Preparation, Characterization and Properties of nHAp/PPC Membrane

Chuanjian Lan\textsuperscript{1,2}*, Xingchen Xiang\textsuperscript{3}*, Shunli Chu\textsuperscript{4}, Wei Wei\textsuperscript{3}, Xi He\textsuperscript{3} and Jiang Li\textsuperscript{1}

\textsuperscript{1}Department of Prosthodontics, School and Hospital of Stomatology, Jilin University, Changchun, China
\textsuperscript{2}Jilin Provincial Key Laboratory of Tooth Development and Bone Remodeling (School and Hospital of Stomatology, Jilin University), Changchun, China
\textsuperscript{3}Department of General Dentistry, School and Hospital of Stomatology, Jilin University, Changchun, China
\textsuperscript{4}Department of Dental Implantology, School and Hospital of Stomatology, Jilin University, Changchun, China

Abstract: Objective: This study aims to synthesize a new biodegradable and biomineralized guided bone regeneration membrane. Experimental Methods: Poly(propylene carbonate) (PPC) and nanohydroxyapatite (nHAp) were prepared by solvent casting/particulate leaching and made into membranes with different compositions (0%, 10%, 20%, 30%, and 40% nHAp/PPC). Their mechanical properties, cell compatibilities and fibroblast barrier functions were studied in vitro. Results: Scanning electron microscopy showed that the nHAp/PPC membrane displayed a smooth and rough surface similar to that of periosteal structure. Tensile test results indicated that the tensile strengths of 10% and 20% nHAp/PPC groups were significantly higher than that of 0% nHAp/PPC group. With the increased nHAp content, the tensile strength of nHAp/PPC membrane and the hydrophilic angle were decreased, and the mineralization ability in vitro was enhanced. After 4 weeks of degradation in vitro, only 40% of the nHAp/PPC group exhibited significantly higher rate of weight loss than that of the 0% nHAp/PPC group. Additionally, scanning electron microscopy showed that MG63 cells adhered to the rough surface of the nHAp/PPC membrane. Five groups of nHAp/PPC membranes showed good fibroblast barrier in vitro. Taken together, this in vitro evaluation suggests that the nHAp/PPC membrane is suitable as a guided bone regeneration membrane.

Key words: Poly(propylene carbonate), Nanohydroxyapatite, Solvent casting/particulate leaching, Guided bone regeneration, Biomaterial

Introduction

Many approaches, which include autologous bone graft, allograft bone transplantation, and the use of bone substitutes or synthetic graft materials, can be used to resolve bone defects\textsuperscript{1,2}. Guided bone regeneration (GBR) is crucial in the repair of bone defects. This technique uses the membrane barrier function to regenerate the bone\textsuperscript{3,4,5}. The barrier membrane can be divided into two types according to degradability: biodegradable and nonbiodegradable biofilms\textsuperscript{6,7}. Biodegradable biofilm can be degraded without secondary surgery, which has attracted the interest of doctors and patients and become a research hot spot.

Poly(propylene carbonate) (PPC) is an aliphatic polyester with good biocompatibility and biodegradability\textsuperscript{8,9,10}. This polyester exhibits many applications, such as medical materials and food bags\textsuperscript{11}. However, the poor mechanical properties of PPC\textsuperscript{11,12} limit its application. Nanohydroxyapatite (nHAp), a major inorganic component in bone tissue with good biocompatibility\textsuperscript{13,14}, is important in osteocyte differentiation and mineralization; it can increase the internal growth of bone\textsuperscript{15,16}. Both in vitro and in vivo experiments demonstrated that inorganic/organic composites can support the adhesion, proliferation, and differentiation of osteoblasts and mesenchymal stem cells, thereby stimulating bone healing\textsuperscript{17}.

In our present study, inorganic nHAp was added to the organic PPC to enhance its mechanical strength and biocompatibility. In a previous study, our group prepared a PPC/poly(butylene succinate) (PBS) GBR membrane by solvent casting/particulate leaching; the prepared membrane exhibits the characteristics of biofilm\textsuperscript{18}, having smooth and rough surface on both sides. Therefore, the present experiment used solvent casting/particulate leaching method to mix nHAp with PPC and produce a new type of GBR membrane. To investigate the effect of nHAp content in PPC, five different types of GBR membranes (0%, 10%, 30%, and 40% nHAp/PPC) were prepared. Their mechanical and physical properties, biocompatibilities, and fibroblast barrier functions were examined.

Materials and Methods

Preparation and characterization of nHAp

nHAp was synthesized according to the precipitation reaction method used by previous studies\textsuperscript{19}. Briefly, 0.3M orthophosphoric acid (H\textsubscript{3}PO\textsubscript{4}) (Beijing Chemical Plant, Beijing, China) was added dropwise to the deionized water suspension of 0.5M calcium hydroxide (Ca(OH)\textsubscript{2}) (Beijing Chemical Plant, Beijing, China) with continuous stirring overnight at room temperature. The pH affects the formation of growth motifs and consequently the grain size\textsuperscript{20,21}. When the pH is alkaline, the growth rate of the growth unit is large, and the grain size is small. Ammonia hydroxide (Beijing Chemical Plant, Beijing, China) was used to maintain the solution pH consistently at more than 10.5 to obtain nHAp. The precipitate obtained was aged for 1 week at room temperature and centrifuged three times with deionized water. Precipitated particles were

*Chuanjian Lan and Xingchen Xiang are equally contributed to this work.
Correspondence to: Dr. Jiang Li, Department of Prosthodontics, School and Hospital of Stomatology, Jilin University, Changchun 130021, China; Tel: +86-0431-88796018; E-mail: 710675401@qq.com
suspended by a magnetic stirrer (IKA C-MAG HS7, Germany) during each wash. Finally, the precipitated particles were placed in a freeze dryer (Shuangjia SJA-10N, Ningbo, China) to obtain nHAp. Scanning electron microscopy (SEM) (FEI Quanta 200, The Netherlands) was used to observe the morphology and size of nHAp. X-ray diffractometer (XRD) (Bruker D8 FOCUS, Germany) was used to determine the crystal structure of the particles (scan angle 2θ = 10–70°, velocity 2°/min). Data were processed using OriginPro 8 software. Fourier transform infrared (FTIR) (Bruker VERTEX 70, Germany) was used to determine the chemical structure of the particles.

**Preparation of nHAp/PPC GBR membrane**

nHAp/PPC GBR membranes were prepared by solvent casting/particulate leaching. First, NaCl (Beijing Chemical Plant, Beijing, China) was crushed with a mortar and sieved through a 150 mesh sieve. The NaCl particle size was controlled at 100 µm and uniformly sprinkled into a 60 mm glass Petri dish for further use. PPC granules (mol.wt. = 5,000) (Changchun Institute of Applied Chemistry Chinese Academy of Sciences) and nHAp powder were weighed, poured into a 100 ml beaker containing the chloroform solution (Haohua Chemical Reagent Co, Ltd, Luoyang, China), and stirred with magnetic stirrer at 50°C for 1 h until fully mixed. The mixed solution was poured into the NaCl-containing glass Petri dish, sonicated for 30 min, and placed in a fume hood for 24 h for complete evaporation of the chloroform solution. The precipitated NaCl-containing nHAp/PPC membrane was subsequently soaked in deionized water (immersion for 7 days at room temperature with daily replacement of deionized water solution) to remove NaCl from the membrane and freeze dried in a lyophilizer. The resulting nHAp/PPC GBR membrane was classified into 0%, 10%, 20%, 30%, and 40% nHAp/PPC, depending on the nHAp content. Their morphologies and colors were also observed. A thickness gauge was used to select three different positions of the membranes randomly and measure their average thickness values.

**SEM observation**

The well-formed nHAp/PPC GBR membranes were observed both at the front and back sides by SEM (FEI Quanta 200, The Netherlands). The nHAp particle distribution in the PPC matrix was also observed.

**Characterization of mechanical properties**

The nHAp/PPC membrane was cut into 60mm-long and 20 mm-wide strips and tested using the material test system (Pantech Industrial Complex Zwick/Z010, Singapore). Tensile strength and elongation at break were measured at 25 °C and 50% relative humidity.

**Dynamic contact angle measurement**

The hydrophilic angle of the material was measured with Data Physics Instruments (KRUSS DSA30, Germany). The contact angles at the front and back sides were also measured.

**Simulated body fluid (SBF) immersion test**

The ability of nHAp/PPC membranes to form apatite in vitro was experimentally tested by immersion in SBF solution. The SBF configuration was based on the method of Kokubo, with the same ion composition in the blood. The experimental procedure was as follows: nHAp/PPC membranes were cut into 1 × 1 cm², placed in a 15ml centrifuge tube containing 10 ml of SBF, and placed in a water bath at 37 °C. The SBF solution was changed daily, soaked for 1 week and 4 weeks, rinsed with deionized water, lyophilized to remove moisture, and sprayed with gold. The rough surface of the membrane was observed by SEM (FEI Quanta 200, Netherlands).

**In vitro degradation**

The nHAp/PPC membrane was lyophilized, cut into a size of 2 × 2 cm², and weighed (W₀). Subsequently, the membrane was placed in a glass vial containing 50 ml of phosphate buffer solution (PBS) (0.01 M, pH 7.4), capped, and placed in a 37 °C incubator. The PBS solution was replaced daily. Then nHAp/PPC membrane was removed at 1, 2, 3, and 4 weeks (the pH of the media was noted at predetermined time points), rinsed three times with deionized water, dried in a lyophilizer, and weighed (W). The degree of degradation was calculated using the following equation:

\[
\text{Weightloss}(\%) = \frac{W_0 - W}{W_0} \times 100
\]

In Eq. (1), W₀ was the dry weight before degradation, and W, was the dry weight at time.

**Cell culture on the nHAp/PPC membrane**

Dulbecco’s modified eagle medium (DMEM, HyClone, Logan, UT), which contained 10% fetal bovine serum (FBS, HyClone, Logan, UT) and 1% penicillin/streptomycin (GIBCO, Grand Island, NY), was used for cell culture. MG63 cells (Chinese Academy of Sciences, China) were grown in an incubator with 5% CO₂ at 37 °C. When the cells grew to approximately 90% of the culture flask, trypsin was digested to harvest cells, centrifuged, and resuspended count.

The nHAp/PPC membrane was cut into 15 mm-diameter discs. The membrane was soaked in 70% ethanol for 10 min, exposed to UV light for 30 min, and rinsed three times with sterile PBS (pH 7.4). The membrane was immersed in a DMEM-containing 24-well plate for 4 h (the rough side up) to allow the cells to adapt. DMEM was aspirated and dried in an incubator at 37 °C with 5% CO₂ for improved cell culture for 2 h prior to inoculation.

Cell suspension (15 µl containing 8×10⁶ cells) was added dropwise onto the rough surface of the membrane and incubated in an incubator with 5% CO₂ at 37 °C for 1 day. The medium was aspirated, washed with PBS (pH = 7.4) three times, and fixed using 2.5% glutaraldehyde for 60 min. Subsequently, the samples were dehydrated for 10 min each in ethanol (30%, 50%, 70%, 80%, 90%, and 100%) and dried at room temperature. SEM was performed to observe cell adhesion.

**Barrier function of nHAp/PPC membrane to fibroblastic cells**

The L929 cell line (American Type Culture Collection, Rockville, MD, USA) was used to examine the barrier function of the smooth surface of nHAp/PPC membranes in accordance with a previous L929 cell barrier function of phase inversion membrane in vitro. The nHAp/PPC membrane was cut into 25mm-diameter disks, sterilized, and immobilized on the outer bottom of a Transwell chamber (24-well plate) (Sigma-Aldrich, St. Louis, MO, USA). Cell-free cell culture medium (1 ml) was added to the well plate from the outside of the cell. L929 cell suspension was added to the upper chamber (8×10⁶ cells), and the medium was supplemented to a total volume of 1 ml (Fig. 1). Ensuring that the nHAp/PPC membrane smooth surface is in contact with the cells rather than with the rough surface is crucial. After culturing for 1 and 3 days in an incubator with 5% CO₂ at 37 °C, the upper chamber was removed. Furthermore, the smooth surface of the nHAp/PPC membrane and the bottom of the 24-well plate were treated with 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, St. Louis, MO, USA). Cells were observed under a fluorescence microscope (TE2000-S, Nikon, Melville, NY, USA).
The existence of C element was partly caused by the CO$_2$ in air. The molar spectrum mainly contains four elements, namely, Ca, P, O, and C. The energy spectrum analysis is displayed in Fig. 3a; the results are shown in Table 1. The SEM images in Fig. 4 further confirmed that the synthesized material was hydroxyapatite with good crystallinity. The characteristic peak of hydroxyapatite appeared at 20 = 26° and 32°, which corresponded to the $\nu_4$ vibration absorption of PO$_4^{3-}$, this result is in agreement with the hexagonal phase hydroxyapatite crystals in space group P6$_3$ in standard XRD cards. FTIR further detected the chemical structure of the synthesized material (Fig. 3c). The peaks at 564 and 601 cm$^{-1}$ corresponded to the $\nu_4$ vibration absorption of PO$_4^{3-}$. The $\nu_3$ vibration absorption peak of PO$_4^{3-}$ was observed at 1036 and 1093 cm$^{-1}$, and the peak at 3750 cm$^{-1}$ corresponded to the absorption of OH$^-$ in hydroxyapatite. The weak absorption peak at 1500–1600 cm$^{-1}$ corresponded to CO$_2^-$, which indicated that a small amount of CaCO$_3$ existed. The CaCO$_3$ existed in the presence of C element in the energy spectrum analysis and the large Ca/P ratio. The presence of a small amount of CaCO$_3$ exerted no effect on subsequent experiments.

**Characteristics of the prepared nHAp/PPC membrane**

nHAp/PPC membranes were macroscopically visible with both rough and smooth surfaces (rough surface was due to NaCl particles). With the increased nHAp content, the nHAp/PPC membrane became rough. The nHAp/PPC membrane thickness is presented in Table 1. The average thickness of the 10% nHAp/PPC membrane was 0.61 mm and that of the 20% nHAp/PPC membrane reached 1.10 mm, which was almost two times that of 0% nHAp/PPC membrane. The average thickness of 40% nHAp/PPC membrane was 1.13 mm, and the increase in nHAp membrane thickness from 20% to 40% was insignificant.

**SEM images of nHAp/PPC membrane**

SEM images of different nHAp/PPC membranes are illustrated in the SEM images of the synthesized nHAp particles. Better connection was observed between the nHAp particles and the PPC matrix. SEM images also directly showed that the nHAp/PPC membrane prepared by solvent casting/particulate leaching presented smooth and rough surfaces similar to that of the periosteal structure. Rough-surface pores were formed by NaCl particles. The pore size (micron size) was consistent with the size of the NaCl particles; large pores were due to the aggregation of multiple NaCl particles. In addition, nHAp participated in the pore formation. In the 0% nHAp/PPC membrane, the roughened surface showed shallow and small pores. With the increased nHAp content, the number and size of pores in the roughened surface increased, and pores clustered together. When the nHAp content reached 40 wt%, observation on an independent hole was nearly impossible because of close linkage.
Mechanical characterization

The stress–strain curve of nHAp/PPC membranes is showed in Fig. 5, which exhibited mechanical properties similar to those of ductile materials under tensile load (except 40% nHAp/PPC). The initial stage obeyed Hooke’s law, that is, the stress is proportional to the strain. With increased stress increased, the curve lost its linear nature. The tensile strength and elongation at breakage of nHAp/PPC membranes are shown in Table 1. The average tensile strength of nHAp/PPC film was 27.54 MPa, and the average elongation at break was 65.78%. The average tensile strengths of 10% and 20% nHAp/PPC membrane were 42.82 and 35.27 MPa, respectively, which were higher than that of 0% nHAp/PPC membrane.

**Table 1. Composition information, average thickness, tensile strength, elongation at break, hydrophilic angle, and goniometer images of nHAp/PPC membrane (mean ± standard deviation, n = 3).**

| Abbreviation of compositions | Description of compositions | Average membrane thickness (mm) | Tensile strength (MPa) | Elongation at break (%) | Mean contact angle (°) with Goniometer image smooth surface | rough surface |
|-----------------------------|-----------------------------|--------------------------------|-----------------------|-------------------------|-----------------------------------------------------------|--------------|
| 0% nHAp/PPC                 | PPC matrix (without nHAp)   | 0.61±0.24                      | 27.54±0.66            | 65.78±2.89              | 95.10±2.07                                                 | 85.36±2.85   |
| 10% nHAp/PPC                | 10% nHAp doped PPC matrix   | 0.82±0.12                      | 42.82±0.37            | 4.66±1.23               | 74.50±1.64                                                 | 61.80±5.24   |
| 20% nHAp/PPC                | 20% nHAp doped PPC matrix   | 1.10±0.27                      | 35.27±0.52            | 5.23±1.06               | 73.83±2.51                                                 | 68.43±1.96   |
| 30% nHAp/PPC                | 30% nHAp doped PPC matrix   | 1.11±0.14                      | 15.63±0.58            | 6.03±0.17               | 70.77±2.67                                                 | 53.97±3.96   |
| 40% nHAp/PPC                | 40% nHAp doped PPC matrix   | 1.13±0.38                      | 4.14±0.49             | --                      | 60.53±4.41                                                 | 48.20±7.05   |

Figure 4. A, a SEM images of 0% nHAp/PPC membrane. B, b SEM images of 10% nHAp/PPC membrane. C, c SEM images of 20% nHAp/PPC membrane. D, d SEM images of 30% nHAp/PPC membrane. E, e SEM images of 40% nHAp/PPC membrane. Among them, A, B, C, D, and E show smooth surface images, and a, b, c, d, and e are rough-surface images. Scale bar = 100μm.

**Figure 5.** nHAp/PPC membranes’ stress–strain curve.

The stress–strain curve of nHAp/PPC membranes is showed in Fig. 5, which exhibited mechanical properties similar to those of ductile materials under tensile load (except 40% nHAp/PPC). The initial stage obeyed Hooke’s law, that is, the stress is proportional to the strain. With increased stress increased, the curve lost its linear nature. The tensile strength and elongation at breakage of nHAp/PPC membranes are shown in Table 1. The average tensile strength of nHAp/PPC film was 27.54 MPa, and the average elongation at break was 65.78%. The average tensile strengths of 10% and 20% nHAp/PPC membrane were 42.82 and 35.27 MPa, respectively, which were higher than that of 0% nHAp/PPC membrane. Correspondingly, their average elongation at break was 4.66% and 5.23% lower than that of 0% nHAp/PPC membrane, respectively. The tensile strength of the nHAp/PPC membrane increased significantly at 10%, which was approximately two times that of 0% nHAp/PPC membrane. The average tensile strengths of 30% and 40% nHAp/PPC membranes were at 15.63 and 4.14 MPa, respectively, which were lower than those of 0% nHAp/PPC membrane. The average elongation at break of 30% nHAp/PPC was 6.03%. The 40% nHAp/PPC membrane was considerably brittle; consequently, its elongation at...
break cannot be measured.

**Contact angle**

Hydrophilicity exerts a crucial effect on the biological activity of the material. The contact angle measurement results of the nHAp/PPC membrane are shown in Table 1. The average contact angles for the smooth and rough surfaces of 0%, 10%, 20%, 30%, and 40% nHAp/PPC membranes were 95.10° and 85.36°, 74.50° and 68.43°, 73.83° and 61.80°, 70.77° and 53.97°, and 60.53° and 48.20°, respectively. The nHAp addition increased the hydrophilicity of the material.

**Mineralization in SBF**

SEM images of rough surfaces of nHAp/PPC membranes for 1 week and 4 weeks in SBF are displayed in Fig. 6. After mineralization for 1 week, mineral deposits were observed on the rough surfaces of nHAp/PPC membranes (Fig. 6A-E), and sediments increased at 4 weeks (Fig. 6a-e). The sediment amount was dependent on time and the nHAp content: long time and high nHAp content resulted in large amount of sediment. The SEM images show that mineralization of 10% nHAp/PPC membranes at 1 week produced a regular network structure similar to that of trabecular bone. With prolonged mineralization time, the sediments became remarkably thick, regular, and closely linked with one another. Mineralization on 20% nHAp/PPC membranes also caused regular precipitation at 4 weeks. However, the precipitate formed on 0%, 30%, and 40% nHAp at 1 week and 4 weeks aggregated into clumps with irregular structure.

**In vitro hydrolytic degradation of nHAp/PPC membrane**

The nHAp/PPC membrane was removed after degradation for a period of time in PBS, lyophilized, and weighed. Weight loss rates are shown in Fig. 7. Results confirmed that the nHAp/PPC membrane is degradable. 0% nHAp/PPC membrane degraded slowly, and the weight
loss rate after 4 weeks was only 6.49% ± 0.55%. The weight loss rate of 40% nHAp/PPC membrane after 4 weeks was high at 23.23% ± 2.73%. The four-week weight loss rates of 10%, 20%, and 30% nHAp/PPC membranes were 4.86% ± 5.59%, 1.87% ± 0.17%, and 0.81% ± 0.17%, respectively. The solution pH was also measured at each degradation point, and the value remain above 7.0 by the 4th week.

**Cell adhesion on the nHAp/PPC membrane**

The SEM images of the coculture of MG63 cells with nHAp/PPC after 1 day are showed in Fig. 8. MG63 cells adhered to the rough surface of 0% nHAp/PPC membrane with extended pseudopodia (Fig. 8a), which indicated that PPC is a biocompatible material. With the increased nHAp content, the adherent MG63 cells became irregularly shaped with increased pseudopodia, which was difficult to discern when the nHAp reached 40% because the cells fused with the membrane (Fig. 8e). This result showed that the nHAp addition can improve the biological properties of the material, which is conducive to cell adhesion.

**Barrier function of nHAp/PPC membrane to fibroblastic cells**

The barrier function of nHAp/PPC membranes was evaluated by stimulation of fibroblast penetration membranes in vitro. Results are shown in Fig. 9. L929 cells were observed on the smooth surface of nHAp/PPC on the 1st and 3rd day, whereas no cell was observed at the bottom of the lower chamber (Fig. 9a-c, f-j). This finding indicated that nHAp/PPC membrane can resist fibroblasts and prevent them from passing through.

**Discussion**

The ideal GBR membrane should be biocompatible and biodegradable; it should also possess a certain mechanical strength to maintain the required space of bone growth. Given its good biocompatibility and biodegradability, PPC has received considerable attention. Many scholars have used this material as a biological scaffold for a large number of experimental studies. In the present study, we used the flexibility of organic materials and the high strength of inorganic materials to construct the GBR membrane, which were combined to compensate for their shortcomings and optimize their advantages.

We first synthesized nHAp by chemical precipitation. At different pH values, calcium phosphate shows different forms. In the experimental synthesis to maintain alkaline environment (pH >10.5), calcium phosphate salts were transformed into hydroxyapatite. SEM results demonstrated that the synthesized hydroxyapatite was nanosized. The XRD and FTIR results showed that the synthesized material was hydroxyapatite with good crystallinity and a small amount of CO$_3$\(^{−}\). The blending of nHAp and PPC by solvent casting/particulate leaching showed that the membrane displayed rough and smooth surfaces as observed with naked eyes. The nHAp addition into the membrane significantly increased the thickness. The SEM images directly demonstrated microscopically that the biofilm possesses smooth and rough surfaces similar to that of natural periosteum. Smooth surface can prevent fibrinolysis from growing into the bone defect area, and rough surface is conducive to osteoblast adhesion and proliferation. With increased surface roughness, biomaterials provide many sites for cell-cell binding, thereby promoting cell adhesion. The nHAp particle addition decreased the smoothness of the film and increased the roughness and thickness.

Furthermore, the nHAp addition changed the mechanical energy of PPC, increased the tensile strength, and significantly reduced the elongation at break. When the nHAp content was 10% and 20%, the tensile strength increased evidently. The nHAp content also increased, but the tensile strength decreased. The tensile strengths of the 10% and 20% nHAp/PPC groups were higher than that of 0% nHAp/PPC groups, and those of 30% and 40% nHAp/PPC groups were lower than that of 0% nHAp/PPC group. Within a certain range, the nHAp particle addition can consume energy to increase the mechanical strength. Molecular dynamics studies attributed this additional dissipation mechanism to nanoparticle mobility. Nanoparticles, when subjected to tensile stresses, are aligned so as to provide temporary crosslinking between polymer chains; consequently, their local strength is enhanced. When the content is beyond this range, the nHAp stacks show less mobility, and they cannot dissipate energy, thereby resulting in a slight decrease in stretch. Elongation at break showed a downward trend with the increased nHAp amount. According to the mechanical strength results, 10% and 20% nHAp/PPC membranes were desired biofilms.

Contact angle can describe the hydrophilicity of the material. The hydrophilic angle of other membrane surface was acute, except that for 0% nHAp/PPC smooth surface. The nHAp addition increased the hydrophilicity of the material because particle addition increased the roughness of the surface. High nHAp content resulted in small contact angle. SBF mineralization experiments were used to assess the ability of nHAp/PPC membranes to form bone. After soaking for 1 week and 4 weeks, a large amount of mineral deposits was observed on the rough surface by SEM. The deposited amount was in the 0% nHAp/PPC group, which indicated that the nHAp addition increased the deposition ability of the material surface. This finding was attributed to that the partial nHAp dissolution releases Ca$^{2+}$, which favors apatite formation. In vitro degradation test, the 40% nHAp/PPC group weight loss rate increased after 4 weeks, and those of the remaining groups decreased due to nHAp addition. This characteristic is conducive to nHAp/PPC membrane used as a GBR membrane, with sufficient time to maintain the degradation of space to ensure new bone formation.

SEM images showed that MG63 cells adhered to the rough surface of the material, with pseudofoot sticking out. High nHAp content resulted in a large number of cells extending pseudofoot and an improved cell compatibility. The in vitro barrier function assay indicated nHAp/PPC membrane can resist fibroblasts and prevent them from passing through at 1 and 3 days.

Overall, results showed that the addition of an appropriate amount of nHAp increased the mechanical strength, biocompatibility, and biomineralization of PPC. According to the results of tensile strength, biocompatibility, and biomineralization, the obtained 20% nHAp/PPC membrane is considered an ideal material. The current in vitro investigations...
serve as a basis for further studies in vivo.

Acknowledgments

The authors would like to acknowledge the Changchun Institute of Applied Chemistry Chinese Academy of Sciences for providing PPC samples freely. This work was supported by Jilin Provincial Science and Technology Department (No.201702040099Y), Changchun Science and Technology Department (No.17YJ001) and Jilin Province Development and Reform Commission (No.2016C048-3). They also thank Fengxiang Gao for technical assistance.

Conflict of Interest

The authors have declared that no COI exists.

References

1. Garcia-Gareta E, Coathup MJ and Blunn GW. Osteoinduction of bone grafting materials for bone repair and regeneration. Bone 81: 112-121, 2016
2. Stanovici J, Nail LRL, Brennan MA, Vidal L, Trichet V, Rosset P and Layrolle P. Bone regeneration strategies with bone marrow stromal cells in orthopaedic surgery. Curr Res Transl Med 64: 83-90, 2016
3. Malmström J, Anderud J, Abrahamsson P, Wälivaara DÅ, Isaksson SG and Adolffson E. Guided bone regeneration using individualized ceramic sheets. Int J Oral Maxillofac Surg 45: 1246-1252, 2016
4. Leung YY. Coronectomy of third lower molars with and without guided bony regeneration: a pilot study. Br J Oral Maxillofac Surg 54: 155-159, 2016
5. Xiao WL, Zhang DZ, Chen XJ, Yuan C and Xue LF. Osteogenesis effect of guided bone regeneration combined with alveolar cleft grafting: assessment by cone beam computed tomography. Int J Oral Maxillofac Surg 45: 683-687, 2016
6. Ma SQ, Chen Z, Qiao F, Sun YC, Yang XP, Deng XL, Cen L, Cai Q, Wu MY, Zhang X and Gao P. Guided bone regeneration with triphosphotyphosphate cross-linked asymmetric chitosan membrane. J Dent 42: 1603-1612, 2014
7. Dziowiecki D, van de Loo S, Gremse F, Kloss-Brandsätter A, Kloss F, Offermanns V, Yamauki K, Kessler P and Lethaus B. Osteoneogenesis due to periosteal elevation with degradable and nondegradable devices in Göttingen Minipigs. J Craniomaxillofac Surg 44: 318-324, 2016
8. Kim G, Ree M, Kim H, Kim IKJ, Kim JR and Lee JJ. Biological affinity and biodegradability of poly(propylene carbonate) prepared from copolymerization of carbon dioxide with propylene oxide. Macromol Res 16: 473-480, 2008
9. Klaus S, Lehenmeier MW, Anderson CE and Rieger B. Recent advances in CO2/epoxide copolymerization-New strategies and cooperative mechanisms. Coordin Chem Rev 255: 1460-1479, 2011
10. Chen LJ, Qin YS, Wang XH, Zhao XJ and Wang FS. Plasticizing while toughening and reinforcing poly(propylene carbonate) using low molecular weight urethane: Role of hydrogen-bonding interaction. Polymer 52: 4873-4880, 2011
11. Dong HP, Kan TG, Yun KL and Kim WN. Effect of multi-walled carbon nanotube dispersion on the electrical and rheological properties of poly(propylene carbonate)/poly(lactic acid)/multi-walled carbon nanotube composites. J Mater Sci 48: 481-488, 2013
12. Peng SW, An YX, Chen C, Fei B, Zhuang YG and Dong LS. Thermal degradation kinetics of uncapped and end-capped poly(propylene carbonate). Polym Degrad Stab 80: 141-147, 2003
13. Fox K, Tran PA and Tran N. Recent advances in research applications of nanophase hydroxyapatite. Chemphyschem 13: 2495-2506, 2012
14. Hench LL and Polak JM. Third-generation biomedical materials. Science 295: 1014–1017, 2002
15. Habibovic P, Kruyt MC, Juhl MV, Clyens S, Martinetti R, Dolcini L, Theilgaard N and van Blitterswijk CA. Comparative in vivo study of six hydroxyapatite-based bone graft substitutes. J Orthop Res 26: 1363–1370, 2008
16. Poinern GE, Brundavanam RK, Mondinos N and Jiang ZT. Synthesis and characterization of nanohydroxyapatite using an ultrasound assisted method. Ultrasom Sonochem 16: 469-474, 2009
17. Wang H, Li Y, Zuo Y, Li J, Ma S and Cheng L. Biocompatibility and osteogenesis of biomimetic nano-hydroxyapatite/polyamide composite scaffolds for bone tissue engineering. Biomaterials 28: 3338-3348, 2007
18. Chu SL, Zhou YM and Zhou QHC. Physical structure characteristics of carbon-dioxide-copolymer biomembrane. J Oral Sci Res 25: 699-702, 2009
19. Yang F, Both SK, Yang X, Walboomers XF and Jansen JA. Development of an electrospray nano-apatite/PCL composite membrane for GTR/GBR application. Acta Biomater 5: 3295-3304, 2009
20. von Hentzen H. Research on mifepristone and levonorgestrel in comparison with the Yuzpe regimen. J Am Med Womens Assoc 53: 222-224, 1998
21. Zhang L, Wang C, Yuan QL and li Z. The factor effecting the crystal and size on synthesis of hydroxyapatite by deposition method. J Log Univ PAP 12: 143-144, 2003
22. Kokubo T and Takada H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 27: 2907-2915, 2006
23. Xue J, He M, Liu H, Niu Y, Crawford A, Coates PD, Chen D, Shi R and Zhang L. Drug loaded homogeneous electrospray PCL/gelatin hybrid nanostructure for anti-infective tissue regeneration membranes. Biomaterials 35: 9395-9405, 2014
24. Vallet-Regi M and González-Calbet JM. Calcium phosphates as substitution of bone tissues. Prog Solid State Chem 32: 1-31, 2004
25. Aoki H. Medical applications of hydroxyapatite: bone mineral drug delivery system cancer & HIV IVH & CAPD dental implant. St Louis: Ishikayu Euro America Inc, 1994
26. Petrov OE, Dylurgerova E, Petrov L and Popova R. Characterization of calcium phosphate phases obtained during the preparation of sintered biphasic Ca-P ceramics. Mater Lett 48: 162-167, 2001
27. Hench LL and Wilson J. An introduction to bioceramics. Singapore World Sci 150-151, 1993
28. Song SL and Zhu SX. Periosteal histological features and ultrastructure. Chinese J Orthop 16: 395-397, 1996
29. Zhao J, Han W, Chen H, Tu M, Huan S, Miao G, Zeng R, Wu H, Cha Z and Zhou C. Fabrication and in vivo osteogenesis of biomimetic poly(propylene carbonate) scaffold with nanofibrous chitosan network in macropores for bone tissue engineering. J Mater Sci Mater Med 23: 517-525, 2012
30. Manavitcharni I, Fathi A, Wang Y, Maitz PK and Dehghani F. Reinforced poly(propylene carbonate) composite with enhanced and tunable characteristics, an alternative for poly(lactic acid). ACS Appl Mater Int 7: 22421-22430, 2015
31. Groot KD, Klein DPAT, Wolke JGC and Blieck-Hogervorst JMAD. Handbook of Bioactive Ceramics. CRC Press, 1990
32. LeGeros RZ. Calcium phosphates in oral biology and medicine. Monogr Oral Sci. Basel, Karger 15: 108-129, 1991
33. Anselme K. Osteoblast adhesion on biomaterials. Biomaterials 21: 667-681, 2000
34. Bhattacharjee P, Naskar D, Maiti TK, Bhattacharya D and Kundu SC. Non-mulberry silk fibroin grafted poly (C-caprolactone)/nano hydroxyapatite nanofibrous scaffold for dual growth factor delivery to promote bone regeneration. J Colloid Interface Sci 472: 16-33, 2016