In Vivo Study of Chitosan-Silica Xerogel Hybrid Membrane for Wound Healing

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Statement of Purpose: Hybridization is considered one of the most useful methods for producing hydrophilic and bioactive materials. Hydrophilic biomaterials have been recognized as potential wound dressing materials due to the fact that the hydrophilicity is known to stimulate epithelial cell proliferation for guided tissue regeneration (GTR) [1]. In our previous study, the chitosan-silica xerogel hybrids were fabricated in form of the membrane and it was demonstrated that they have an excellent cell-affinity because of a good bioactivity and hydrophilicity of the silica xerogel [2]. Moreover, since the flexible and biodegradable chitosan has been widely studied as a wound healing material, the chitosan-silica xerogel hybrid membrane is expected to exhibit desirable properties for tissue regeneration applications [3]. Therefore, in this study, the chitosan-silica xerogel hybrid membrane was fabricated and the *in vivo* test was executed to evaluate their feasibility as wound dressing materials.

Methods: The silica xerogel synthesized by sol-gel process and chitosan solution dissolved in 5 wt % HCl were mixed with 3:7 volume ratios (30 % silica xerogel-70 % chitosan) [3]. This chitosan-xerogel hybrid mixture was poured into methanol solution to obtain fine hybrid fibers. The as-prepared fiber slurry was filtered into membrane form. The membranes were dried using a freeze-dryer and warm-pressed at 40 °C. The wettability of the membranes was examined by measuring the contact angles of water droplets on the surface of the membranes using a Phoenix 300 contact angle analyzer (Surface Electro Optics Co. Ltd., Korea). The *in vivo* tests were performed to investigate the effect of the hybrid membrane for wound healing using a hairless mouse. Two rectangular defects of 10 mm^2 were created symmetrically on the bilateral sides of the back of the mice. One defect was covered with the hybrid membrane and the other was covered without membrane as a control. The defects were sutured with an adhesive bandage in order to prevent the wounds exposure and detachment of the membrane, and then the wound healing was monitored by optical microscope as a function of time.

Results: Fig. 1 shows the contact angles of the different membranes with respect to distilled water. The pure chitosan membrane, with an average contact angle of approximately 95 º, was remarkably hydrophilicized through the addition of the silica xerogel. The GTR property of the hybrid membrane assessed by the *in vivo* animal test was presented in Figs. 2 and 3. After healing for 3 days [Fig. 2], the wounds covered with the hybrid membrane showed regeneration of the dermis, while the control (no membrane) have no change. Moreover, the hybrid membrane induced the nearly complete regeneration of the wounds for 8 days [Fig. 3]. In the control group after 8 days, the size of the wounds significantly decreased, but the blood clot observed, resulting in inhibition of the perfect-wound healing. Hence, these results indicate that the hybrid membrane possess a good guided tissue regeneration ability.

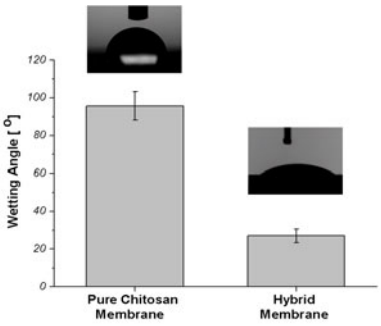

Fig. 1. The wettability of the different membranes with respect to distilled water.

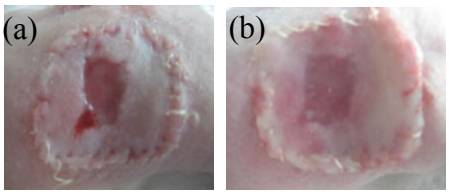

Fig. 2. The wounds observed by optical microscope after healing for 3 days: (a) no membrane (control), (b) the hybrid membrane.

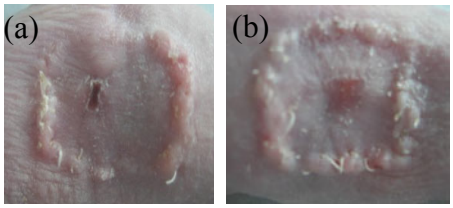

Fig. 3. The wounds observed by optical microscope after healing for 8 days: (a) no membrane (control), (b) the hybrid membrane.

Conclusions: The chitosan-silica xerogel hybrid membrane was fabricated through the sol-gel process, and their potential as guided tissue regeneration (GTR) membranes was examined by *in vivo* animal test. The chitosan-silica xerogel hybrid membrane which is highly hydrophilic induced effective wound healing without blood clotting. This experimental finding suggested that the chitosan-silica xerogel hybrid membrane could be used for guided tissue regeneration.

Reference

1.Gilchrist T et al. Biomaterials 1993;4:317-320.
2. Lee EJ et al. Biomaterials 2009;30:743-750.
3. Paul W et al. Trends Biomater Artif Organs 2004;18:18-23.