

Biological Evaluation of Biodegradable PCL-BCP/PCL Bi-Layered GBR Membrane

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Abstract

Guided bone regeneration(GBR) can be described as the use of a barrier membrane to provide a space available for new bone formation in a bone defect. By placing a membrane between fast-growing fibrous tissue covering a bone defect and the defect itself, slow-growing osteoblasts can migrate into the bone defect and lead to the reossification of this area. This work is aimed to study bi-layer GBR membrane, considered as a substitute for bone formation, following addition of biphasic calcium phosphate(BCP) nanofibers for the mineralization of osteoblasts for bone regeneration.

Introduction

Bone healing is an important matter in the clinical field, and one which requires ideal materials and techniques to accomplish^{1,2}. Guided bone regeneration (GBR) method is a well established therapy for mandible and alveolar bone defects infected by periodontal diseases. GBR membranes also have an important function which encourages bone growth into bone defect sites. GBR membranes are occasionally utilized with dental implant³ or bone grafting materials^{4,5}.

In general, the GBR membranes are expected to satisfy the following general requirements: good biocompatibility, appropriate biodegradability, suitable adhesiveness between the membrane and surrounding bone tissue to prevent the movement of the membrane, high flexibility to provide surgical facility, and sufficient mechanical strength to maintain their barrier function long enough for bone regeneration⁶⁻⁸.

Recently, an electrospinning technique has been introduced and allows the preparation of thin fibrous membranes^{9,10}. The electrospun nanofibrous membrane is produced by the electrospinning and drying of polymer solutions/melts. It has already been shown that electrospun membranes have the potential to promote osteoblastic cell function and bone regeneration^{11,12}.

The aim of the current study was to develop a biodegradable bi-layered composite GBR membrane comprising of the honeycomb-patterned film of PCL(polycaprolactone) and the nanofiber mat of BCP(biphasic calcium phosphate) and PCL to enhance the bone regeneration. The mechanical properties, bioactivity and *in vitro* osteoblast-like cellular behavior of the bi-layered composite GBR membrane were examined.

Materials and Methods

Fabrication of honeycomb-patterned films

Honeycomb-patterned films were fabricated by applying moist air to spread the polymer solution containing a biodegradable polymer (polycaprolactone: PCL, M/W.=80,000) and an amphiphilic polymer (polystyrene-block-poly(N,N-dimethylacrylamide: PS-b-PDMA). PCL

and PS-b-PDMA were mixed with 10:1 wt% and dissolved in chloroform at a concentration of 5 mg/ml. The polymer solution was poured into a round glass dish with simultaneous blowing of highly humid air. The honeycomb patterned structure with a pore diameter of 5 μm was formed.

BCP sol preparation

BCP precursor sols with different calcium to phosphorus molar ratios (Ca/P) were prepared by mixing calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) and triethyl phosphate ($(\text{C}_2\text{H}_5\text{O})_3\text{PO}$) in 2-methoxy ethanol to produce hydroxyapatite (HA) and β -tricalcium phosphate (TCP). Each sol was prepared in Ar atmosphere and the composition of the sol is given in Table 1. As-prepared precursors were closely capped, and then placed in a drying oven at 35°C for 72 hrs for aging. In order to examine the stoichiometry, the precursors were dried and then calcined at 800-1100 °C using a ramp of 3°C/min.

Table 1. Compositions of precursor sols for calcium phosphate phases

| Ca/P | Chemicals (g) | | Obtained phases |
|------|--|---|-------------------------------|
| | $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ | $(\text{C}_2\text{H}_5\text{O})_3\text{PO}$ | |
| 1.67 | 1 | 0.6 | hydroxyapatite |
| 1.49 | 1 | 0.7 | β -tricalcium phosphate |
| 1.58 | 1 | 0.63 | biphasic calcium phosphate |

BCP/PCL nanofibers

BCP/PCL composite nanofibers were prepared by electrospinning a precursor mixture of BCP and PCL sol. For BCP/PCL composite nanofibers, PCL sol was dissolved in a mixture of 75 wt% chloroform and 25wt% methanol, and then BCP sol were dissolved in the mixed solvent solution. The concentration of PCL solution in BCP/PCL composite solution was varied in the range from 3 to 7.5 wt%. BCP/PCL nanofibers were fabricated by an electrospinning process with a feed rate of 1mL/hr and an applied voltage of 20kV using a high voltage power supply. The collection plate of aluminum foil was located around 13 cm from the needle tip. During electrospinning process, the solvents were evaporated in the air and obtained nanofibers mats were randomly oriented. The fabricated samples were dried for one night at room temperature under vacuumed condition.

Results and Discussion

Mechanical characterization GBR membranes

Composite Honeycomb-pattern films and nanofiber GBR membranes were carefully cut into the rectangular dimension of 10mm width and 60mm length. Tensile test of GBR membranes was conducted by Instron 3343 microtester with 2mm/min cross-head speed. As shown in Fig. 1, the average tensile strength of membranes was about 2.57 MPa. This value can be compared with previous study of Ueyama et al¹³. They reported that the tensile strength of their calcium alginate GBR membranes was 0.017MP. Although BCP is has brittle property, this tensile strength is much higher than the expected value. It is believed that the tensile strength of our GBR membranes may be sufficient high enough for clinical application.

MTT assay

The cytotoxicity of membranes was tested by quantitative analysis using the MTT test. After exposure to the various dilutions of extract (i.e., 25%, 50% and 100%) for 1, 3 7days, the proliferation of MC3T3-E1 cells was measured. As a control, no extract added cell cultures were used. The extracts of the membranes showed no inhibition of the cell metabolism

compared to the control group. From the quantitative scores, it was concluded that the extracts of the membranes demonstrated no cytotoxic reactivity in this test, as seen in Fig. 2.

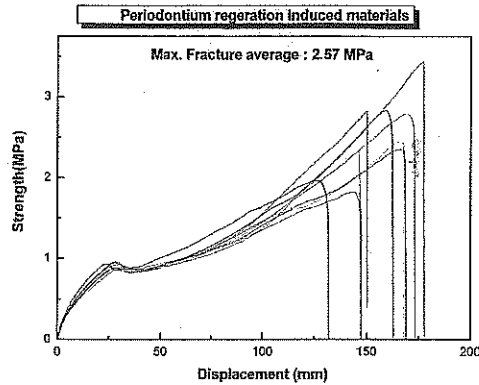


Fig. 1 Tensile curves for the bi-layered PCL film and BCP/PCL nanofiber membranes

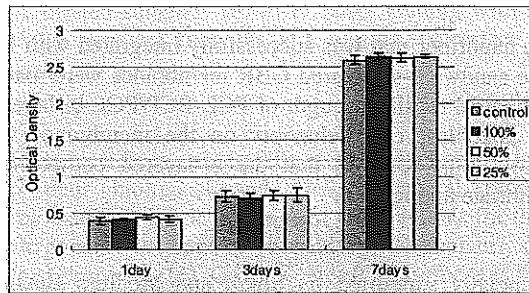


Fig. 2 MTT results of the cytotoxicity test of bi-layered composite GBR membrane.

ALP staining

For the analysis of cell differentiation on the membranes, the activity of ALP was examined after a 2-week incubation in the aforementioned culture medium. The ALPase activity was visualized using naphthol AS-BI phosphate, with fast red violet and sodium citrate as couplers according to the manufacture's protocol. The MC3T3-E1 cells cultured on the membranes showed comparable ALP activity to those on a culture dish. The cells on the membranes were presented more active ALPase activity than on the culture plate (Fig. 3).

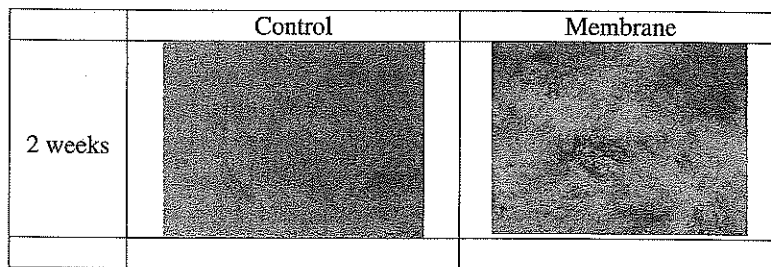


Fig. 3 ALP staining and osteoblastic differentiation of MC3T3-E1 cells on membrane

Biodegradation test

The extent of the *in vitro* degradation was also monitored through sample weight change before and after immersion. To further study the immersion-induced degradation process, a series of weight change measurements were performed for all immersed samples (Fig. 4).

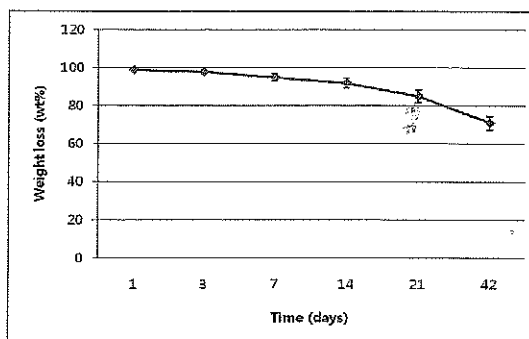


Fig.4 Weight loss of membranes after immersion in hank's solution

Conclusions

In this study, BCP/PCL nanofiber mat was successfully integrated with PCL to honeycomb-pattern porous film. The sandwich-structured GBR membranes were made using a layer-by-layer method. The mechanical tensile strength of PCL-BCP/PCL bi-layered membranes was high enough and the osteoblast cell behaviors on the membranes were also excellent compared to the control. It is believed that the bi-layered composites may be a good candidate for GBR membrane application.

Acknowledgements

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