Ca-DEFICIENT HYDROXYAPATITE SCAFFOLDS FROM INJECTABLE CALCIUM PHOSPHATE/CHITOSAN CEMENTS

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Abstract

Calcium phosphate cement (CPC) has been successfully used in bone tissue regeneration for many years. However, poor mechanical properties and low biodegradation rate limit any further applications. R-cement has a higher solubility than conventional CPC and its reaction products (CDHA) are similar to the mineral phase of bone.

In this work we have developed new CPC composition which consists of a mix of cement R, glycerol as a liquid phase carrier and a biodegradable hydrogel (chitosan) which acts as a binder and was incorporated into R-cement to strengthen this biomaterial. The cement past was found to be stable in a syringe (even after two month of storage in the freezer) and hardened only after being exposed to biological fluids.

Keywords: *calcium phosphate cement, injectability, chitosan*

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Introduction

Calcium phosphate cement (CPC) can be molded or injected to form a scaffold in situ, it has excellent osteoconductivity, and can be resorbed and replaced by new bone [1]. Because of the apatitic nature of the set cement, it is highly compatible with soft and hard tissues [2]. The advantages an injectable CPC cement include: shortening the surgical operation time, minimizing the invasive surgery, reducing postoperative pain and scar size, achieving rapid recovery, reducing cost [3]. For implantation of CPC many surgeons want a material which after implantation in bone is completely resorpted within the shortest possible time, at least when this resorption is compensated by the growth of the new bone [4]. Although most of calcium phosphate cements are more resorbable than sintered hydroxyapatite ceramics, they still show relatively slow resorption kinetics and in many cases the cement remains stable in the implanted site during years. K, Na, Ca - Phosphate (cement R) has a higher solubility than conventional CPC and its reaction products are similar to the mineral phase of bone (CDHA). However, pure mechanical properties limit any further applications. The biopolymer chitosan was incorporated into R-cement to strengthen this biomaterial. Chitosan and its derivatives are natural biopolymers that are elastomeric, biocompatible and resorbable [5]. Chitosan incorporated into CPC, can yield higher flexural strength, toughness, and strain-to-failure [6]. In this work a cement R/chitosan blend was developed with the advantage of being moldable (the CPC paste intimately adapts to the bone cavity) and capable of in situ setting to form calcium deficient hydroxyapatite (CDHA) under physiologic conditions (in an aqueous environment at body temperature).

Materials and methods

R-cement was prepared by mixing two powders: $(Ca_2KNa(PO_4)_2)$ and $(H_2PO_4)_2{\cdot}H_2O$ as a catalyst in the correct stoichiometric ratio. As a liquid phase we have used a gel mixture of dehydrated glycerol and chitosan prepared by dissolution of the chitosan powder in glycerol under constant stirring during few hours. The optimum required quantity of the polymer in the formulation was chosen by estimating the improvement in cohesion and injectable properties of the paste. The powder and liquid were manually mixed with a spatula to form a cohesive past that was filled into a stainless steel mold of 10 mm diameter and 3 mm deep and immersed in a Ringer's solution. The time measured from the powder-liquid mixing to the time after which the cement paste reached a point of stiffness where it becomes unworkable, was used as the setting time for the specimen.

In order to determine the stability of the paste after two months, syringes with and without chitosan paste compositions were stored in the freezer at -18°C.

Injectability tests were performed at a constant injection speed of 15 mm/min using a MTS 858 BIONIX. The injectability was reported as a weight loss percentage between the cement extruded from the syringe and the cement remained inside the syringe. XRD analysis (Bragg-Brentano PANalytical X'Pert PRO MPD) was used to examine the effect of chitosan on the R cement conversion to hydroxyapatite. To evaluate the compressive strength of the cement, the paste was moulded into cylinders (6 mm in diameter, 12 mm in length). Then the samples were incubated in Ringer's solution for 6 hours, 1, 3, 7 and 14 days and then, compressive strength was measured, using a universal material testing machine (Adamel Lhomargy, DY 32/34). Each measurement was repeated five times. The compressive strength was calculated by using the fracture load divided by specimen's cross-section area. Immediately after the samples had been tested in compression, water of specimens was eliminated by dipping them in acetone and then dried in an oven at 60°C to stop hydrolysis reaction. The microstructural development was investigated by scanning electron microscopy (SEM Jeol JSM 6400) on the broken surfaces of specimens previously tested in compression.

Results and Discussions

Cement setting time is listed in TABLE 1. R cement with water had a relatively slow setting time. The setting time increased with the presence of glycerol reaching 50 min. The samples stored in the freezer for two months showed a significantly lower setting time of 29-32 min. The addition of chitosan into the cement composition had minimal increase in the initial setting time of samples after storage in the freezer.

Injectability tests were performed on time zero samples as well as on the samples after two month of storage in the freezer. The injection force is presented in FIG. 1.

TABLE 1. R-cement setting time for time-zero and two-month samples.

	Time-zero samples		Two-month samples	
	Initial time	Final time	Initial time	Final time
CR/water	<10 min.	16-20 min.		
CR/glycerol	<50 min.	4.00 h	<29 min.	3.30 h
Cr/glycerol/ chitosan	<50 min.	4.00 h	<32 min.	3.30 h

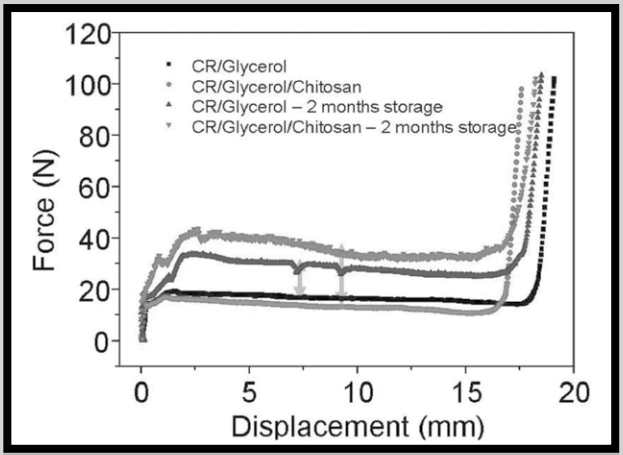

FIG. 1. Injection force vs. syringe displacement for R-cement and R-cement/chitosan pastes before and after storage in the freezer.

Typical curves for injectable paste are shown for both: time zero samples and two-month samples. However, a small difference is observed in the applied force in the second case, which is about 20 N higher. Probably, it is due to the small reaction with water from ambient from the residual impurity of glycerol. It is not significant and the blend can be easily injected anyway. The cement was extruded and a maximum force of 100 N was achieved, because higher forces may not be practical in manual injection during surgery. The percentage of extruded paste was determined as the mass of the paste that could be extruded from the syringe divided by the original mass of the paste inside the syringe, which was about 96% for both CR/glycerol and CR/glycerol/chitosan samples.

FIG. 2 presents the X-ray diffraction patterns of the cements. After 1 day of immersion, characteristic peaks of CSPP were still present. After 7 days CSPP's peaks disappeared, and peaks around 2□ = 26° and 32° attributable to hydroxyapatite were observed, suggesting the totally convertion of R cement into CDHA. Basically, both types of R-cement (CR/glycerol and CR/glycerol/chitosan) showed the same XRD pattern before and after two month stored in the freezer.

The effects of chitosan on mechanical properties of the R cement after different immersion times in Ringer's solution are shown in FIG. 3a. For CR/glycerol/chitosan the maximum compressive strength was attained after 3 days of immersion, while the highest value of compressive strength was observed after 1 day for time-zero samples and after 3 days for samples which were stored in the freezer (FIG. 3b). The addition of chitosan seemed to increase the compressive strength of the cement after storage in Ringer's solution for 14 days.

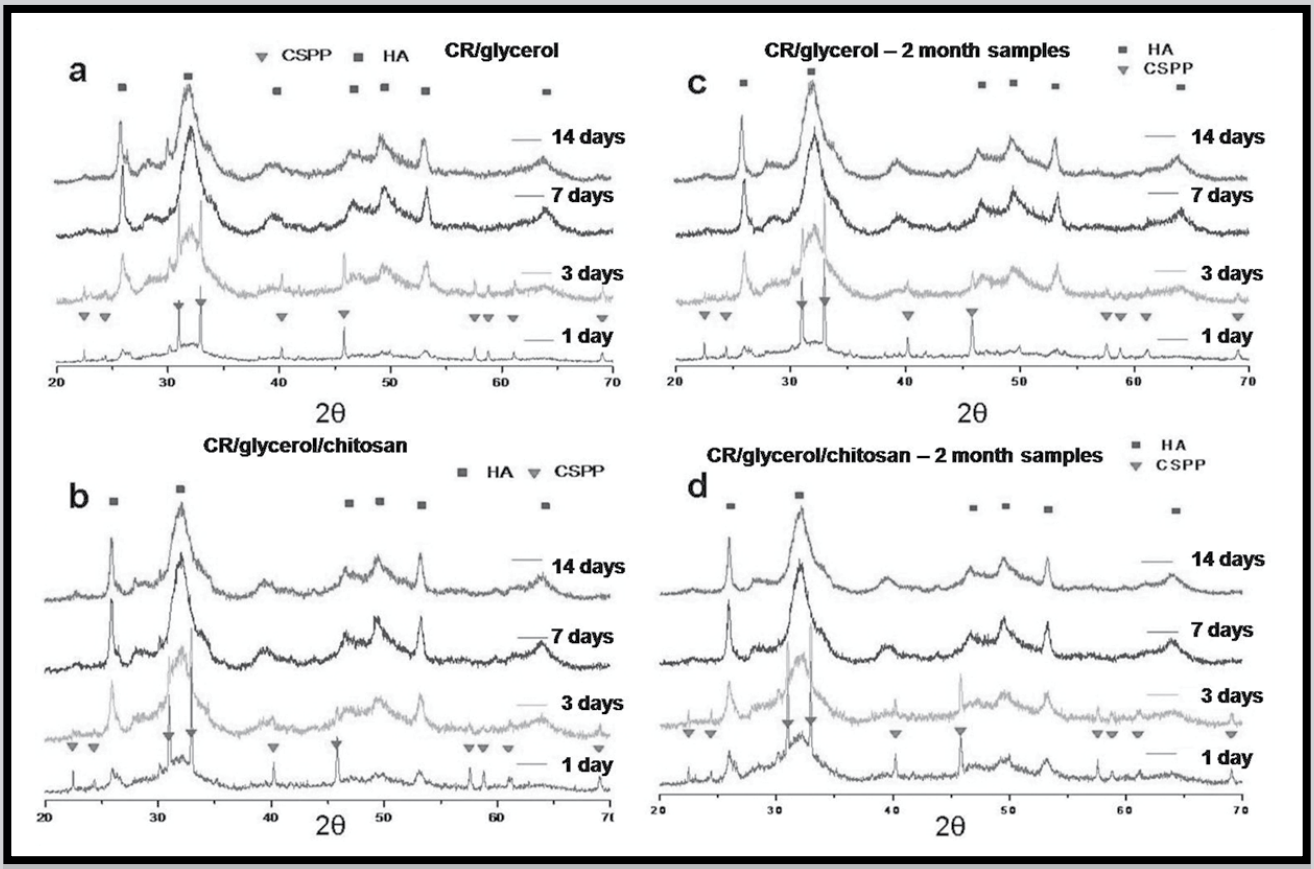

FIG. 2. The X-ray diffraction spectrum of the (a) R cement/glycerol and R cement/glycerol/chitosan (b) samples after different times (1, 3, 7 and 14 days) of immersion in Ringer's solution. (a-b) time-zero samples, (b-c) samples after two month of storage in the freezer.

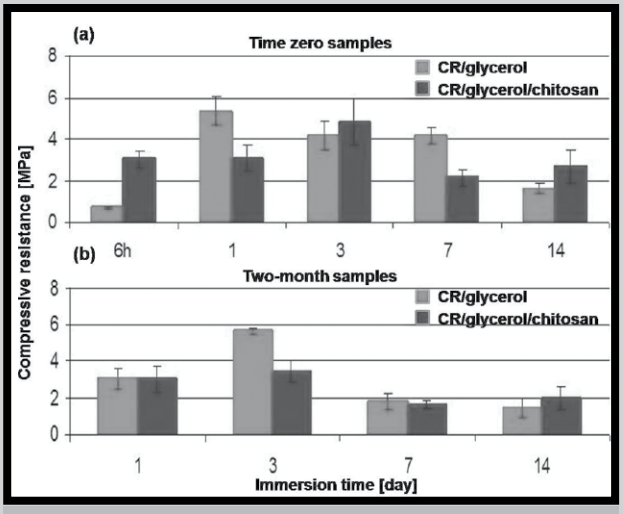

FIG. 3. Mechanical properties of (a) time zero samples after different times (6h, 1, 3, 7 and 14 days) of immersion in Ringer's solution and (b) samples after two-month of storage in the freezer.

Typical scanning electron microscopic pictures of the fractured surfaces are shown in FIG. 4. It can be seen that granular crystals appeared on the surface of specimens soaked in Ringer's solution for three days. In both cases SEM micrographs show the formation of nanosized hydroxyapatite crystals in flowery-like morphologies. There were no significant changes in the morphology of R-cement before and after two-months storage in the freeze.

Conclusions

The cement setting is the result of a dissolution and precipitation process, and the entanglement of the precipitated crystals is responsible for cement hardening [7]. The set samples consisted of calcium deficient hydroxyapatite (CDHA), as determined by X-Ray diffraction, where no compositional change was produced by the chitosan addition. XRD results showed no difference between R cement before and after two month of storage in the freezer. R cement/glycerol/chitosan blends were produce with good injectability and cement setting features in a physiological fluid. R cement paste composition was developed to shorten the surgical time by avoiding the one-side powder-liquid mixing and to improve the cement mechanical properties (addition of chitosan). The addition of chitosan seemed to increase the compressive strength of R cement after 2 weeks of immersion in Ringer's solution but further studies are needed to investigate the long term resorption rate of R cement/chitosan blend.

Acknowledgments

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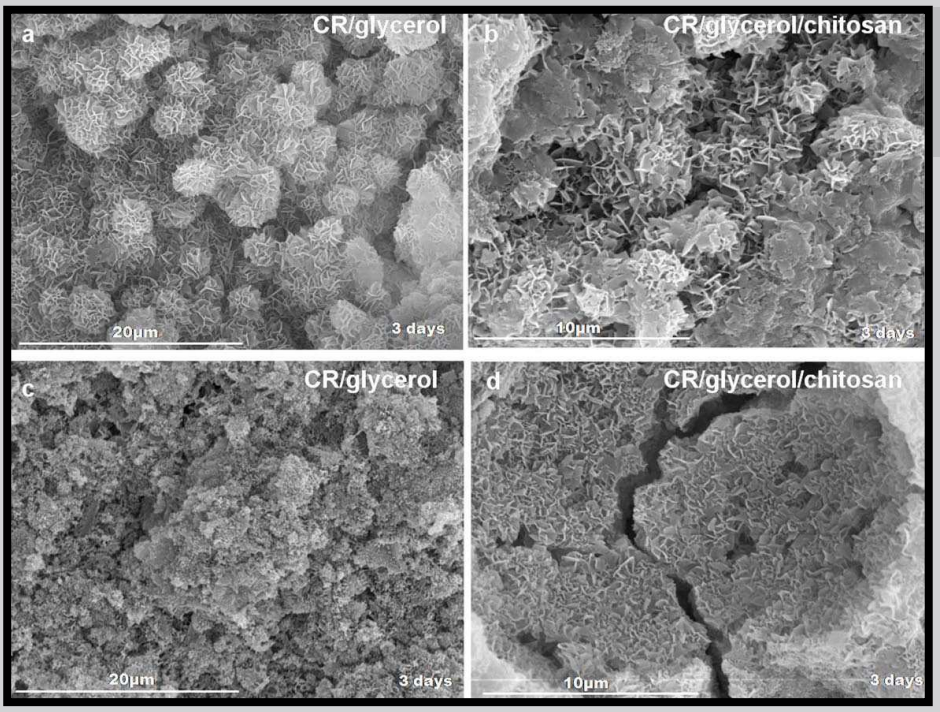

FIG. 4. SEM micrographs of R cement scaffold: (a-b) time zero samples; (c-d) two-month samples.

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