Thermo-responsive artificial extracellular matrix protein for nerve regeneration

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Introduction: Seriously injured nerves have been treated with autologous nerve grafts. However, the resection of the normal nerve results in permanent loss of the donor function, and size mismatch between the injured nerve and the graft nerve is also an important problem. Therefore, artificial nerve conduits have been also widely accepted to bridge the gap between severed nerve stumps. Poly (D, L-lactic acid) (PLA) is recently used as the substrate for nerve conduits because it is non-enzymatically hydrolyzed to low-toxic lactic acid *in vivo* and has excellent mechanical, shaping and molding properties. However, PLA does not possess any biological activities and is preferred to be modified with bioactive molecule such as bioactive peptides. A wide variety of bioactive peptides such as RGD has been selected for tissue engineering. Although various modification techniques for PLA have been reported, so far they are prone to adverse chemical reactions. It is then necessary to develop simpler and more effective modification techniques. The purpose of this study is to introduce the nerve regenerative ability onto PLA-based nerve conduits by use of a thermo-responsive artificial extracellular matrix (aECM) which is composed of elastin-like repetitive sequence $(VPGIG)_n$ [1, 2] and neurite outgrowth promoting sequence AG73 (RKRLQVQLSIRT) [3].

Methods: Thermo-responsive aECM, AG73-$(VPGIG)_{30}$, was synthesized by the genetic engineering process. The DNA encoding $(VPGIG)_{30}$ sequence was self-ligated with the non-palindromic *Ban I* sticky-end in order to avoid the sequence inversion and the insertion of unnecessary amino acids. *E. coli* was transformed with an expression vector containing insert DNA for AG73-$(VPGIG)_{30}$ and then AG73-$(VPGIG)_{30}$ expression was induced by adding 0.1 M β-isopropyl thiogalactoside. After purification using the His-tag affinity column, AG73-$(VPGIG)_{30}$ was characterized CD spectroscopy and DLS. AG73-$(VPGIG)_{30}$ was adsorbed onto PLA film and nanofiber nerve conduits via its thermo-responsive property. The nerve regenerative property of the AG73-$(VPGIG)_{30}$-modified PLA scaffolds was evaluated using PC12 cells.

Results: The solution of purified AG73-$(VPGIG)_{30}$ showed a temperature-dependent phase transition at about 16 ºC in PBS which is very different from the case of the animal-derived water soluble elastin (Figure 1). That is, the conformational transition of AG73-$(VPGIG)_{30}$ from random-coil to β-spiral with temperature occurs even the physiological salt concentration. This thermo-responsive property below the body temperature is important and useful to be adsorbed stably onto PLA scaffolds. Furthermore, the differentiated PC12 cells attached and were promoted neurite outgrowth on the AG73-$(VPGIG)_{30}$ modified PLA scaffolds at 37 ºC more effectively than on non-coated or free AG73-adsorbed surfaces (Figure 2).

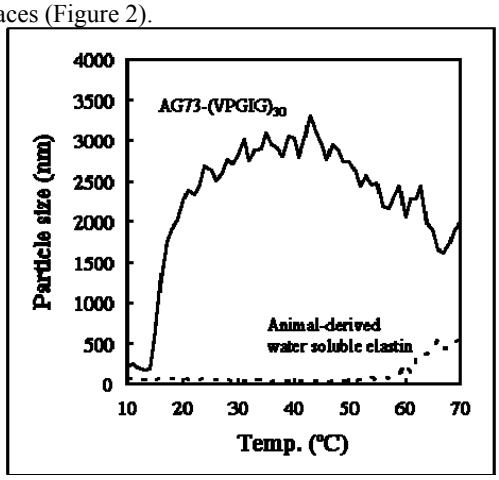

Figure 1. Thermo-responsive phase transition of AG73-$(VPGIG)_{30}$ and animal-derived water soluble elastin in PBS with temperature.

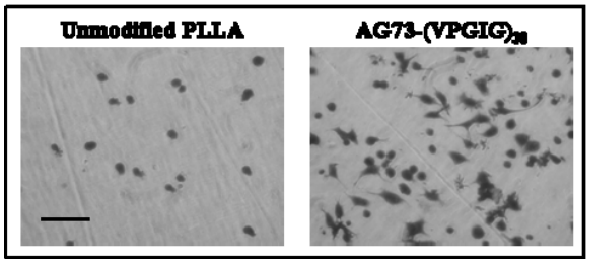

Figure 2. Neurite outgrowth of PC12 cells on unmodified and AG73-$(VPGIG)_{30}$ adsorbed PLA films. (Scale bar; 100 μm)

Conclusions: In this study, thermo-responsive aECM AG73-$(VPGIG)_{30}$ was designed, biosynthesized, and applied as surface modifier of PLA scaffolds. Neurite outgrowth activity was imported to the PLA scaffolds easily by thermal adsorption of AG73-$(VPGIG)_{30}$, which is useful for the surface modification of nerve conduits.

References:

1. Panitch A. Macromolecules. 1999: 32: 1701-1703.
2. Yamaoka T. Biomacromolecules. 2003: 4: 1680-1685.
3. Richard BL. Exp Cell Res. 1996: 228, 98-105.