time. CS has been shown to be most effective as an implant material if allowed to set outside of the body (as opposed to unset material being placed directly in a surgical site) because blood proteins interfere with setting to some degree [5]. A recent study showed that medical grade injectable CS reached a compressive strength of 2.4 MPa after 7 min of mixing and then increased to approximately 10 MPa after 1 hr [18]. There have been few controlled studies on how the various loading concentrations of additives, such as viscous polymers, affect the mechanical and degradation behavior of the material in a pre-set form.

4.2. Sample preparation

The production of a moldable putty with the use of CMC and HY is not only possible, but a relatively simple process. CMC concentrations of 4% or greater yielded a workable material, with the degree of workability increasing directly with concentration. For each concentration, the consistency of the material can be further manipulated by varying the amount of water in the formulation. At a single concentration of CMC, it is possible to produce materials with a consistency ranging from a dry dough-like material to a sticky paste. It should be noted, however, that decreasing the amount of mixing water may prevent the material from completely setting, leading to a non-uniform crystalline structure and therefore a weaker material. This is particularly true of samples containing higher polymer concentrations, because these polymers absorb water. Lower water volumes do not allow uniform and complete dissolution of the CS hemihydrate.

4.3. Flexural testing

There was a concentration-dependent increase in the flexural strength of CS with the addition of CMC. Without CMC, failure of CS flexural test specimens begins as a crack forms at one of the stress concentrators on or within the side of the sample that is in tension. The first points to yield to this stress are the higher energy, less stable crystal boundaries, so the crack propagates along these boundaries until failure occurs. In CS containing CMC, this process begins in a similar fashion, but propagation is interrupted by discontinuities in the structure presented by the macromolecules. Cracks begin, but their progress through the material is retarded, and the samples maintain structural integrity for a longer period. Furthermore, ionization of the carboxylic acid groups of CMC enables formation of ionic bonds with cations, such as the abundant calcium ions present in CS. Thus, additional reinforcement of the composite material may have resulted from the increased energy needed to debond ionically bound CMC from the CS matrix during crack propagation. The presence of CMC also appeared to stiffen the material, as all concentrations of CMC produced materials with higher flexural moduli than CS alone. Reinforcement of ceramic composites for flexural strength is well documented in literature. For example, addition of 30 wt% poly(L-lactide) to hydroxyapatite greatly improved the tensile and compressive properties of the material [19]. Similar results have been seen in other dental materials, such as Artglass resin (used for making dental crowns), which achieved significant increases in flexural strength of between 124% and 490% when augmented with polyethylene, Kevlar, or glass fibers [20].

There was a significant change in strength and stiffness of CS composites when the water used for mixing was varied between the minimum and maximum values that produced a putty-like material. The flexural strength and modulus appear to be exhibiting a concentration-dependent increase with added mixing water. This is both counterintuitive and contrary to the behavior noted in literature [5, 13], where the prevailing view is that the use of a larger amount of water than is strictly necessary causes interruptions in the precipitation of CS and leaves a matrix with less structural integrity. Extra water also can result in greater porosity as the excess water evaporates. However, low water concentrations made it difficult to produce a uniform consistency using our hand-mixing method, and it is possible that because of the hygroscopic polymers CS was unable to achieve adequate wetting to allow it to completely dissolve and reprecipitate.

The pronounced decrease in density of the CS samples with the addition of CMC is particularly interesting when compared with the improved mechanical properties. The resulting composite materials are stronger, stiffer, and lighter (less dense). Although the additives could be a source for crack initiation that could be detrimental to the strength of the material, this was not observed. The increased strength of composite material shows that the CMC or HY chains must be slowing crack propagation to an extent that overcomes any deleterious effects that may have occurred.

4.4. Compression testing

Additional testing showed that addition of polymers also can significantly improve the compressive strength of CS, especially at higher CMC concentrations. Some of this increase likely is due to the augmentation the macromolecules provide against the propagation of cracks along crystal boundaries. As described previously for the flexural strength, cracks running along these boundaries are forced to change direction or stop altogether when encountering an obstacle in the form of the polymer. The reason for the reduction in strength at 5% CMC still is not understood, but it is possible that at low concentrations the disruption of the crystalline matrix is greater than any augmentation that can be provided, or the porosity produced by the presence of the CMC molecules creates stress concentrators that serve as the beginning of cracks rather than as sites to stop their propagation.

Hyaluronan provided even greater augmentation to compressive strength than CMC and provided it at lower concentrations. Although 5% HY was the only concentration tested because of a limited supply, it produced some of the strongest samples in our study. Samples with 5% HY were stronger than those with 5% CMC and were comparable those with 10% CMC. Perhaps incorporation of HY resulted in a more uniform distribution within the CS matrix or resulted in few internal defects that initiated cracks. These results merit further investigation.

4.5. Degradation testing

Degradation testing revealed the major weakness of the composite CS materials. Over the first two to six days of immersion in physiological saline, there was a clear increase in the weight of the samples as the materials absorbed water. Samples augmented with CMC showed a concentration-dependent increase in the amount of water they absorbed, which was associated with a respective swelling of the material. At 10% CMC, samples took on a spongy appearance and swelled to about twice their original size. Paired with this increase in size and water content was an increase in degradation rate. Swelling and penetration of PBS into the interior of the samples increased the surface area available for erosion of the material, and there was a distinct concentration-dependent relationship between the amount of CMC added and the rate of degradation. The swelling may be desirable when filling more complex bone defects.

High temperature treatment was examined as a method for maintaining strength gains from the addition of CMC while slowing the degradation rate of the composites. At 150°C, CS is converted to the anhydrite form, which is much less soluble than the hemihydrate [13]. Heat treatment was successful at reducing water uptake and slowing the rate of degradation, and it appeared that the treatment was successful at slowing the rate of water penetration to the interior of the samples. For this reason, heat-treated samples also did not exhibit the swelling seen in samples with equivalent CMC concentration without heat treatment. Unfortunately, heat-treated samples lost all gains in mechanical strength and were prohibitively brittle for mechanical testing.

Interestingly, CS samples loaded with HY absorbed less water than samples loaded with an equal amount of CMC. Addition of HY also resulted in a slower degradation rate than an equivalent amount of CMC. It was expected that hyaluronic acid samples would absorb larger amounts of water than the CMC samples, as a high water holding capacity is a significant characteristic of HY, but the opposite was observed.

5. Conclusion

Addition of CMC or HY to CS resulted in composite materials with better handling characteristics and improved mechanical properties after set. However, addition of these viscous polymers proved to be a compromise, as the degradation rate of the augmented materials was increased. The samples have been strengthened to allow the insertion of the devices, the swelling of the material keeps it in contact with bone, and the addition of an appropriate amount of polymer may allow manipulation of the degradation rate to assure that degradation occurs at a rate comparable to that at which bone is formed. These properties suggest that the enhanced CS composite materials may be useful in certain clinical situations, such as filling non-uniform bone defects and situations that require mechanical integrity of the bone graft substitute during implantation.

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