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Recellularized nerve allografts with differentiated mesenchymal stem cells promote peripheral nerve regeneration

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ABSTRACT

Chemical-extracted acellular nerve allografting, containing the natural nerve structure and elementary nerve extracellular matrix (ECM), has been used for peripheral nerve-defect treatment experimentally and clinically. However, functional outcome with acellular nerve allografting decreases with increased size of gap in nerve defects. Cell-based therapy is a good strategy for repairing long nerve defects. Bone-marrow-derived mesenchymal stem cells (BMSCs) and adipose-derived mesenchymal stem cells (ADSCs) can be induced to differentiate into cells with Schwann cell-like properties (BMSC–SCs or ADSC–SCs), which have myelin-forming ability *in vitro* and secrete trophic nerve growth factors. Here, we aimed to determine whether BMSC–SCs or ADSC–SCs are a promising cell type for enriching acellular grafts in nerve repair. We evaluated axonal regeneration distance by immunofluorescence staining after 2-week implantation. We used functional and histomorphometric analysis to evaluate 3-month regeneration of the novel cell-supplemented tissue-engineered nerve graft used to bridge a 15-mm-long sciatic nerve gap in rats. Introducing BMSC–SCs or ADSC–SCs to the acellular nerve graft promoted sciatic nerve regeneration and functional recovery. Nerve regeneration with BMSC–SCs or ADSC–SCs was comparable to that with autografting and Schwann cells alone and better than that with acellular nerve allografting alone. Differentiated bone-marrow-or adipose-derived MSCs may be a promising cell source for tissue-engineered nerve grafts and promote functional recovery after peripheral nerve injury.

The treatment of peripheral nerve defects remains a clinical challenge. However, the use of autologus nerve grafting, the "gold standard" for reconstructive strategies, is limited by tissue availability, secondary deformities, potential difference in tissue size and structure, donor-site morbidity and loss of function [4,16,17]. Furthermore, the clinical outcomes of functional reinnervation after grafting with cutaneous nerve grafts are not always satisfactory. Only a few motor or mixed nerves are suitable for clinical use in nerve repair. Recent reports showed that sensory isografts are inferior to motor and mixed nerve isografts for the repair of mixed nerves in rodent animals [14,16]. Therefore, promising strategies and alternatives to autologus nerve grafts need to be developed for both sensory and motor nerve regeneration.

Acellular nerve allografts have been verified experimentally and clinically as an alternative for repairing both sensory and motor peripheral nerve defects [5,8,22,26]. These acelluar nerve grafts remove the immunoreactive Schwann cells (SCs) and myelin but preserve the internal structure of the native nerve and contain important extracellular matrix (ECM) components such as collagen I and laminin and growth factors related to nerve repair and regeneration [26]. However, with the increasing length of the nerve defect, the regenerative effects of acellular nerve grafts are not satisfactory [9,22], perhaps because of lack of viable cells [23], which normally help to degenerate the debris and remodel the nerve regeneration environment of the graft. Supplementing acellular grafts with cells may improve large-gap models [15].

Cell-based therapy can create a favorable environment for peripheral nerve regeneration. Many groups have supplemented acellular nerve grafts with cultured SCs [2,3]. SCs are the most important and successful seed cells for tissue-engineered grafting, but autologous SCs are difficult to obtain and quickly expand in large numbers. Therefore, some easily accessible sources of seed cells with SC characteristics are in demand. Mesenchymal stem cells (MSCs) from bone marrow (BMSCs) and adipose tissue (ADSCs) can differentiate into SCs [7,10]. MSC-derived SC-like cells have the

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myelin-forming ability *in vitro* [24]; they express myelin-related markers [13] and can re-myelinate when transplanted into injured sciatic nerves in rats. MSC-derived SCs were safe and effective when transplanted into non-human primates for 1 year; they showed no local tumor formation [21]. As well, they showed the same trophic influences as SCs [10,22]. Therefore, MSC-derived SCs could be a candidate source for cell therapy for peripheral nerve injury.

We fabricated a novel tissue-engineered nerve graft using an acellular nerve graft and MSC-derived SCs and explored its feasibility for repair of a long peripheral nerve gap. We used a classical chemical decellularization protocol to obtain the acelluar nerve allograft [20], isolated and cultured BMSCs and ADSCs and differentiated them into SC-like MSCs, then evaluated the nerve regeneration and functional recovery with the acellular nerve allograft combined with derived and authentic SCs in bridging a 15-mm sciatic nerve defect in rats.

All the experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee

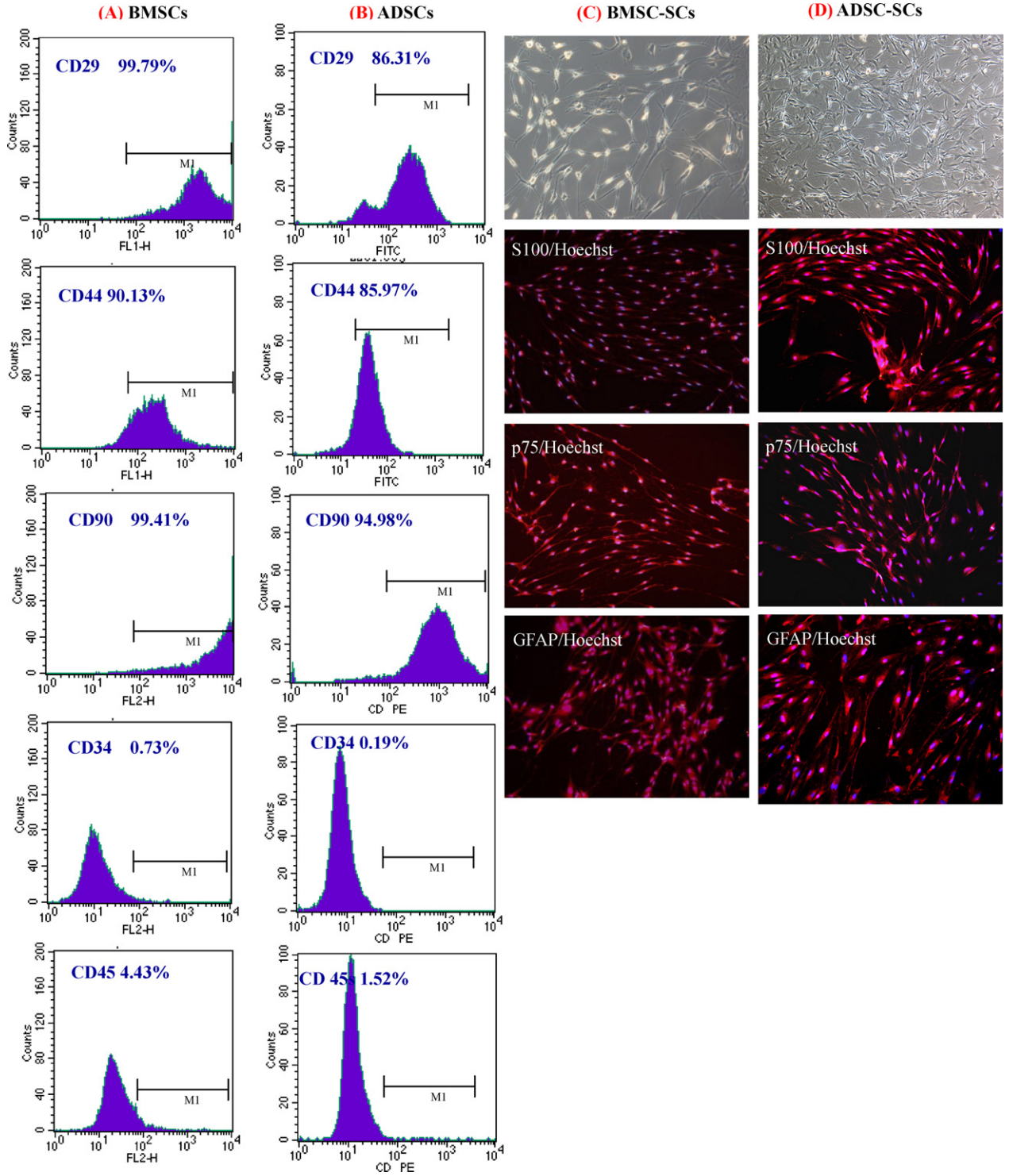

Fig. 1. Flow cytometry of the expression of cell surface markers in rat bone marrow-derived mesenchymal stromal cells (BMSCs, (A)) and adipose-derived MSCs (ADSCs, (B)). Phase-contrast microscopy and immunocytochemistry for S100, p75 and glial filament acidic protein (GFAP) of BMSC–SCs (C) and ADSC–SCs (D). Nuclei were counterstained with Hoechst 33258 (blue). (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)