Fibrous membranes consisting of siloxane-poly(lactic acid)-vaterite hybrid (Si-PVH) were prepared by an electrospinning method for bone reconstruction. The resulting membranes have high porosity and flexibility. Ionic silicon species have been reported to enhance proliferation and differentiation of osteoblast-like cells. The Si-PVH fibrous membranes had a hydroxyapatite-forming ability in simulated body fluid and released the silicon species gradually in a cell-culture medium. Murine osteoblast-like cells were seeded to proliferate on the membranes. The membrane was bi-layered with a poly(lactic acid) fibrous membrane and then implanted on a defect formed in the calvaria of rabbits for 12 weeks. The bone formation in the implanted membrane was better than that in a control one.

1. Introduction

Biodegradable polymer scaffolds have been developed for application in biomaterials for bone reconstruction, tissue engineering, and drug delivery systems. 3-D scaffolds with high porosity are common in the biomaterials. Cellular compatibility and bone formation were reported to be enhanced in/on porous materials. A trace amount of ionic silicon species has been recently reported to stimulate cellular activities, such as the proliferation and differentiation of bone osteoblasts and the mineralization of human osteoblasts and the osteogenic differentiation of mesenchymal stem cells [1-3]. A novel hybrid consisting of poly(L-lactic acid) (PLLA) and calcium carbonate particles (vaterite) containing siloxane was developed [4]. Vaterite is one of the polymorphs of calcium carbonates and shows higher solubility in aqueous solution than the other polymorphs, calcite and aragonite. We designed that ionic silicon species are gradually released from the materials through the degradation of PLLA matrix and vaterite and that the materials are able to show the chronic effects of the species on cellular activities.

Electrospinning is an advantageous process that can generate polymer fibrous membranes with high porosities and various fiber diameters. In the present work, fibrous membranes consisting of siloxane-poly(L-lactic acid)-vaterite hybrid (Si-PVH) were prepared by an electrospinning method. To enhance the cellular adhesion, the hybrid fibers were coated with hydroxyapatite (HA) using simulated body fluid (SBF). The cellular compatibilities of the membranes were evaluated by in vitro tests using murine osteoblast-like cells. The bone formation in the membranes was evaluated by in vivo tests using rabbits.

2. Materials and Methods

SiV powders were prepared with a carbonation process using aminopropyltriethoxysilane. Their particle size was ~1 μm and the silicon content was estimated to be ~3 wt% by X-ray fluorescence analysis.

PLLA (PURASORB®, Mw; 260 kDa) was mixed with the SiV powders at 200°C using a kneader, resulting in the formation of Si-PVH containing 60 wt% of the powders. Electrospinning was carried out using a chloroform solution containing Si-PVH under 20 kV of an impressed voltage and ~100 μA of applied current.

The resulting Si-PVH membrane was soaked in 10 ml of an aqueous solution with ion concentrations 1.5 times those of SBF (1.5SBF) at 37°C for 24 hours to be coated with HA layer.

Ion release of the HA-coated Si-PVH was evaluated. The membrane was soaked in 4 ml of a culture medium (α-MEM) containing 10% fetal bovine serum (FBS) and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO2 for 5 days. The amount of silicon species in the culture medium after the soaking was measured by inductively coupled plasma atomic emission spectrometry.

Murine osteoblast-like cells (MC3T3-E1 cells) were seeded onto the HA-coated Si-PVH membrane in 24-well plates, at a density of 30,000 cells/well. The culture medium was replaced after 1 day-culturing and then replaced every other day. The cells were chemically fixed and dried. The resulting samples were morphologically observed by scanning electron microscopy (SEM).

A bi-layered membrane consisting of HA-coated Si-PVH and PLLA membranes was prepared by pressing the them at 15 MPa with a stainless steel mesh heated at 150°C. The PLLA membrane was applied to improve the mechanical strength of the Si-PVH one. The resulting bi-layered membrane was used for in vivo tests using rabbits. An 8-mm diameter hole was drilled into the front midline of the animal’s calvaria using a bone cutter and then covered with the prepared bi-layered membranes. The bi-layered membranes were implanted with the HA-coated Si-PVH layer on the side in contact with the hole and PLLA one on the side in contact with the skin. After 4, 8, and 12 weeks of implantation, the tissues were stained with Villanueva-Goldner and observed by optical microscopy.

3. Results and Discussion

Figure 1 shows SEM micrographs of the resulting membrane, which is a porous nonwoven cloth of ~200-μm thickness; the sizes of pores formed by entangling the fibers in the cloth are less than 100 μm. The surfaces of the fibers were coated completely with HA.

From the membrane, ionic silicon species were continuously released into the culture medium at the rates of 0.2-0.5 ppm/day. Although these values are very small, they may be effective in the gene-activation of cells.

The cellular compatibility of the HA-coated Si-PVH membrane compared to the PLLA one was evaluated in culture using MC3T3-E1 cells. The numbers of the cells cultured on the Si-PVH membrane were higher than that on the PLLA one at all time points until 2 weeks. This may be due to the effects of the silicon species on cell functions and the HA-coating which shows high cellular compatibility. The cells entered the porous membrane and elongated on the fiber surfaces. The large-sized pore spaces in the HA-coated Si-PVH membrane are expected to cause the cells to proliferate.

The histological observation on in vivo tests showed that mineralized tissues formed in and around the HA-coated Si-PVH layer in the bi-layered membranes. The histology of the sample after 4 weeks of implantation indicated that the mineralization started inside of the HA-coated Si-PVH layer. The area of the mineralized tissues was found to expand in the inside of the layer by the histology after 8 weeks of implantation. After 12 weeks, the mineralized tissues almost completely filled the inside of the layer. No mineralization was observed in a PLLA layer in the bi-layered membrane. The HA-coated Si-PVH layer in the bi-layered membranes are expected to induce the cells to infiltrate into the inside of the membranes and enhance the mineralization of the cells.

![Fig. 1. SEM micrographs of the Si-PVH membrane coated with HA.](image-url)

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