Surface Modification of Poly(lactic acid)-based Nerve Conduit with Oligo(lactic acid)-Oligo peptide Amphiphilic Conjugates

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Statement of Purpose:

Tissue regeneration using biodegradable scaffolds has been attracting great attention as a alternating strategy to the artificial organs and organ transplantation. Among various biodegradable materials poly(lactic acid) or poly(glycolic acid) are safe and promising materials but the adhesion, proliferation, and differentiation of cells on them are not necessarily satisfactory. Therefore, great efforts have been put forth in modifying the surface characteristics of the PLLA scaffolds which do not possess any side functional groups for surface modification.

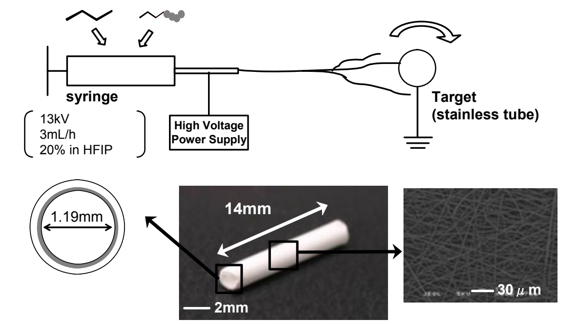
In the present study, we developed a novel immobilization method of bioactive molecules onto PLLA scaffolds using novel oligo(lactic acid)–oligo peptide amphiphilic conjugates. A solution containing PLLA homopolymer and a small amount of the amphiphilic conjugates were cast, freeze dried, and electrospun, and their characteristics were evaluated especially as the nonwoven nonofiber-type nerve conduit.

Methods:

Oligo(lactic acid)-oligopeptide amphiphilic conjugates were synthesized by using 9-fluorenylmethoxycarbonyl (Fmoc)-based stepwise solid-phase system on a Fmoc-PAL-PEG-PS resin. The peptides with the functional sequences (IKVAV) were synthesized by the general protocol and the amino terminus was deprotected with 20% piperidine/DMF without cleaved from the resin. The deprotected amino terminus was reacted with the oligo(lactic acid) with the degree of polymerization is about 19, whose hydroxyl terminus was capped with acetic acid anhydride. Resultant oligo(lactic acid)-oligo peptide amphiphilic conjugates on the resin were washed with DMF, DCM, and methanol and were cleaved from the resin using 85% trifluoroacetic acid (TFA), giving amphiphilic conjugate, OD19-IKVAV. The purity of the conjugates was confirmed by HPLC.

The nerve guide has double layered structure of the nonwoven fabric layers. Both layers were prepared by the electrospinning method as shown in Figure 1.

For preparing the inner layer, 20 % of PLLA solution in hexafluoroisopropanol (HFIP) containing 1 wt % of OD19-IKVAV was spun at the spinning rate of 3 mL/h and at 13kV. The distance between the syringe and target was 10 cm. After 30 sec spinning, the thickness of the inner layer became 20μm. Then, PLLA/PEG mixed solution in HFIP at the mixing ratio of 9:1 was spun for 10min resulting in the outer layer with the thickness of 180 μm. The appearance and the SEM microphotographs of the nonwoven fabrics were also shown in Figure 1.



Inner layer :1wt% OD19-IKVAV/PLLA (20μm)
Outer layer :10wt% PEG/PLLA(180μm)

Figure 1. Electro spinning equipment and the dable layered nerve guide

Rat sciatic nerve model was used for evaluating the nerve conduit. Female Wister rats weighing 180-210 g were anesthetized with 50mg/kg pentbarbital. The left sciatic nerve was exposed through a gluteal muscle-splitting incision. The sciatic nerve was transected and 10 mm of nerve was remobed. Continuity was reestablished by a 14-mm- long nerve guide. Various conduit made of PLLA/ OD19-IKVAV, PLLA, and silicone was evaluated 1 month postoperation.

Results:

Amphiphilic conjugates including oligo(lactic acid) segment and the hydrophilic peptides such as RGD or IKVAV were found to be effective modification moieties for the PLA-based biodegradable scaffolds. Especially, the conjugates were uniformly distributed in the PLLA matrices. The release rate of the distributed conjugates was greatly suppressed depending on the hydrophobic-hydrophilic balance of the conjugates.

When nonwoven nanofiber conduit containing OD19-IKVAV amphiphilic conjugates were applied to the rat injured sciatic nerve model, accerelated tissue regeneration in comparison with the case of PLA original conduit has been observed. The regenerated axon seems to grow along with the luminal surface of the nerve conduit. These results suggest that the amphiphilic peptides migrated to the surface of the scaffolds and were stablely immobilized due to the hydrophobic interaction.