Human Schwann cell derivation from small skin biopsies

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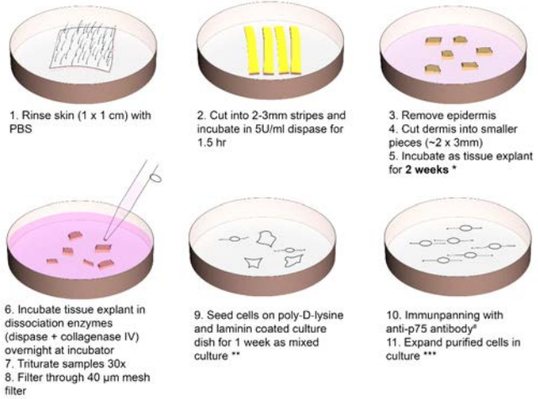
Introduction

Skin is an easily accessible tissue, which can be harvested with minimally invasive approaches from patients. We have previously shown that human Schwann cells (SCs) can be selected and expanded in culture from autopsy-derived adult skin[1]. Towards clinical application of autologous SC therapies, we aim to improve the specificity and reliability of our protocol to obtain SCs from fresh skin biopsies, processed using clinical-relevant techniques including small starting sample (1 cm^2) , short in vitro processing durations (4 weeks) and minimal manipulation.

Methods



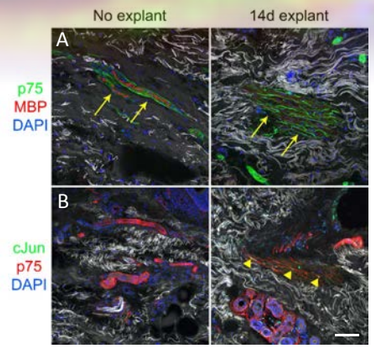
Medium used:
*: DMEM + 10% FBS + 50 ng/ml neuregulin + forskolin (5 µM) + plasmocin (0.02%)
**: DMEM + 2% FBS + 50 ng/ml neuregulin + forskolin (5 µM)
***: DMEM + 10% FBS + 50 ng/ml neuregulin + forskolin (5 µM)
#: in-house made hybridoma supernatant from 200-3-G6-4 cell line (20.4)
Dissociation cocktail: DMEM + 10% FBS + 1.25U/ml dispase + 0.125% Col IV

Reference

1. Stratton JA, Kumar R, Sinha S, Shah P, Stykel MG, Shapira Y, Midha R, Biernaskie J. Purification and Characterization of Schwann Cells from Adult Human Skin and Nerve. eNeuro, 2017

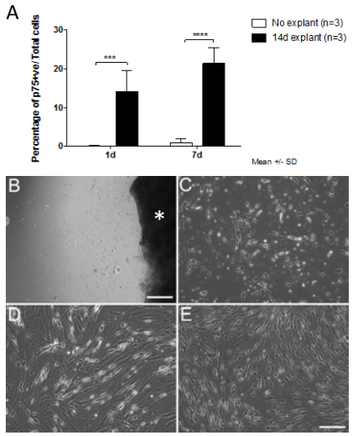
Results

1. *Changes in ex vivo tissue explants*



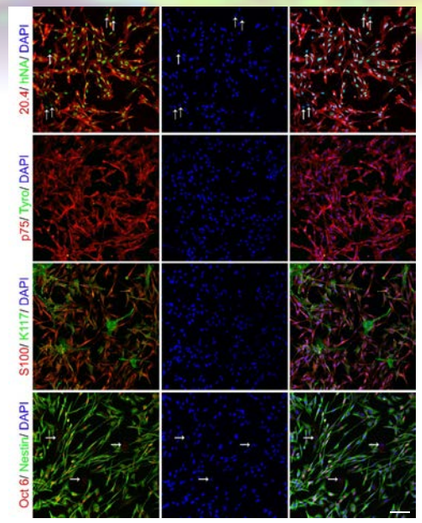
▲ Immunostaining of sectioned skin samples showed changes in morphology (arrowsheads, A) and up-regulation of c-Jun in $p75^+$ Schwann cells (arrows, B) after 14 days tissue explant incubation. Collagen fibres appear as white. Scale bar= 100 µm.

2. *Expansion of Schwann cells in culture*



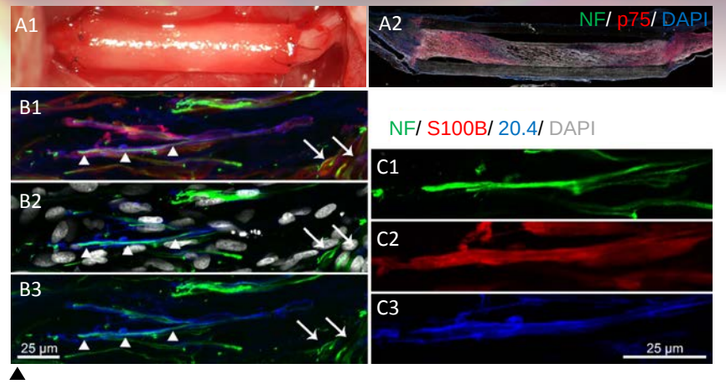
◀ Comparison of $p75^+$ cells harvested from no explant (white bars) *vs.* 14 day tissue explants (black bars) in 1- and 7-days in cultures after dissociation (A). Approximately one-fifth of cells in mixed cultures were Schwann cells after 7 days in culture (A; 0.8% in no explant vs. 21.4% in 14d explant, $p<0.0001$). Brightfield images (B-E) show cells migrating out from explant (asterisk, B), bipolar cells in mixed cultures at 1 day (C) and 7 days (D) after dissociation; and Schwann cell-like cells at 7 days after immunopanning (E). Scale bar = 100 µm.

3. *Skin derived cells express Schwann cell markers*



▲ Immunopanning with primate specific p75 antibody (20.4) increased purity to over 90%. Immunostaining showed that of Schwann cells were positive for human nuclear antigen (hNA), S100, Oct6 and nestin while negative for tyrosinase (Tyro) and thy1 (K117). Scale bar= 100 µm.

4. *Human Schwann cells ensheath rodent axons*



▲ Seeding of skin derived Schwann cells into a 1 cm long Integra NeuroGen 3D tube and implantation into immunosuppressed SD rat after sciatic nerve transection (A1). Immunostaining with p75 (red) and neurofilament (green) shows Schwann cells and axons in the tube (A2). Confocal images of axons (NF, green), pan-Schwann cell marker (S100B, red); human specific Schwann cell marker (20.4, blue) and DAPI (grey) show human derived Schwann cells ensheath axons (arrowheads) (B-C). Endogenous rodent Schwann cells only appear as S100B positive (arrows in B). Scale bar in B-C= 25 µm.

Conclusions

We have identified that 2-week of explant incubation before dissociation iss a critical step in reliably isolating human Schwann cells from a small biopsy of skin. Schwann cells can then be purified using immunopanning for Schwann cell surface markers such as p75. We routinely obtain 2-3 million purified Schwann cells in 4 weeks using this technique. These cells are positive for Schwann cell markers and are able to ensheath rodent axons when transplanted into a rodent peripheral nerve injury model.

Future directions

We are currently investigating the transcriptomic differences between nerve and skin derived Schwann cells.

Acknowledgments

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