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Research Article

WISTAR VS SPRAGUE-DAWLEY: THE INFLUENCE OF RAT STRAIN ON SCHWANN CELL ISOLATION

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ABSTRACT. Schwann cell is associated with axon by forming either myelin sheath or Remak bundles. Studies on Schwann cell biology usually employ primary culture as an experimental model that is established by isolating Schwann cells from rodent nerves. This study was to compare whether Wistar and Sprague-Dawley strains can influence Schwann cell isolation from the sciatic nerve tissue using the D-valine selective media method. Sciatic nerve tissue was dissected and the epineurium with blood vessels was stripped off. The tissue was teased and then incubated in either 0.05% (w/v) or 1% (w/v) type I collagenase for 90 minutes and 60 minutes, respectively. The digested tissue was filtered, washed, and centrifuged. The cell pellet was resuspended and finally plated in laminin/poly-L-lysine coated well plate. Schwann cell growth was observed weekly until day 28 and images were captured using an inverted microscope. Stronger enzymatic digestion resulted with significant fibroblast contamination in both strains, leading to unsuccessful Schwann cell isolation. Schwann cell growth derived from low enzymatic digestion was comparable between both strains but beyond 21-day, Sprague-Dawley culture was observed to be overtaken by fibroblasts. Furthermore, Sprague-Dawley Schwann cells assumed a differentiated phenotype, greatly reducing their proliferative capacity and eventually fibroblast growth surpassed Schwann cell growth. Wistar Schwann cells managed to outgrowth fibroblasts because they maintained proliferative phenotype. Meanwhile, Wistar fibroblasts were generally at the state of senescence. Although some Sprague-Dawley fibroblasts appeared to be senescent, presence of proliferating cells, typically possess small, elongated morphology was evident. Due to this, Schwann cells were able to thrive in Wistar culture whilst fibroblasts eventually managed to outgrow Schwann cell in Sprague-Dawley culture. In summary, isolating Schwann cell from Wistar rat was comparatively superior to that from Sprague-Dawley rat using D-valine selective media technique.

Keywords: *D-valine selective media, Schwann cell, primary cell isolation, rat strain*

INTRODUCTION

Schwann cell is the primary glial cell in the peripheral nervous system and is closely associated with axon through the formation of either the myelin sheath [3,6] or the Remak bundles [5]. Myelin sheath is a thick and compact insulating membrane formed by myelin Schwann cells enwrapping their cell membrane around a single large-calibre axon(≥1μm) multiple times [3, 6]. The saltatory nerve conduction is made possible by the myelin sheath which drastically accelerates the propagation of action potential. On the other hand, the Remak bundle is formed by Remak Schwann cell which encloses several small-calibre axons (0.5-1.5μm) in its mesaxon [3, 9]. Besides facilitating nerve conduction, Schwann cells also play supportive functions in diverse neurobiology processes ranging from development [14], neuropathic pain mechanism [22] to nerve injury response and regeneration [13].

The investigation on the mechanisms of Schwann cell biology commonly employ primary cell cultures as experimental model which is established by isolating Schwann cells from rodent nerves. Rat is a common rodent species for isolating Schwann cells and previously, several different rat strains have been used for this purpose such as Lewis, Fisher, Sprague-Dawley and Wistar rats [2, 15, 16, 21]. There are also several different techniques for isolating Schwann cells such as in vivo and in vitro nerve tissue pre-degeneration methods, cold-jet method, antimitotic treatment, antibody-mediated cytolysis, immunoselective methods and D-valine selective media [15]. Therefore, there is no standardized isolation techniques and rat strains used vary from one laboratory to the other. It is argued that the feasibility of abovementioned isolation methods might be dependent to or at least affected by the strain of rat used. This study hypothesized that rat strain can influence the efficacy of the D-valine selective media method in isolating and purifying Schwann cells. To test the hypothesis, this study compared two common rat strains, namely Wistar and Sprague-Dawley, to evaluate whether the strain can influence the isolation of primary Schwann cell from sciatic nerve tissue using the D-valine selective media method. Firstly, we compared the dissociation of sciatic nerve tissue between the strains by studying the effect of collagenase concentration on tissue digestion. Then, we evaluated the degree of Schwann cell growth and fibroblasts contamination between the two strains.

MATERIALS AND METHODS

Reagents and Chemicals

DMEM L-valine was obtained from Nacalai Tesque (Japan). Foetal bovine serum, glutamax, penicillin-streptomycin, amphotericin B, N-2 supplement and bovine pituitary extract were purchased from Gibco (USA). Collagenase type 1, Clostridium histolyticum, Poly-L-Lysine and laminin were procured from Merck (Germany). Forskolin was purchased from Chemfaces (China).

The dissection and extraction of rat sciatic nerve tissue

Adult male rats of both Wistar (Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia) and Sprague-Dawley (KRK Enterprise, Malaysia) strains were used in the study, in accordance with the approval granted by the Institutional Animal Ethics Committee. The sciatic nerve tissue was used for the isolation of primary Schwann cells. Rats were euthanised using carbon dioxide (CO_2) asphyxiation and followed by

cervical dislocation. Method for isolating primary Schwann cells was adapted from a previously published method [15]. Firstly, the rat was placed on a working surface in the prone position. The rat skin was sterilised with 70% alcohol. The skin was cut along the midline and retracted laterally to expose the deep fascia. A small cut was made obliquely along the deep fascia of the thigh from the spine to the knee of both sides. The muscles of the posterior thigh were split to expose the entire length of sciatic nerve. The sciatic nerve was gently lifted using forceps and it was dissected by cutting at its proximal and distal ends. The nerves of the both sides were placed into chilled medium with serum (DMEM L-valine supplemented with 10% foetal bovine serum, 1% glutamax, 1% penicillin-streptomycin and 0.5% amphotericin B).

Primary Schwann cell isolation from rat sciatic nerve

Firstly, the epineurium and blood vessels were stripped off by holding the proximal nerve section using the fine forceps and carefully, using another mounted needle, pulling the connective tissue towards the distal segment, like taking off a sock. The epineurium-free nerve was then teased using a mounted needle into separated nerve fascicles until the tissue resembled a cotton ball. The tissue was processed under a stereomicroscope and kept in a small amount of culture medium containing antibiotics throughout the process. After that, the tissue fragment was transferred into either 0.05% (w/v) or 1% (w/v) type I collagenase (both dissolved in culture media without any supplements) and incubated at 37°C, 5% CO_2 with intermittent agitation for 90 minutes and 60 minutes, respectively. The tissue dissociation procedure was summarized in Table 1. The digested tissue was filtered through a 40-um cell strainer, mixed with a fresh serum containing medium to stop the enzymatic activity, and centrifuged at 400g for 6 minutes. After removing the supernatant, the cell pellet was resuspended in 1 ml of Schwann cell culture medium (DMEM D-valine supplemented with 10% (v/v) foetal bovine serum, 1% glutamax, 1% penicillin-streptomycin, 0.5% amphotericin B, 5μM forskolin, 1% N-2 supplement and 20μg/ml bovine pituitary extract). The cell suspension was divided and plated into 2 wells of a 6-well cell culture plate that has been precoated with poly-L-lysine and laminin. Then, 4.5 ml of Schwann cell culture medium was added into each well. The cells were maintained in a humidified condition at 37°C, 5% CO_2 for 28 days. 2 ml of fresh Schwann cell culture medium was added into the wells at day 7. At day 11, the culture medium was changed and thereafter, the medium was changed every 2-3 days.

Table 1. *Sciatic nerve tissue dissociation*

	Wistar rat		**Sprague Dawley rat**	
Collagenase concentration	0.05%	1%	0.05%	1%
Duration of incubation	90 minutes	60 minutes	90 minutes	60 minutes
Complete dissociation	No	Yes	No	Yes

Observation of Schwann cell growth using phase contrast microscopy

Schwann cell growth was observed at day 7, 14, 21 and 28. Two to four areas were captured using the inverted microscope (Olympus, CKX31SF) at 100x and 200x magnification. Cultured fibroblasts were characterised by their flattened and polygonal

shaped with an irregular cellular outline while cultured Schwann cells were relatively smaller, having an elongated bipolar or multipolar shape which were arranged in a unique mosaic pattern.

RESULTS

Comparison of sciatic nerve tissue dissociation using collagenase digestion between Wistar and Sprague-Dawley rats

The study first compared the two strains with respect of sciatic nerve tissue dissociation by studying the effect of collagenase concentration on the tissue digestion. Sciatic nerve tissue extracted from both rat strains were digested in 0.05% (w/v) and 1% (w/v) type I collagenase for 90 minutes and 60 minutes, respectively. Fig. 1 shows processed sciatic nerve tissue in collagenase solution with different concentrations before and after incubation at 37°C, 5% CO_2. In 0.05% (w/v) concentration, the tissues from both strains were observed to be completely digested with no apparent tissue fragments present in the collagenase solution after incubation. Similar observation was also recorded in 1% (w/v) concentration with the absence of large tissue fragment in the solution for both strains. However, upon filtration with 40 µm cell strainer, small tissue fragment was collected from digestion using 0.05% (w/v) collagenase solution and this observation was true for both strains (Fig. 2). On the other hand, no significant tissue fragment was collected for both strains when digested using 1% (w/v) collagenase solution, suggesting that the tissue from both strains were completely dissociated (Fig. 2).

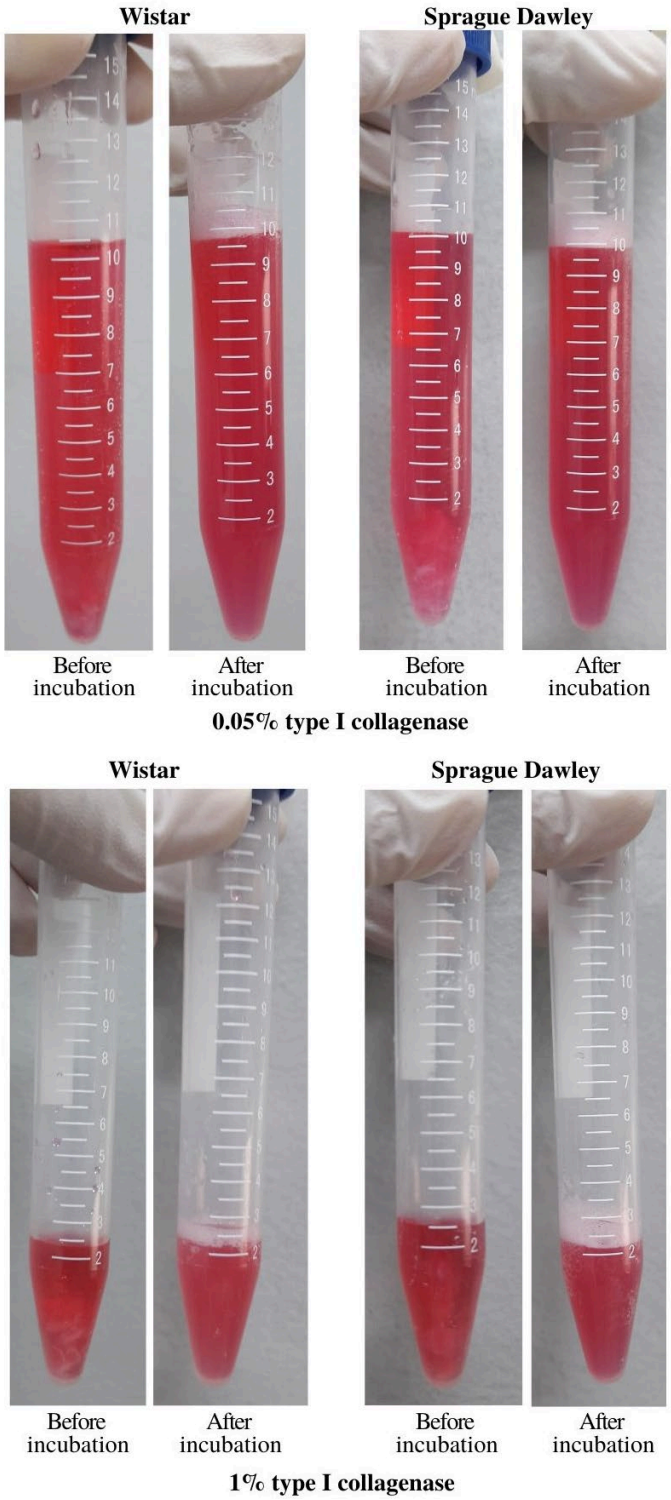

Fig. 1. *Sciatic nerve tissue digestion in type I collagenase*

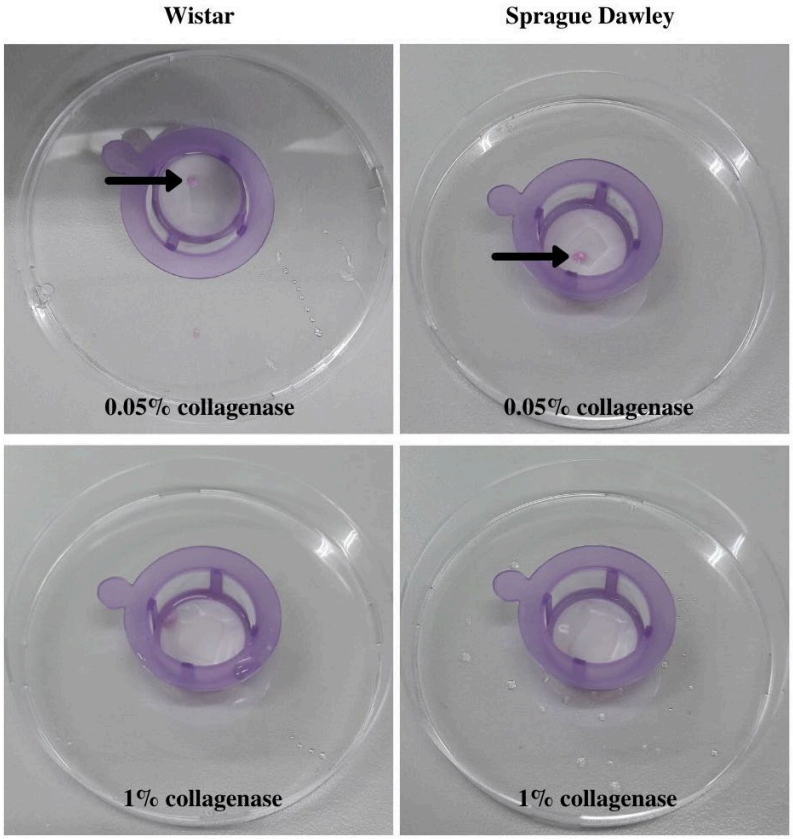

***Fig. 2**. Undigested sciatic nerve tissues (arrow) following filtration using 40 µm cell strainers*

Comparison of Schwann cell growth and fibroblast contamination between Wistar and Sprague-Dawley rats

We then compared Sprague-Dawley and Wistar rats with respect of the feasibility to isolate Schwann cells by studying Schwann cell growth and fibroblasts contamination. Following tissue dissociation, the resultant cell suspension was seeded onto well plates and maintained in culture for 28 days. Schwann cell growth and fibroblasts contamination were observed through phase contrast microscopy at day 7, 14, 21 and 28. In both strains, initial cell outgrowth was relatively low when using 0.05% (w/v) collagenase digestion (Fig. 3) as compared to 1% (w/v) collagenase digestion (Fig. 4). Despite low outgrowth, colonies of Schwann cells started to form at day 14 in both Wistar and Sprague-Dawley strains (Fig. 3). Meanwhile, for 1% (w/v) collagenase digestion, Schwann cells were virtually absent and fibroblasts growth was pervasive throughout the culture duration in both strains (Fig. 4). In culture derived from 0.05% (w/v) collagenase digestion, fibroblasts were present in culture from both strains at day 14 and 21 although fibroblasts growth was significantly lower than Schwann cells. By day 28, Schwann cells had virtually overgrown fibroblasts in cultures from Wistar rat with fibroblasts were virtually absent in the cultures. Meanwhile, in Sprague-Dawley cultures, the presence of Schwann cells was discernibly less than in Wistar cultures and fibroblasts remained observable in the cultures (Fig. 3).

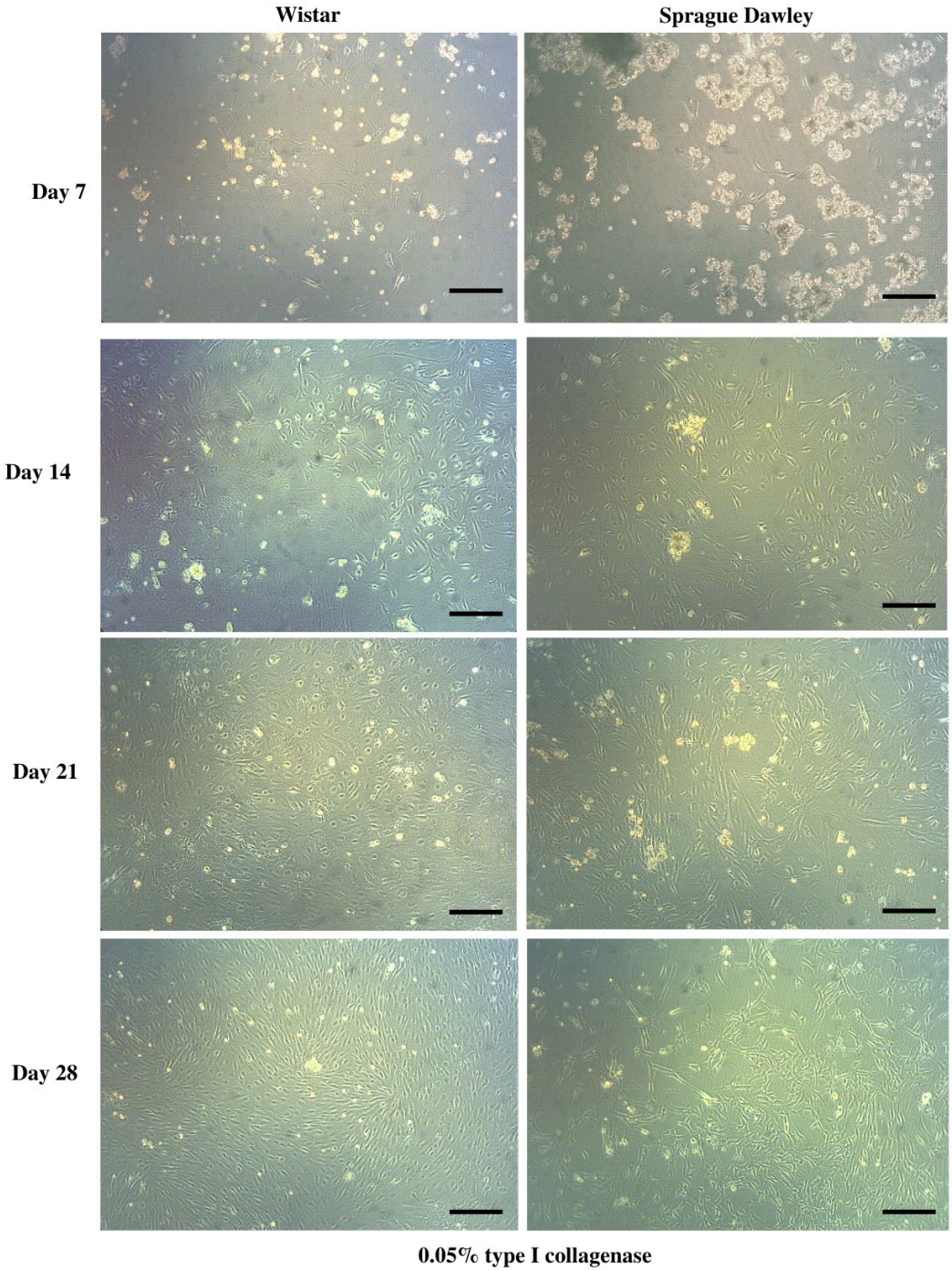

Fig. 3. *Phase contrast micrographs of Schwann cells isolated from Wistar and Sprague-Dawley rats at day in vitro 7, 14, 21 and 28 after plating. Sciatic nerve tissues were dissociated using 0.05% type I collagenase. Scale bar = 200 μm.*

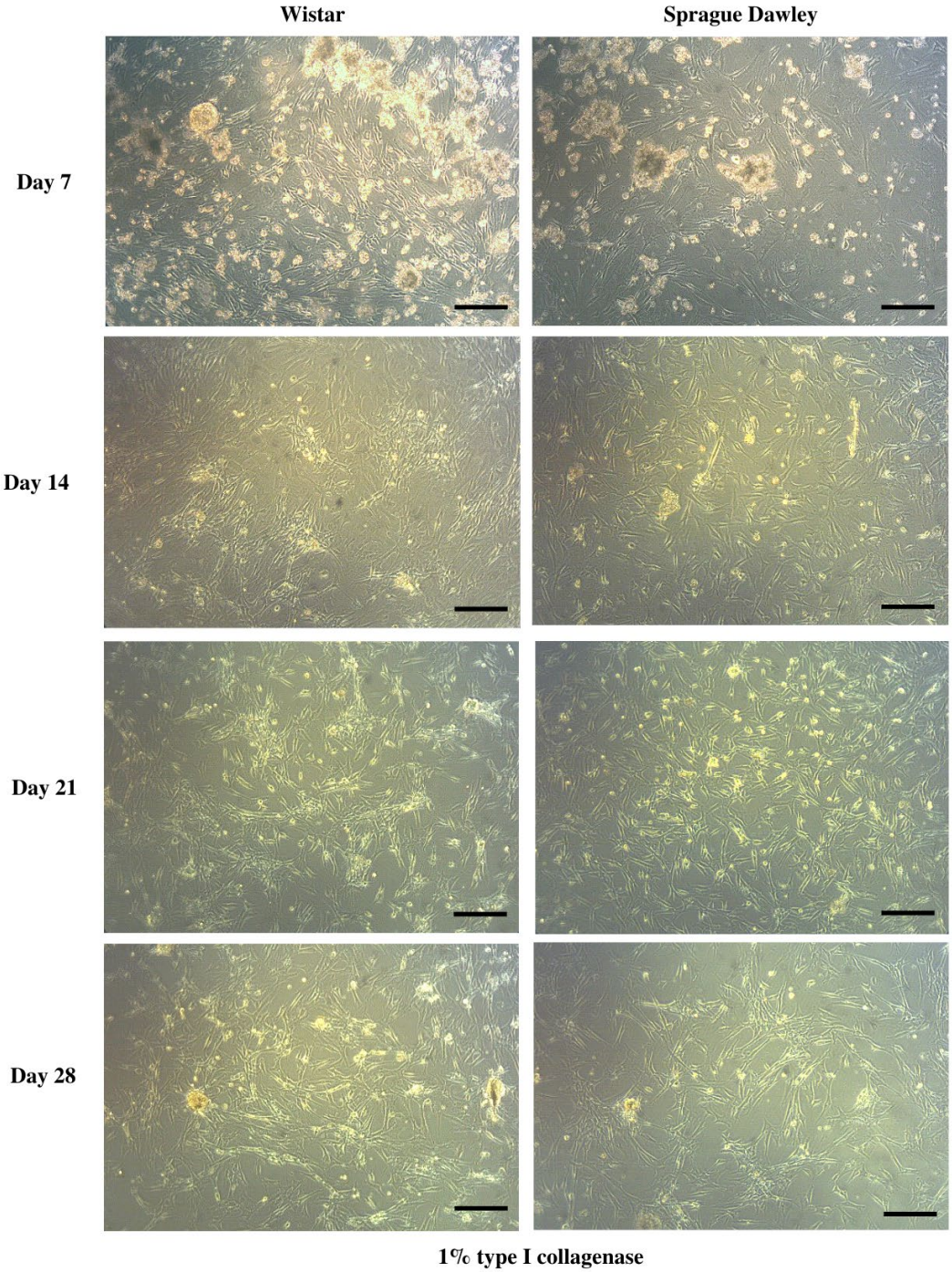

Fig. 4. *Phase contrast micrographs of Schwann cells isolated from Wistar and Sprague-Dawley rats at day in vitro 7, 14, 21 and 28 after plating. Sciatic nerve tissues were dissociated using 1% type I collagenase. Scale bar = 200 µm.*

Observation on Schwann cell morphology in culture derived from Wistar and Sprague-Dawley rats

In this section, only observation from 0.05% (w/v) collagenase digestion was recorded because no Schwann cell growth was observed in cultures from 1% (w/v) collagenase digestion. At day 7, Schwann cells in cultures derived from both strains exhibited similar morphology i.e. bipolar, spindle-like morphology (Fig. 3). However, at day 21, the typical bipolar morphology of Schwann cells became less prominent in Wistar Schwann cells with the cells appeared rounded without clear cytoplasmic processes (Fig. 5). On the other hand, Sprague-Dawley Schwann cells retained their spindle-like morphology (Fig. 5). At day 28, as cultures became confluent, Wistar Schwann cells assumed spindle-like morphology, however, they appeared short and broad at the central body. Comparatively, Sprague-Dawley Schwann cells exhibited elongated bipolar morphology with slender cell body (Fig. 3).

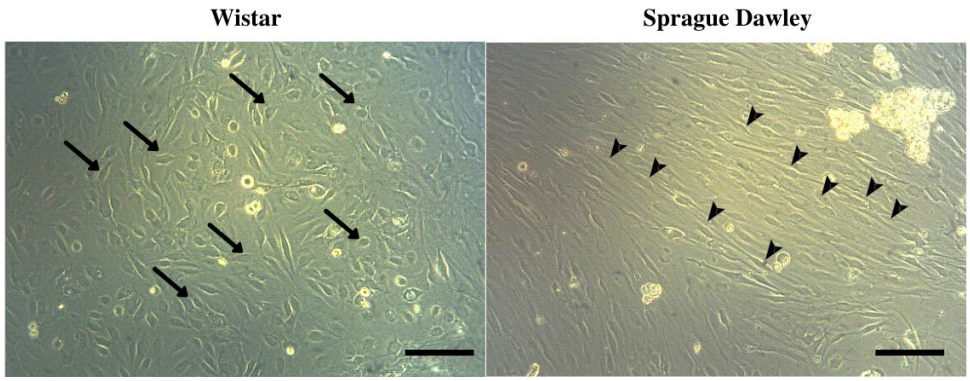

Fig. 5. *Phase contrast micrographs of Schwann cells isolated from sciatic nerve tissues of two different rat strains at day in vitro 21. Note that Wistar Schwann cells (arrow) appeared rounded without clear cytoplasmic processes while Sprague Dawley Schwann cells (arrowhead) retained their spindle-like morphology. Scale bar = 100 um.*

Observation on fibroblast morphology in culture derived from Wistar and Sprague-Dawley rats

The study also compared the morphology of fibroblasts between Wistar and Sprague-Dawley strains. In both strains, fibroblasts isolated using 0.05% collagenase digestion exhibited with an irregular morphology and they were larger than typical fibroblasts. The cytoplasm was extensive with long slender cytoplasmic processes and undistinguishable cell boundary (Fig. 6 (a) and (b)). Meanwhile, fibroblasts isolated from 1% collagenase presented with a prominent cell boundary and elongated morphology with smaller size (Fig. 6 (c) and (d)). This suggests that the former fibroblasts underwent extensive cell spreading unlike in the latter. Of note, Wistar fibroblasts exhibited a much more extensive cell spreading features than Sprague-Dawley because there were still some fibroblasts with elongated morphology in the Sprague-Dawley culture.

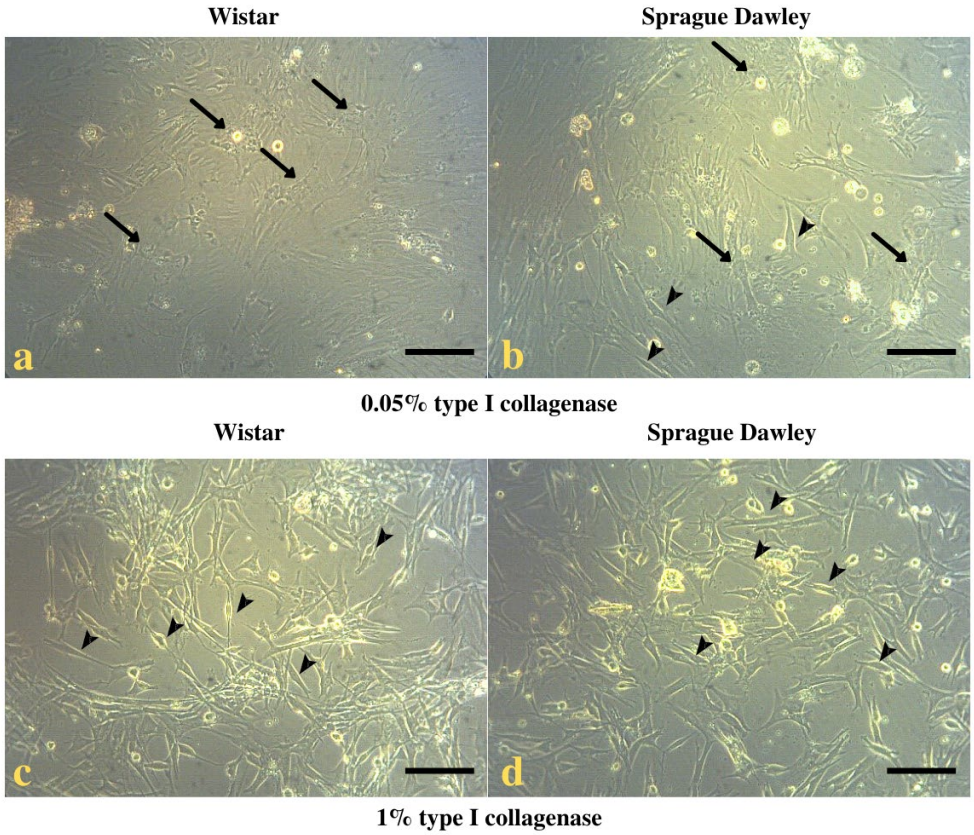

Fig. 6. *Phase contrast micrographs of fibroblasts isolated from sciatic nerve tissues of two different rat strains at day in vitro 21. In 0.05% type I collagenase, Wistar fibroblasts were predominantly presented with extensive processes morphology (arrow) while Sprague-Dawley fibroblasts were showing both extensive processes morphology (arrow) and elongated morphology (arrowhead). In 1% type I collagenase, virtually all fibroblasts of both strains were presented with elongated morphology (arrowhead). Scale bar = 100 um.*

DISCUSSION

Schwann cells are commonly isolated from the sciatic nerve as this peripheral nerve is easily extracted due to its large size [23]. In this study, the sciatic nerves were dissected from two different strains of adult rats i.e. Wistar and Sprague Dawley which are among the common strains used in culturing Schwann cells [1,15,19,20]. Adult rats were selected as large size of peripheral nerve is preferable to obtain more Schwann cells. However, some studies have used neonatal rats or mice [1,11,20].

Enzymatic digestion is a crucial process in primary cell isolation in which the process helps to dissociate tissue to release desired cells. The present study examined the dissociation profile of the sciatic nerve tissue from Wistar and Sprague-Dawley rats by digesting the tissue using high and low concentrations of type I collagenase. Previous findings had indicated that there were differences between Wistar and Sprague-Dawley rats in term of the morphometric features of the sciatic nerve tissue [10,12]. The epineurium, a connective tissue layer surrounding peripheral nerves, in Sprague-Dawley

rat was reported to be at least 50% thicker than in Wistar rat. Grounded on these findings, we postulated that tissue dissociation of Sprague-Dawley sciatic nerve would require stronger enzymatic digestion in comparison to Wistar nerve. Nonetheless, the study discovered that there was no significant difference in tissue dissociation profile between the two strains when digesting using both low and high concentrations of type I collagenase. Thus, rat strain does not affect the tissue dissociation process in isolating primary Schwann cells from the sciatic nerve. It is also interesting to note that stronger enzymatic digestion resulted with significant fibroblast contamination in both strains, leading to unsuccessful Schwann cell isolation. Thus, tissue digestion using low concentration of type I collagenase is recommended for isolating Schwann cells from both rat strains as the method is practically effective to dissociate sciatic nerve tissue with minimal fibroblast contamination.

Analysis on Schwann cell growth showed that it was comparable between Wistar and Sprague-Dawley rats (in digestion using low concentration of type I collagenase) up until 21-day in culture, however Sprague-Dawley culture was observed later to be overtaken by fibroblasts (data not shown). Of note was the stark difference in Schwann cell morphology between the two strains i.e. Sprague-Dawley Schwann cells were elongated whereas Wistar cells were comparatively shorter. The different cellular morphology may serve as a clue on why the differential outcomes were generated. Recently, it has been shown that Schwann cell elongation is associated with cellular differentiation [8]. Mature Schwann cells such as Remak and myelin cells were discovered to be at least twice longer than immature Schwann cells while, repair Schwann cells can elongate by 7- to 10-fold the length of immature cells. Therefore, we infer that beyond 21-day in culture, Sprague-Dawley Schwann cells assumed a differentiated phenotype, greatly reducing their proliferative capacity [4,17] and eventually fibroblast growth surpassed Schwann cell growth. Wistar Schwann cells, on the other hand, managed to outgrowth fibroblasts because they maintained a proliferative phenotype which is characteristic of immature Schwann cells.

Fibroblasts' state of proliferativeness is also a contributing factor for the differing outcomes between Wistar and Sprague-Dawley rat strains. We speculate that Sprague-Dawley fibroblasts were more proliferative as compared to Wistar fibroblasts. The morphological analysis suggested that Wistar fibroblasts were generally at the state of senescence which is typically characterized by flattened and enlarged morphology [18]. Although some Sprague-Dawley fibroblasts appeared to be senescent, presence of proliferating cells, typically possess small elongated morphology [18], was evident. Due to this, Schwann cells were able to thrive in Wistar culture whilst fibroblasts eventually managed to outgrow Schwann cell in Sprague-Dawley culture.

In this study, D-valine, instead of L-valine, was used in the culture medium to selectively inhibit fibroblast growth due to its low expression level of D-amino acid oxidase. In contrast, Schwann cells express a higher level of D-amino acid oxidase to metabolize D-valine into L-valine which is essential in cell survival and proliferation [7]. Depleted of L-valine, fibroblasts may undergo cell senescence, potentially induced by oxidative stress [18]. The fact that Sprague-Dawley fibroblasts can overcome the inhibitory effect of D-valine, may suggest that the level of D-amino acid oxidase may be higher than that in Wistar fibroblasts. Nevertheless, further study is needed for confirmation.

This study reports findings that were mainly based on qualitative analysis such as cell morphological analysis. Although the analytical method is still useful to differentiate

between Schwann cell and fibroblast, quantitative study such as cell purity analysis using immunofluorescence or flow cytometry techniques may provide a much more conclusive data in measuring the number of Schwann cells in culture. This analysis will be explored in a future study.

CONCLUSION

Isolating Schwann cell from Wistar rat was comparatively superior to that from Sprague-Dawley rat using the D-valine selective media technique. Fibroblasts contamination was better controlled in Wistar cultures than in Sprague-Dawley cultures using the D-valine selective media and in turn suggesting the adult Wistar as a preferred rat strain to use in isolating Schwann cells. Although complete tissue dissociation did not occur in 0.05% collagenase in both strains, Schwann cells still can be obtained after 28-day in culture. Despite complete tissue dissociation, tissue digestion using 1% collagenase is not recommended due to significant fibroblasts contamination. Further study is needed to compare the Schwann cell purity between Sprague-Dawley and Wistar cultures as well as to analyse the level of D-amino acid oxidase in fibroblasts of both strains.

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