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Evaluation of chitosan/β-tricalcium phosphate microspheres as a constituent to PMMA cement

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Two methods, a traditional emulsion technique and a high voltage electrostatically modified encapsulation system, were used to fabricate degradable chitosan/β-tricalcium phosphate (β-TCP) microspheres. The two distinct kinds of microspheres both exhibited good sphericity and the β-TCP was trapped well inside the chitosan gel. The microspheres prepared by high voltage electrostatic system exhibited a rougher outer surface and narrower size distribution. These microspheres were then used as an added constituent to commercially available PMMA bone cement. Four modified cement composites that were prepared with different composition ratios of the two kinds of chitosan/β-TCP microspheres that were made from emulsion technique (C1P1 and C2P1) and from a process by a high voltage electrostatic system (EC1P1 and EC2P1) were compared with the PMMA cement (Pure P). The characteristics of these materials indicate that with the addition of chitosan/β-TCP microspheres as a constituent into the PMMA cement significantly decreases the curing peak temperature. Furthermore, the setting time increases from 3.5-min to 9-min, as compared to the PMMA cement. These changes could be beneficial for the handling of the bone cement paste and causing less damage to the surrounding tissues. Understandably, the presence of chitosan/β-TCP microspheres in the prepared composites reduced the ultimate compressive strength and bending strength. From the degradation test and SEM observations, the modified chitosan/β-TCP/PMMA composites could be degraded gradually and create rougher surfaces that would be beneficial to cell adherence and growth.

1. Introduction

Polymethylmethacrylate (PMMA) cement has widely been used as a fixation filler that is injected into the gaps between the prosthesis and the surrounding bone [1, 2]. Despite its wide acceptance as a grouting material in dentistry and orthopedic applications, PMMA cement has some inherent problems. Firstly, the high heat generated during its hardening polymerization process, which has been shown to cause damage to the surrounding bone tissue. Secondly, poor compatibility with bone: PMMA does not adhere to the bone nor induce bone formation and may contribute to mobility of the implant and, later on, aseptic loosening may occur after long-term implantation [3]. Several attempts to improve the situation were made by the addition of small quantities of ingredients such as bioactive tricalcium phosphate (TCP) or hydroxyapatite (HA) into the matrix of PMMA bone cement [4, 5]. Other researchers have found that the addition of hydroxyapatite (HA) can enhance the mechanical properties of bone cement and the extent of changes varied depending on the type of bone cement and the amounts of HA added [6]. HA has also shown to reduce the potentially harmful heat generated during the polymerization of PMMA bone cement [7]. Although HA is a bio-resorbable calcium phosphate and has good biocompatibility with bone, the resorption rate is much too slow in the physiological environment and thus results in an undesirable long-term HA ceramic inter-surfacial effect to the implant. The microscopic form of β-tricalcium phosphate (β-TCP) is another bio-resorbable and biocompatible ceramic with similar characteristics to the inorganic moiety of natural bone. From clinical practice and experience, β-TCP has a more favorable resorption pattern and osteotransduction property than HA. Besides, β-TCP can also be gradually adsorbed following new bone formation and is often incorporated as an additive to bone-grafting and dental materials.

When β-TCP is considered as a component of bone cement material, conditions such as proper injectability,

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appropriate setting time and mechanical strength, bioactivity and low setting temperature, all need to be monitored closely during the treatment of bony fractures or related applications. There are many formulations of in-situ moldable cements commercially available that could meet these requirements. However, for some formulations, the resorption patterns and rates are not compatible with the bone remodeling process and thus often cause secondary failure after long-term period of implantation [8–10]. To improve the situation, we attempt to incorporate β-TCP as an ingredient into the commercial PMMA bone cement powder for preparation of the cement composite. Particulate or porous β-TCP ceramic has proven successful as resorbable hard tissue replacements when low loads are applied to the system. With the degradation of this ceramic, the bone tissues can grow on or into the pores of this material and thus replacement may follow. However, the β-TCP ceramic in particulate form, being incoherent to each other, may be easily washed off from the wound area. It seems that to group the particulate ceramic into manageable packages by a process of capping or encapsulating would be highly desirable.

Chitosan is a biopolymer with high potential and often used in orthopedic applications to provide and temporary mechanical support in the regeneration of bone cell due to its biocompatible, biodegradable, osteoinduction, and non-toxic characteristics [11, 12]. Furthermore, it can be fabricated into various shapes and forms such as film, fiber, microsphere or powder to improve its potential applications in biomedical field [13–15].

Our aim of this study is to prepare a chitosan/β-TCP containing PMMA bone cement composite system to outline a rough application domain of interest by characterizing a few formulations for their thermal, mechanical and degradable properties. We prepared the chitosan/β-TCP into two forms of microspheres: one by using of emulsion technique and another by high voltage electrostatic system. The resulting chitosan/β-TCP microspheres were incorporated as an additional constituent to commercial PMMA cement [4, 16] in a series of formulations. By bringing a biodegradable material into a "barren" PMMA resin we hope that a modified bone cement may become biocompatible. Consequently, under controlled hydration over the interface of chitosan/β-TCP/PMMA cement composite, the modified material could degrade partially in physiological environment and induce bone tissues or cells adherence and growth. We examined a series of composites for their properties of curing temperature, setting time, compressive strength, compressive modulus, bending strength and bending modulus. In addition, degradation behaviors were examined. Furthermore, osteoblast cell cultures and animal studies are currently underway in our lab.

2. Materials and methods

2.1. Materials

The bone cement used in this study was purchased from Howmedica Int. Ltd. (Ireland). Chitosan was purchased from TCI, Tokyo, Japan, with the molecular weight of 300,000, deacetylation degree 83%. β-tricalcium phosphate was purchased from Merk-Schuchardt (Germany). Glutaraldehyde (50% w/v) and sodium tripolyphosphate ($Na_5P_3O_{10}$, 5%) were purchased from SHOWA (Tokyo, Japan). Acetic acid, mineral oil, span-80 and acetone were purchased from Sigma (St. Louis, MO). All chemicals used in this study were of reagent grade.

TABLE I Compositions of composites[a]

Item	Chitosan/β-TCP microspheres	PMMA cement powder	Abbreviation
A	0	100	Pure P
B	50[b]	50	EC1P1
C	66.7[b]	33.3	EC2P1
D	50[c]	50	C1P1
E	66.7[c]	33.3	C2P1

[a]Number represents part (in weight percent) for each component.
[b]Microspheres prepared by electrostatic system.
[c]Microspheres prepared by emulsion method.

2.2. Preparation of chitosan/β-TCP microspheres

The chitosan/β-TCP microspheres were prepared by the water/oil emulsion method and by a high voltage electrostatic system that developed by our lab [16]. To describe the procedure briefly: a 2% (2 g) solution of

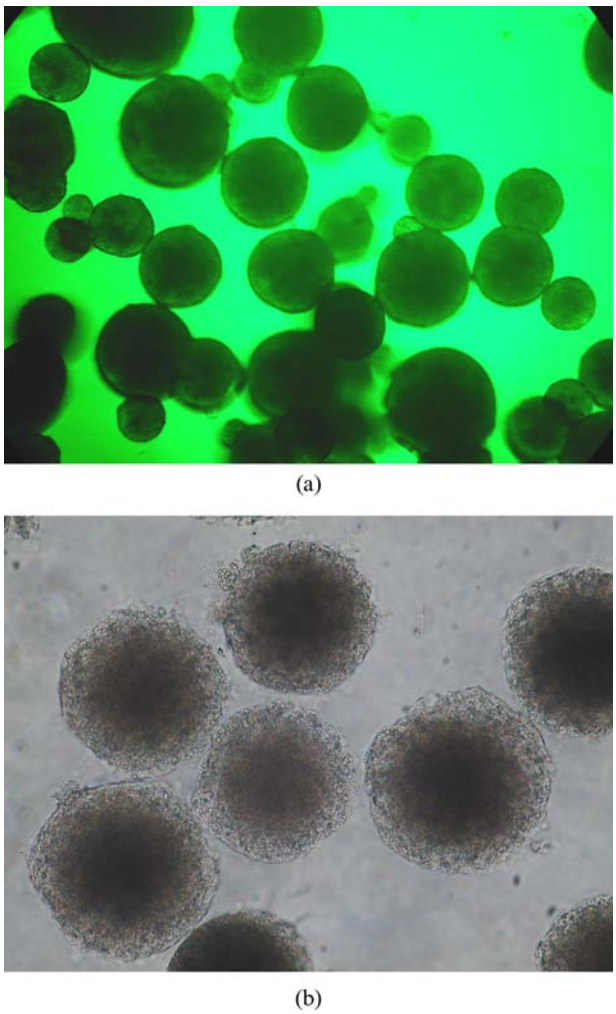
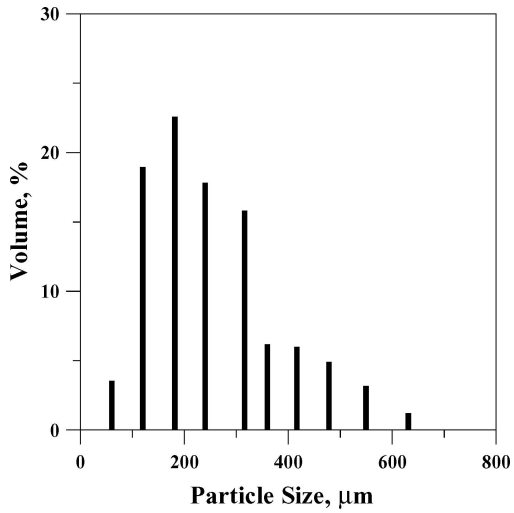


Figure 1 Photomicrographs of chitosan/β-TCP microspheres prepared by: (a) emulsion method and (b) electrostatic system.

chitosan was dissolved in 0.1 N acetic acid solution (100 mL). β-TCP powder (3.72 g) was then poured into this solution and stirred for 8 h to allow β-TCP powder to be well dispersed. A portion of this mixture was then put into mineral oil to prepare chitosan/β-TCP microspheres (emulsion method) and the remaining was put into a syringe pump by extruding chitosan/β-TCP droplets into a 5% $Na_5P_3O_{10}$ solution to prepare chitosan/β-TCP microspheres. The preparation parameters were set according to our previous research results [16]. At the end of preparation period, the microspheres were collected and dried in vacuum and kept in a desiccator for future use.

2.3. Evaluation of chitosan/β-TCP microspheres

A Laser Diffraction Particle Size Analyzer (Coulter LS-230, USA) was used to analyze the size distribution of microsphere samples. The β-TCP entrapment efficiency was examined by micrographs taken from an optical microscope (Olympus, IX-70, Japan). The SEM (Hitachi, S-2700, Japan) micrograph was also used to evaluate the surface morphology of these microspheres.



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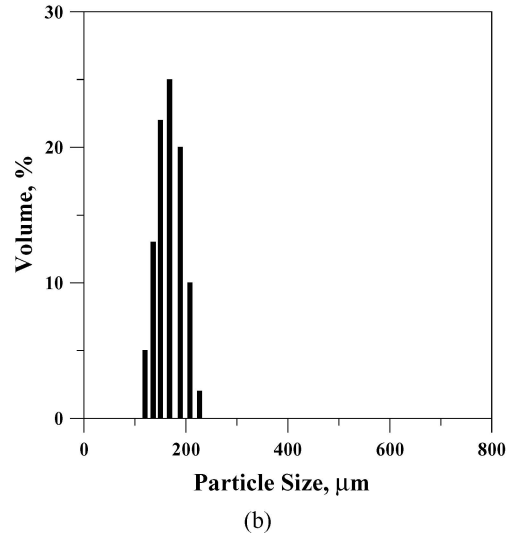


(b)

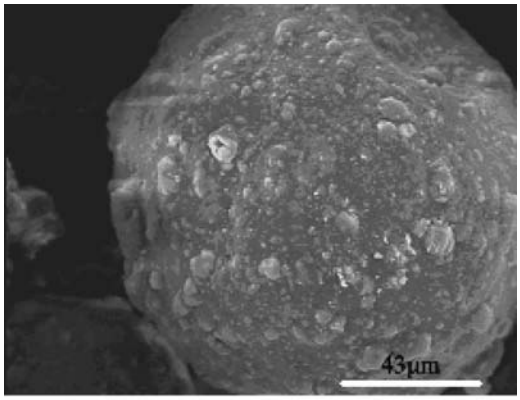
Figure 2 The size distribution of chitosan/β-TCP microspheres prepared by: (a) emulsion method and (b) electrostatic system.

2.4. Preparation of chitosan/β-TCP/PMMA cement composites

PMMA bone cement powder was mixed with the prepared chitosan/β-TCP microspheres at predetermined weight ratios (Table I). The powder mixture and liquid monomer were mixed at room temperature of the weight ratio 2:1 (according to the cement manufacturer's direction) to prepare chitosan/β-TCP/PMMA composites. After the mixing, the mixed dough was poured into a Teflon cylindrical mold (5 mm in diameter and 4 mm in depth). The setting time and curing temperature of this hardening polymerization were recorded and determined (Digital thermometer, Model 305, Taiwan). In order to evaluate the influences of different preparation methods of chitosan/β-TCP microspheres that may impact to the properties of these composites, we monitored and examined their changes between these composites and classical PMMA bone cement.

2.5. Mechanical test of chitosan/β-TCP/PMMA cement composites

The mechanical properties of these composites (cylindrical specimens with a height of 25 mm and a diameter of 10 mm) were measured by using an MTS System (Eden Prairie, USA). These specimens were compressed or bent at a speed of 10 mm/min and the mechanical parameters were recorded automatically by the computer system.



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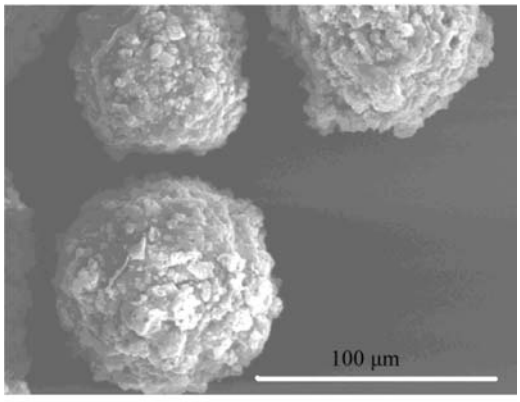


(b)

Figure 3 SEM of chitosan/β-TCP microspheres prepared by: (a) emulsion method and (b) electrostatic system.

2.6. *In vitro* degradation measurement

The prepared samples were immersed in a beaker that contained 50 mL of phosphate buffer saline solution (pH 7.4) and were then put into a 37 °C shaker for slow shaking. The degradation ratio was expressed by the weight loss of these samples after a particular period. The SEM micrographs were also utilized to observe the morphological changes of these composites after degradation test.

3. Results and discussion

3.1. Microspheres morphology

The chitosan/β-TCP microspheres prepared by emulsion method and electrostatic system showed good sphericity and β-TCP was well entrapped inside the chitosan membrane (Fig. 1). The microspheres prepared by emulsion method ranged in diameter from 60 to 600 μm, whereas the size distribution of microspheres prepared by electrostatic system was narrower, from about 120 to 220 μm (Fig. 2). Observed from the microphotographs, microspheres prepared by the electrostatic system exhibited a rougher boundary between the chitosan membrane and entrapped β-TCP, whereas microspheres prepared by the emulsion method had a thinner chitosan membrane layer. These results apparently were caused by the difference in fabricating methodologies and crosslinking reagents of microspheres utilized in this study. Actually, microspheres prepared and crosslinked with glutaraldehyde in oil phase (emulsion process) exhibited a smoother surface as compared to microspheres prepared and crosslinked with $Na_5P_3O_{10}$ in aqueous phase (electrostatic system). SEM micrographs also confirmed these observations and revealed that microspheres prepared by electrostatic system exhibited a rougher surface with wrinkles (Fig. 3).

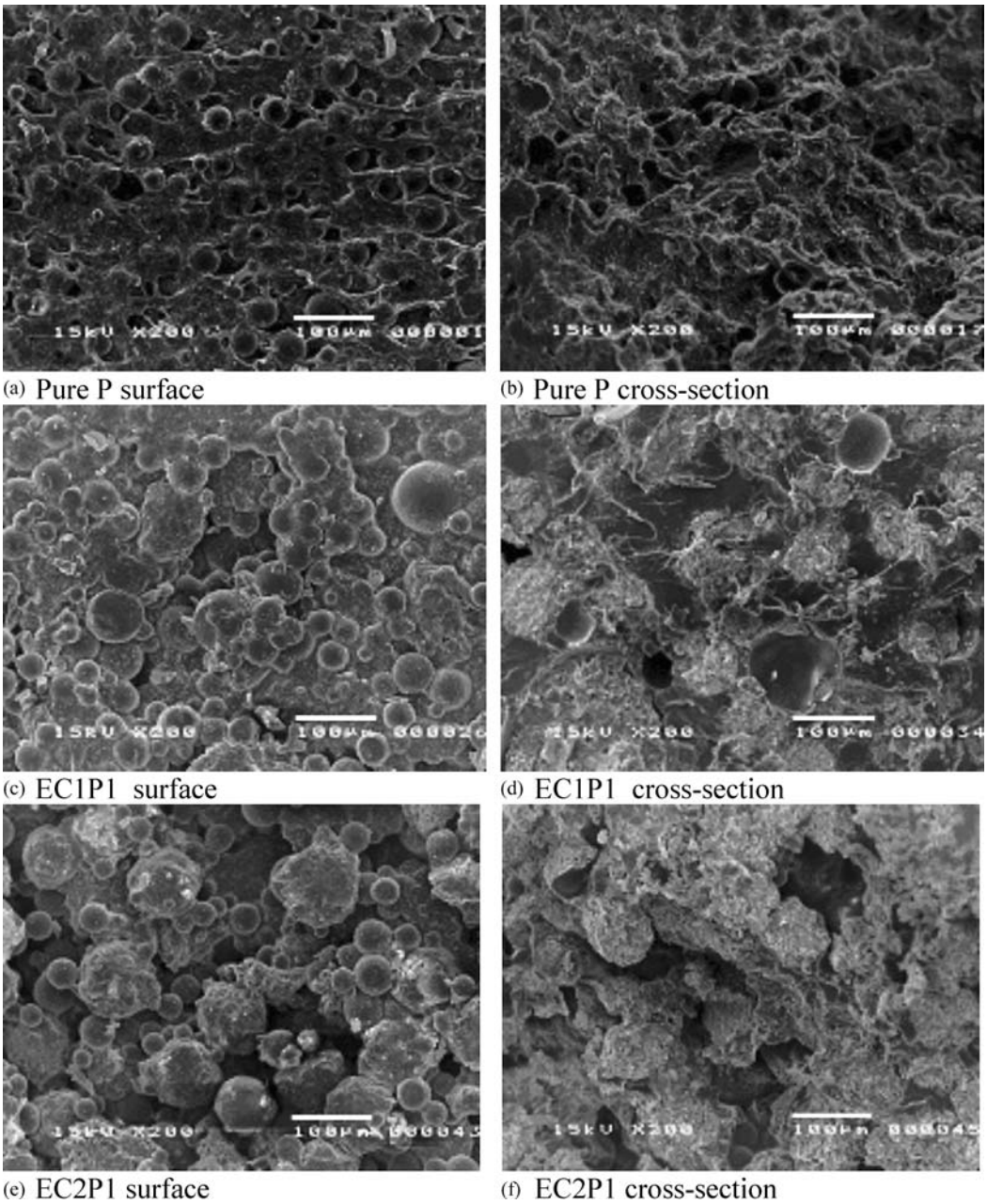
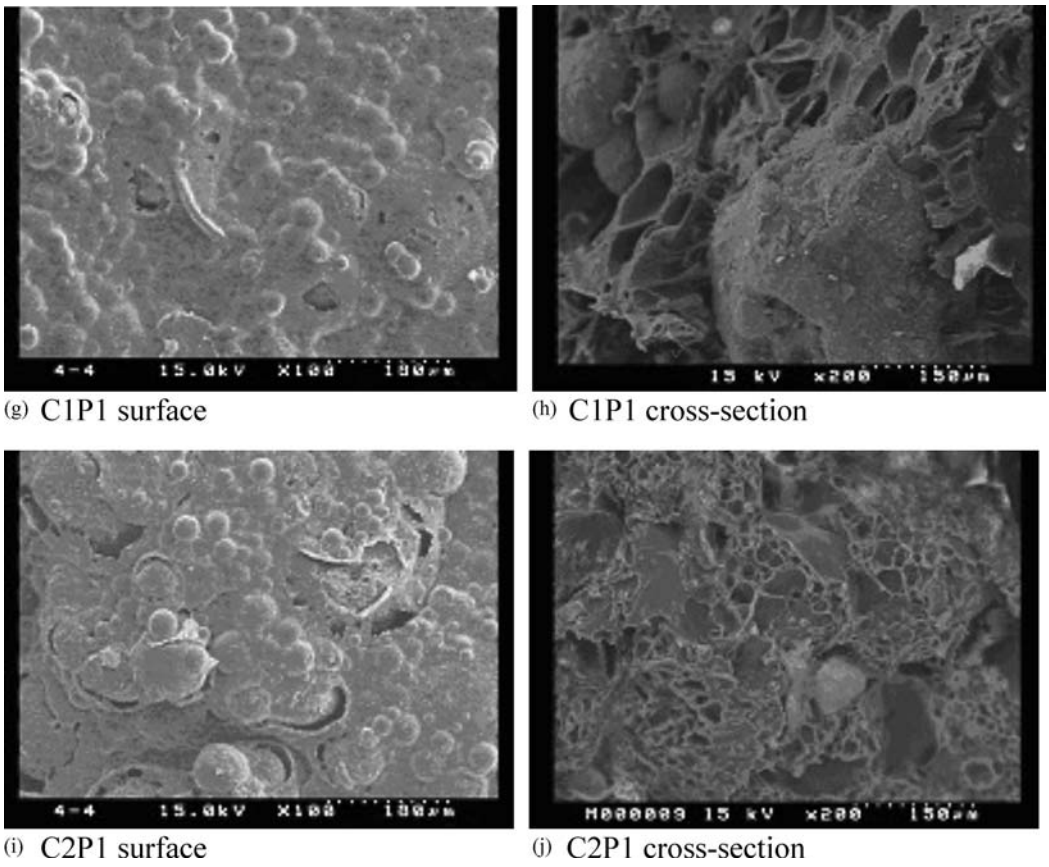


Figure 4 SEM of the composites: (a) surface morphology of Pure P, (b) cross-sectional morphology of Pure P, (c) surface morphology of EC1P1, (d) cross-sectional morphology of EC1P1, (e) surface morphology of EC2P1, (f) cross-sectional morphology of EC2P1, (g) surface morphology of C1P1, (h) cross-sectional morphology of C1P1, (i) surface morphology of C2P1 and (j) cross-sectional morphology of C2P1. (*Continued*)



(g) C1P1 surface (h) C1P1 cross-section

(i) C2P1 surface (j) C2P1 cross-section

Figure 4 (*Continued*).

3.2. Morphological and physical characteristics of chitosan/ β-TCP/PMMA cement composites

Fig. 4 shows the SEM micrographs of surface and cross-section morphology of chitosan/β-TCP/PMMA cement composites. As shown, the prepared chitosan/ β-TCP microspheres mixes well with PMMA powder and thus cause rough fractures of unique morphological structures in the surface and cross-section areas of chitosan/ β-TCP/PMMA cement composites. The higher the ratio of chitosan/ β-TCP microspheres, the rougher the structure that can be prepared. In brief, the presence of these microspheres altered the structure of the cured PMMA cement and hence influences the overall properties of the cement.

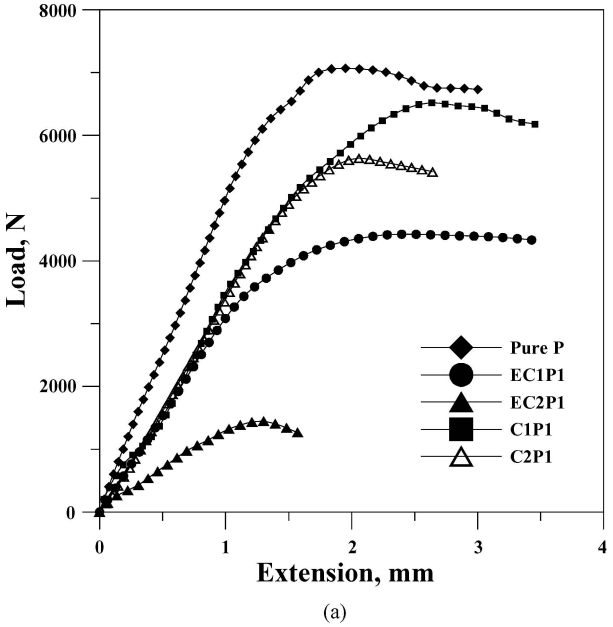
Some physical characteristics of prepared chitosan/ β-TCP/PMMA cement composites are given in Table II. As compared with pure PMMA cement, addition of chitosan/β-TCP microspheres decreased the ultimate compressive strength (UCS) and ultimate bending strength (UBS). The UCS decreased from 89.6 MPa (Pure P) to 83.5 and 73.5 MPa for C1P1 and C2P1, respectively, and to 44.3 and 14.6 MPa for EC1P1 and EC2P1, respectively. The UBS decreased from 37 MPa (Pure P) to 23 and 22.5 MPa for C1P1 and C2P1, respectively, and to 6 and 8 MPa for EC1P1 and EC2P1, respectively. In addition, the compression modulus and bending modulus also decreased with the presence of chitosan/β-TCP microspheres as compared with pure PMMA cement (Fig. 5). Due to the smaller size of microspheres prepared by the electrostatic system, greater numbers of microspheres were added to prepare a composite with the same weight ratio. Hence, these microspheres probably provided much greater surface area that might interfere with the polymerization of PMMA powder and liquid monomer and thus cause significant decreases in the mechanical strength of EC1P1 and EC2P1.

In addition, the presence of chitosan/β-TCP microspheres also increased the setting time of the composites (Table II). The increased setting time was proportional to the weight ratios of chitosan/β-TCP

TABLE II Physical properties of Chitosan/β-TCP/PMMA cement composites

Item	Ultimate compressive strength (MPa)	Modulus of compression (MPa)	Ultimate bending strength (MPa)	Modulus of Bending (MPa)	Setting time (min)
Pure P	90.6 ± 0.7	1163.1 ± 73.9	38 ± 5.7	628.4 ± 48.9	3.5
EC1P1	44.3 ± 16.7	779.4 ± 221.8	6.1 ± 1.1	130.6 ± 23.4	7
EC2P1	14.6 ± 5.5	419.9 ± 29.8	8.1 ± 1.5	155.6 ± 29.2	9
C1P1	83.5 ± 0.3	1099.7 ± 179.6	23 ± 1.4	555.7 ± 15.3	6
C2P1	73.5 ± 1.4	1136.3 ± 24.4	22.5 ± 0.7	558.9 ± 12.6	8

Average ± S.D, $n = 3$.



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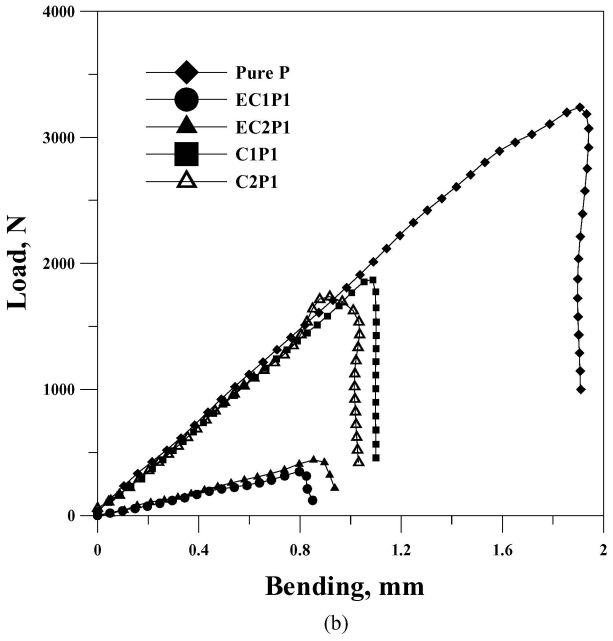

(b)

Figure 5 Ultimate compressive strengths and ultimate bending strengths of prepared Pure P, EC1P1, EC2P1, C1P1 and C2P1 composites.

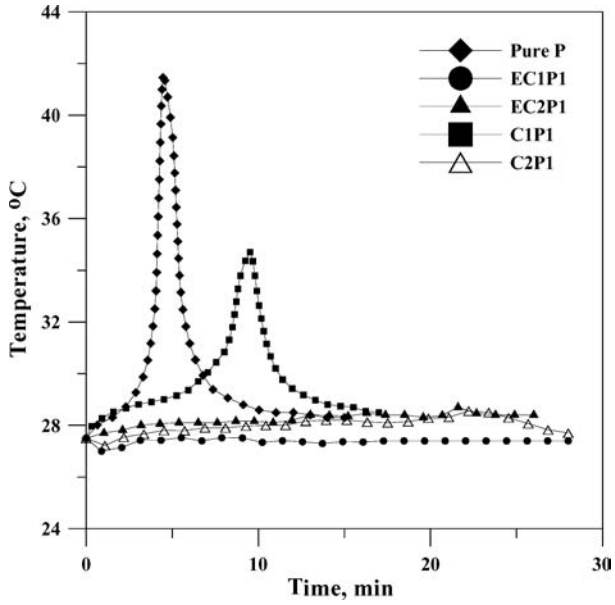

Figure 6 Curing temperature profiles of prepared Pure P, EC1P1, EC2P1, C1P1 and C2P1 composites.

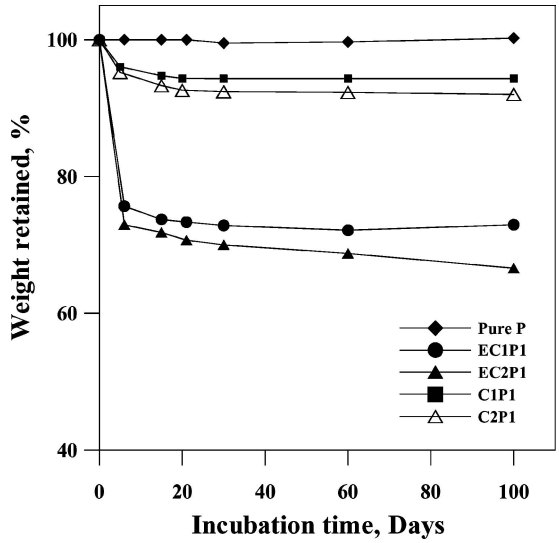

Figure 7 The degradation curve of Pure P, EC1P1, EC2P1, C1P1 and C2P1 composites in PBS, pH 7.4 solution.

microspheres. This increased setting time could be helpful to the manipulation of the cement 'dough' and improve the handling characteristics during the operation. These properties are desirable in surgical procedures, for cement can be easily filled into the bony defects and reduces the chance of having void space between bone and cement. The other important improvement of these prepared composites was substantial reduction of peak curing temperature during the PMMA hardening polymerization, as compared with the pure PMMA bone cement. As can be seen in Fig. 6, the decreased curing temperature for all prepared composites will not necrose surrounding bond tissues during in situ operation and hence increase the biocompatibility of these composites significantly.

The degradation test of these prepared composites was shown in Fig. 7. As can be seen, EC2P1 had greater weight loss (about 33.5 %) than EC1P1, C1P1 and C2P1 (27, 8.9 and 5.7%, respectively) after 100 days of shaking. When glutaraldehyde was used as a crosslinker in oil emulsion method, the microspheres exhibited a thinner outer layer of chitosan membrane by higher crosslinking efficiency and thus provided better mechanical strength (Fig. 1). Furthermore, all these layers of chitosan membranes have shown a variety of degradation characteristics. The SEM micrographs showed the surface morphologies of these composites (Fig. 8). The ruptured chitosan/β-TCP microspheres appearing in these composites revealed and confirmed the degradation behavior was an ongoing process and thus caused a rougher morphology as compared with pure PMMA cement. The more chitosan/β-TCP microspheres added the rougher the morphology that was generated. And this rough morphology could be beneficial for cell adhesion and in-growth, and in turn, provide a better fixation

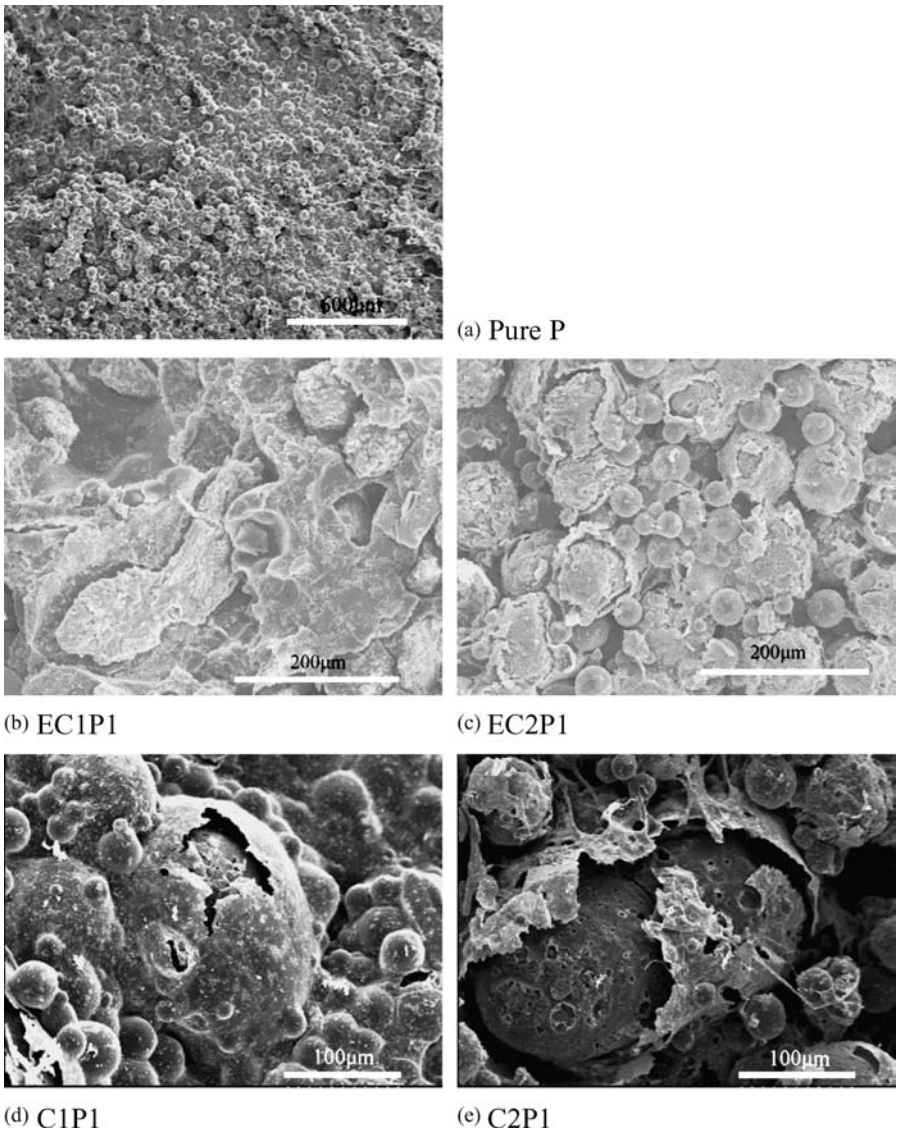

Figure 8 SEM of (a) Pure P, (b) EC1P1, (c) EC2P1, (d) C1P1, and (e) C2P1 composite after 100-day shaking (surface morphology).

between the implant and surrounding tissues. In real applications, the resorption process of these degradable composites could be facilitated by enzymatic digestion. Thus, we expect that one could engineer a composite system with different weight ratios of these chitosan/β-TCP microspheres to the PMMA powder for the preparation of a set of specimens with different degradation behaviors to fulfill the clinical requirements in new bone formation and remodeling.

4. Conclusions

In this study, we proposed two kinds of chitosan/β-TCP microspheres prepared by emulsion method and electrostatic system and then used them as a constituent to PMMA powder to prepare a series of chitosan/β-TCP/PMMA composites. From the preliminary results, we found that the presence of microspheres would alter the properties of composites, including the decreased mechanical strength and curing temperature, increased setting time and different degradation rate, as compared with classical PMMA cement. Addition of chitosan/β-TCP microspheres into PMMA powder to prepare the modified composites would result a longer setting time to final cure which would be helpful to dough manipulate during operation. On the contrary, the short setting time of pure PMMA cement can hamper its manipulation in clinical applications. The curing temperatures of these composites were significantly lower than that of pure PMMA cement. This decreased curing temperature was less harmful to the surrounding tissues and thus could provide better biocompatibility. But, the diminished mechanical strength is a major limitation of these composites when considering as a load-bearing material. SEM observations indicated that these composites could degrade gradually and provide rough and porous spaces for cell growth even long after chitosan/β-TCP was dissolved away. Rough and porous chitosan/β-TCP/PMMA cement enabled bone in-growth, which was desirable because it provided a more stable structural anchorage of the cement to the surrounding tissues. Another studies have shown that the pore size required for successful bone cells ingrowth was at least 150 μm [17], which made these four composites promising for and consistent to clinical considerations and applications.

Acknowledgments

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