INTRODUCTION:
Successful repair of severed tendons is a major challenge faced by hand surgeons. The most common complication of tendon repair is loss of digital motion as a result of restrictive adhesions between the tendon and the surrounding tissues after the repair. As a possible adhesion resistance material, we have developed a biocompatible phospholipid polymer containing 2-methacryloyloxyethyl phosphorylcholine (MPC) called poly MPC-co-o-butyldimethylacrylate-co-p-vinylphenylboronic acid (PMBV). The PMBV polymer can be formed into a hydrogel that can be easily applied to the sutured tendon by mixing with another polymer poly(vinyl alcohol) (PVA). Aiming at clinical application of this hydrogel (MPC polymer hydrogel), the present study initially determined the optimal concentration of the PMBV polymer. We then investigated the effect of the local application of the MPC polymer hydrogel on peritendinous adhesions and tendon healing using animal models. Finally, we looked into the underlying mechanism using a cell culture system.

METHODS:
The hydrogel formation from various concentrations of PMBV was examined in vitro with a fixed concentration of PVA (2.5 wt%). The stability of the hydrogel in vivo was examined by subcutaneous implantation of a diffusion chamber containing the hydrogel in the back of rats. After 1 and 3 weeks, the viscoelastic properties and the microstructure of the extracted hydrogel were assessed using rheometer and scanning electron microscope, respectively. To evaluate the peritendinous adhesions and tendon repair, rabbit flexor digitorum profundus (FDP) tendons were cut in zone II, sutured by Kessler suture, subjected to local application of the MPC polymer hydrogel or the control distilled water, and then fixed by cast for 1, 3, and 6 weeks. The harvested tendons underwent HE staining and immunohistochemistry for type I and type III collagens, and were scored by grading of histological adhesion and inflammation. For the mechanical analyses, work of flexion (WOF) and maximal tensile strength were measured using a rheometer to evaluate the peritendinous adhesions and tendon healing, respectively. To learn the underlying mechanism, fibroblastic NIH3T3 cells were cultured in the upper chamber of a double chamber dish, and the cell migration was determined by the number of cells that moved to the lower chamber due to the difference in the serum concentration between the upper (2%) and lower (10%) chambers (Figure 4, left); and cell viability in the absence of the concentration difference was evaluated by MTT assay (Figure 4, right) after 24 h of the culture.

RESULTS:
The hydrogel formation with 2.5 wt% PVA in vitro was achieved by 2.5 through 5.0 wt% of PMBV. Between MPC polymer hydrogels containing this range of concentrations of PMBV in a diffusion chamber, the dissociation was decreased and the viscoelasticity was increased in a dose-dependent manner 1 and 3 weeks after the implantation. Since only the MPC polymer hydrogel containing 5.0 wt% of PMBV could sufficiently achieve a honeycomb structure with pores of 400-800 nm in diameter even 3 weeks after the implantation, we determined this to be the PMBV concentration to use for the subsequent experiments.

Three weeks after the suture of the rabbit FDP tendon, there were severe peritendinous adhesions that prevented the passage of a vessel loop under the tendon in the control group, while few or no adhesions were observed in the MPC polymer hydrogel applied tendon (Figure 1). Histological analyses confirmed the suppression of adhesion grade by the MPC application, especially 3 weeks after surgery, while inflammation grade was not affected (Figure 2). The mechanical analyses also revealed that peritendinous adhesions determined by the WOF were decreased in the MPC group (Figure 3A). Interestingly, the maximal tensile strength representing the tendon healing was not impaired but instead was enhanced by the MPC polymer hydrogel application at 6 weeks (Figure 3B).

Migration of cultured fibroblasts was inhibited by the MPC hydrogel that covered the bottom of the upper chamber (Figure 4, left). Meanwhile, cell viability was comparable in the presence and absence of the hydrogel in the cultures, indicating that the hydrogel is biologically inert (Figure 4, right).

DISCUSSION:
A local application of the MPC polymer hydrogel immediately after the tendon suture prevented peritendinous adhesions without impairing the tendon healing. This effect may be due to suppression of cell migration from the surrounding tissues by formation of a honeycomb microstructure with nanometer-sized pores which blocked passage of cells but allowed that of cytokines and growth factors for the tendon repair. In addition, the MPC polymer hydrogel was shown to be bioinert, providing excellent biocompatibility, probably because the MPC polymer is composed of phosphorylcholine mimicking the neutral phospholipids of biomembranes. Hence, the MPC polymer hydrogel may be an ideal material for prevention of peritendinous adhesions, in that it is easy to use, biocompatible, and remains during key phases of tendon repair. Considering the urgent need for successful repair of severed tendons, this nanotechnology would improve the quality of care of patients with tendon injury, especially at the zone II digital flexor tendon.