NEW ARTIFICIAL NERVE CONDUITS MADE WITH HYALURONIC ACID FOR PERIPHERAL NERVE REGENERATION

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Introduction:
The achievement of peripheral nerve regeneration through biodegradable tubular conduits is at the frontier of nerve repair surgery. An ideal biodegradable conduit should maintain its structural integrity, nano-structured porous scaffold with pore and large surface area permitting cell adhesion and infiltration, and subsequent tissue ingrowth during nerve regenerative process. Thus many conduits using various biodegradable materials, such as polyglycolic acid, collagens, chitosan, poly(lactic acid) and polycaprolactone have been developed, however, no biomaterials act as an agent to aid nerve growth and repair. We fabricated new artificial nerve conduits made with hyaluronic acid (HA) that facilitate a pathway for cellular and axonal ingrowth during peripheral nerve regeneration, identifying viability of disseminated Schwann cells and neuron cells into HA conduits.

Materials and Methods:
All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the national and international laws and policies, and were approved by ethics committee of Nagoya University.

Cell preparation: Schwann cells were harvested from dorsal root ganglia (DRG) and sciatic nerves of Sprague-Dawley (SD) female rat aged 6 weeks and weighing about 200g. The harvested DRG and sciatic nerves were cultured in DMEM supplemented with 10% fetal bovine serum for 1 week and following 1 week with serum-free medium. Total of 1.0×10^6 Schwann cells were obtained and examined with immunostaining for anti-S100 antibody.

Neurospheres were prepared from the hippocampus of SD rat fetus at the specified gestational age of 16 days. The dissociated cells were cultured in DMEM/F12 replenished with N2 supplement and bFGF in 2 weeks. Total of 1.0×10^5 neurospheres were obtained and examined with immunostaining for anti-Nestin antibody.

Fabrication of HA conduits: HA (8×10^6 molecular weight) purified from rooster comb (Seikagaku Corporation, Japan) was used to conjugate cinnamic acid during photo-crosslinking reaction. The photo-crosslinked HA was poured into mold and exposed to UV light. The product was lyophilized and stored with desiccant at −20°C. HA tubular conduit has porous nano-structure of 50μm with inner diameter of 1.2mm. (Fig.1)

In vitro cell culture studies: Into HA conduits, 0.8% collagen gel was filled with Schwann cells (1.0×10^5) and neurosphere (1.0×10^5) separately. Each cell in the HA tube were cultured three-dimensionally in DMEM with 2.5% FBS for 3 weeks.

SEM analysis: To examine the morphology of the HA conduits and cells viability with scanning electron microscopy (SEM, JSM-5200, JEOL, Ltd, Japan), the samples were fixed in 2.5% glutaraldehyde and post-fixed with 1% osmium tetroxide, dehydrated with graded concentration of ethanol. The tissue was then cut to 5-μm thickness using a microtome. Micrographs were obtained at an accelerating voltage of 5kV.

Results:
Throughout the 3 week experimental periods, the HA conduits remained circular with a round lumen, and construct of cell-conduits maintained the size and shape of the original architecture of the tube. SEM micrographs of both Schwann cells and neurosphere cells in the HA conduits demonstrated cell adhesion to the conduit surfaces and migration into the porous matrix. Cell growth and neurite outgrowth were observed serially and obviously after 3 weeks. (Fig.2, 3)

Discussion:
HA has been implicated in wound healing and tissue repair occurs in the absence of acute inflammation, and collagen is deposited in a highly organized, scarless manner. Currently Seckel et al. demonstrated that HA enhances the peripheral nerve regeneration process that explained by the facts that HA organized the extracellular matrix into a hydrated open lattice, thereby stimulating the cellular ingrowth. In addition, other investigation has suggested a role for HA in synaptogenesis. Thus, an ideal artificial nerve conduit made with the biomaterials involving the remodeling of the pathway for the migration of support cells and axons could be fabricated by HA, a naturally derived, nonimmunogenic, enzymatically degradable and nonadhesive glycosaminoglycan photocrosslinked. The in vitro cell culture study showed that the 3-D porous scaffold seeded Schwann cells and neurospheres have the significant role in nerve engineering as evidenced by support of cell differentiation and neurite outgrowth. These findings provide the feasibility of using the HA conduits for better cell adhesion and differentiation, leading to axonal regeneration in peripheral nerve reconstruction.

References

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Fig.1

Fig.2 Schwann cell

Fig.3 Neurosphere

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