

Prevention of Peritendinous Adhesions with Electrospun Poly(ϵ -caprolactone)-graft-chitosan Nanofibrous Mats

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Introduction: Post-surgical adhesion is one of the urgent problems facing surgeons. To improve current problems facing anti-adhesion products, we propose to use electrospinning to fabricate a porous nanofibrous membrane (NFM) as an anti-adhesion barrier. The membrane will have microporous pores to allow diffusion nutrient diffusion while block cell penetration to prevent post-surgical adhesion. The membrane will also have good mechanical properties and flexibility for application at the surgical site. In this study, we prepare and characterize electrospun polycaprolactone (PCL) NFM surface-grafted with chitosan (PCL-g-CS NFM) and assess its effectiveness in prevention of peritendinous adhesion *in vivo*.

Methods: PCL NFM was prepared by electrospinning of 10% PCL solution in organic solvents. The NFM was modified by oxygen plasma treatment, followed by surface grafting of chitosan (CS). Plasma treatment of electrospun NFM was performed between two symmetric parallel plate electrodes in a glow discharge stainless steel reactor. The sample was placed in the reactor with 5 cm distance to each electrode and the reactor was vacuumed until reaching 4 Pa, followed by purging with high purity oxygen. Plasma-treated membranes were reacted with 10 mg/ml CS solution at pH 5 for 24 h in the presence of an activating reagent. The CS-coated NFMs were washed with DDI water extensively and dried overnight in a vacuum oven for storage in a desiccator. To confirm the effects of the NFMs, we used the rabbit flexor digitorum profundus tendon model with a flexor mechanism analogous to human digits. The zone-II area of the flexor digitorum profundus tendon of the second and third toes of three-month-old New Zealand white rabbits were released from the tendon sheath, divided, cut with the scalpel just distal to the chiasm and proximal to the vincula, and sutured with the modified Kessler stitch with 6-0 braided polyester. The animals were divided into four groups. Groups 1 to 3 were subjected to local applications of PCL NFM, PCL-g-CS NFM, and a commercial anti-adhesion film (SeprafilmTM), and group 4 (control group) received applications of normal saline. After skin closure, the operatively treated leg was immobilized in a cast to limit the movement of the interphalangeal joints. After two, four, and eight weeks, the animals were sacrificed and the peritendinous adhesions and tendon healing were compared with those in the control group.

Results: From SEM, FTIR, TGA, and ESCA experiments, we confirm successful grafting of CS to PCL NFM. The nanofibrous morphology could be maintained with slight increase of diameter from 432 to 481 nm (Figure 1). From cell penetration and protein permeation experiments, the NFM can exclusively block penetration of fibroblasts without influencing the diffusion rates of bovine serum albumin. Cell culture experiments also verify PCL-g-CS NFM could reduce cell attachment but does not hinder

cell proliferation. Figure 2 shows the gross views of representative tendons receiving different treatments. The untreated tendon in the control group exhibit dense adhesion formation with the surrounding tissue, characterized by a large bundle of fibrous tissue bridging between the tendon and the surrounding tissue (Figure 2A). For the groups where tendons were wrapped with SeprafilmTM and PCL NFM, there are small bundles of fibrous tissue loosely bridging between the tendon and the surrounding tissue (Figures 2B, C). In contrast, no adhesions are evident between the tendon and the surrounding tissue in the group treated with PCL-g-CS NFM (Figure 2D). Further analysis from joint activities angle, tendon gliding distance, pull-out force, and histology indicate statistical difference between the PCL-g-CS NFM group from the control or other treatment groups.

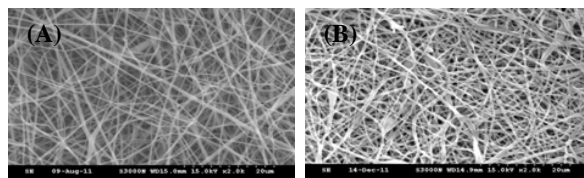


Figure 1. SEM micrographs of electrospun nanofibers. (A) PCL NFM, (B) PCL-g-CS NFM.

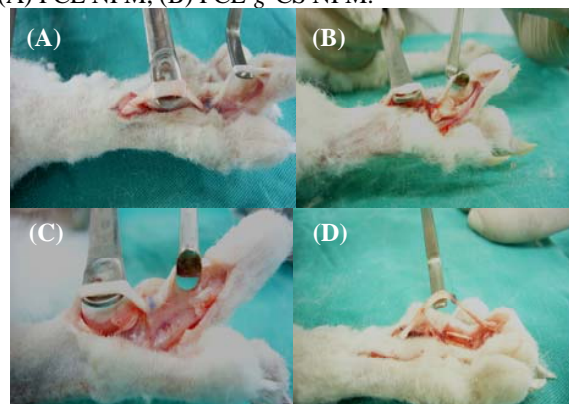


Figure 2. The extent of tendon adhesion 8 weeks post surgery. (A) Control, (B) PCL NFM, (C) SeprafilmTM, (D) PCL-g-CS NFM.

Conclusions: Chitosan could graft to PCL NFM while maintaining its nanofibrous structure. Taking advantage of the microporous structure of NFM, the anti-adhesion NFM could allow unhindered nutrients and wastes permeation while block cell penetration at the surgical site. The surface-grafted CS layer can reduce fibroblasts attachment, but not influence the tendon repair, and provide a long term lubricating effect to further improve the effectiveness of anti-adhesive PCL NFM after tendon surgery. PCL-g-CS NFM can effectively reduce the generation of peritendinous adhesion and is expected to enhance the healing of the tendon.