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On the Relationship Between Fascicle Diameter and Perineurium Thickness in the Ulnar Nerve of Pigs

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***Abstract*—The anatomical structures of peripheral nerves significantly influence the performance and selectivity of neural interfaces. Among these structures, the perineurium thickness, because of its high resistivity, is important in shaping the electric field distribution within a nerve. However, data on the perineurium thickness of somatic nerves in animals is sparse. As animal models are the first step towards developing novel implantable nerve interfaces, this study characterises the perineurium thickness in the ulnar nerve of pigs. Distal and proximal sections of the ulnar nerve (n = 6) were extracted, stained with haematoxylin and eosin, and the perineurium thickness and fascicular diameters were measured. In total, 129 fascicles were quantified, and the average fascicle diameter was 269 ± 73.3 µm and 277 ± 81.1 µm for the distal and proximal nerves, respectively (p = 0.59). A linear relationship was observed between fascicle diameter and perineurium thickness (R^2 = 0.69). This relationship was affected by the location of the nerve section, with distal sections having a greater perineurium thickness than proximal segments. Finally, equations were provided to estimate the perineurium thickness based on fascicle diameter. This information can be used to develop realistic peripheral nerve models, which can reduce variability and improve the selectivity of neural interfaces.**

Keywords—ulnar nerve, nerve morphology, perineurium thickness, nerve stimulation

I. Introduction

The application of electrical stimulation to modulate peripheral nerve activity can be used to restore motor and sensory function in impaired systems [1]. One of the main challenges limiting the clinical applicability of peripheral nerve neuromodulation is the inability to a priori obtain the neuronal activation patterns created by an interface [2], therefore resulting in non-selective activation of muscle groups in the case of functional electrical stimulation [3] or activation of unwanted fibre groups causing adverse side effects in the case of bioelectronic medicine [4].

Peripheral nerves have a complex structure that can be summarised as individual fibres surrounded by a layer of connective tissue (i.e., the endoneurium). Bundles of fibres are tightly packed together in fascicles. Each fascicle is then surrounded by dense layers of cells and collagen (i.e., the perineurium), which provides most of the mechanical strength of the nerve and maintains the blood-nerve barrier. Finally, the epineurium is the outermost part of the nerve, holding the fascicles together [5].

The anatomical structure of peripheral nerves (e.g., nerve and fascicle diameter, number of fascicles, and the spatial location of fascicles and fibres) significantly influences the electrical potentials inside the nerve that result from the external application of stimulating currents. One of the most important structures for nerve modelling is the perineurium, which because of its high resistivity, causes a significant decrease in the gradient of the potential field inside the fascicle, thereby increasing electrical stimulation thresholds [6]. As the perineurium resistance is a function of the thickness and resistivity of the tissue layer, models of peripheral nerves that consider the perineurium a constant value independent of fascicle diameter may result in inaccurate electrical stimulation thresholds [7].

Several studies have shown a linear relationship between perineurium thickness and fascicle size in human and animal peripheral nerves [8]–[11], leading to the implementation of this relationship in computational models of peripheral nerves [12]–[14]. However, evidence indicates that this relationship, at least for somatic nerves in humans, is not a constant function throughout the length of the nerve. In a study by Sunderland and Bradley [10], the authors reported greater thickness of the perineurium at the wrist than at the axilla for the ulnar and median nerves. In contrast, in a study by Abdalbary et al. [15], greater perineurium thickness was observed in the forearm compared to the arm and wrist.

Most studies investigating the relationship between perineurium thickness and fascicular diameter have been

performed with human nerve specimens. Studies performed in animals, however, primarily explored this relationship in autonomic nerves, and information on this association in somatic nerves in animals is still insufficient. As animal models are the first stage towards the development of implantable neural interfaces, and the use of pigs in preclinical studies for neural interface development has increased because of the pig's nerve size and polyfascicular structure [16] that resembles the human counterpart, making them a suitable animal model, this study aimed to investigate the relationship between fascicular diameter and perineurium thickness in the ulnar nerve of pigs and, in addition, evaluate if this relationship is different across proximal or distal sections of the nerve.

II. Methods

A. Nerve Dissection

The experiment was approved by the Danish Veterinary and Food Administration under the Ministry of Food, Agriculture, and Fisheries of Denmark (protocol number: 2017-15-0201-01317). Six Danish Landrace pigs were used for this study (mean weight: 35.4 ± 3.2 kg). The animals were anaesthetised and placed in a supine position where an incision of roughly 20 cm was performed on the anterior forelimb. The ulnar nerve (UN) was exposed and freed from the neighbouring tissue. A protocol to obtain excitation thresholds of the ulnar nerve was carried out [17] (not reported in this manuscript), and the animals were then euthanised with an overdose of pentobarbital.

The nerve samples were obtained at two locations in the forelimb (see Fig. 1), using the branching of the UN to the dorsal cutaneous branch of the UN as reference: (1) four cm proximally to the branching point (proximal UN) and three cm after the branching point (distal UN). The samples were 2 mm in length.

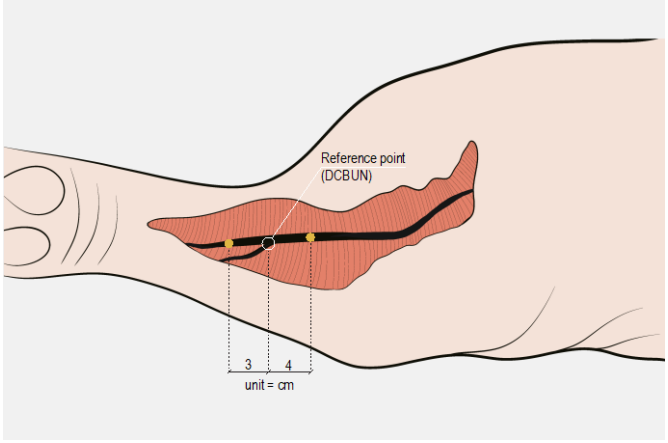

Fig. 1. Illustrative image of the two nerve sections extracted for the histology. The reference point refers to the dorsal cutaneous branch of the ulnar nerve (DCBUN).

B. Histology and Image Analysis

The nerve samples were fixed by immersion in formaldehyde solution (4% for at least 24 h), embedded in paraffin, and stained with haematoxylin and eosin. Slices of 2.5 µm were digitised under 40x magnification (NanoZoomer S360, Hamamatsu Photonics, Hamamatsu, Japan). The images were then visualised with viewer software NDP.view2 (Hamamatsu Photonics, Hamamatsu, Japan), where the individual fascicles were identified. For each fascicle, the inner and outer perineurium boundaries were manually quantified. The area of the fascicles was converted to effective diameters, and a correction factor of 1.25 was applied to the diameters. This step was performed because tissue samples immersed in formalin shrink, and comparisons between formalin-embedded samples and frozen samples showed that multiplying the fascicle diameter by 1.25 can correct for this effect [18]. Finally, the perineurium thickness was defined as half of the difference between the effective diameters of the boundaries, as in [11]. The inner perineurium was considered to measure the fascicular diameter, and only fascicles with clear perineurium boundaries were included in the analysis.

C. Statistical Analysis

Firstly, a linear model was used to relate the fascicle diameter with the perineural thickness (M1). Then, to assess if this relationship depended on the nerve location (proximal vs distal), a second linear model was fitted to the data, considering the nerve's location as an explanatory variable (M2). M1 and M2 were compared with the Chi-square test and the coefficient of determination (R^2) to investigate if the nerve location had an influence on the relationship between fascicle diameter and perineurium thickness. If M2 was found to be significantly superior (higher R^2 and significant *F*-test) to M1, two linear models were used: one for the distal UN and one for the proximal UN. On the contrary, if M2 and M1 were equivalent, a single model was used to fit the whole data without considering the location of the nerve section. The linear model assumptions of homoscedasticity and normality were verified through residual analysis. Finally, a two-sample independent *t*-test was used to compare fascicle diameter and perineurium thickness between proximal and distal segments of the UN. The adopted significance level was 0.05, and the statistical analysis was performed in R [19].

III. Results

In total, 129 fascicles were identified and measured across the six animals corresponding to 55 fascicles for the distal UN and 74 fascicles for the proximal UN. For all animals, both sections of the nerve were polyfascicular; the median number of fascicles observed for the proximal UN was 13.5 (range: 7 – 15), whereas the median number of fascicles for the distal UN was comprised of 7 (range: 7 – 13) fascicles. Fig. 2 shows a proximal UN containing 15 fascicles with the outer and inner perineurium segmented in yellow and red, respectively.

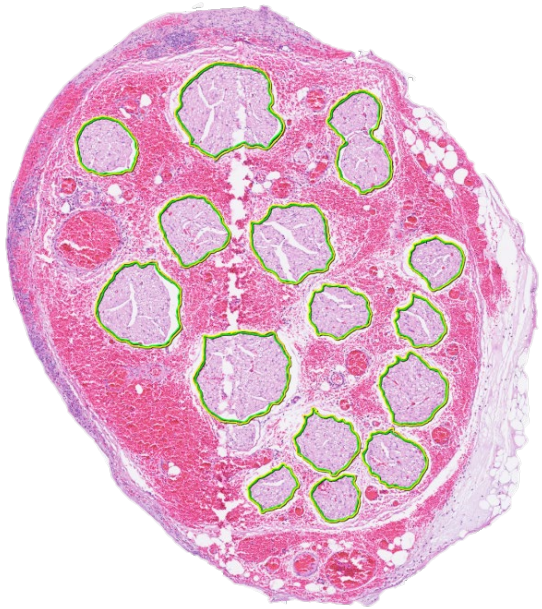

Fig. 2. Pig proximal ulnar nerve containing 15 fascicles stained with haematoxylin and eosin. The yellow and green traces depict the outer and inner perineurium boundaries.

The average fascicle diameter was 269 ± 73.3 µm and 277 ± 81.1 µm for the distal and proximal nerves, respectively. An independent samples *t*-test showed no significant difference in fascicle diameter between distal and proximal UN ($p = 0.59$). These fascicles also showed highly heterogeneous diameters, ranging from 134 µm to 455 µm. Moreover, the perineurium thickness was greater for the distal UN than the proximal UN, with averages of 7.9 ± 1.9 µm and 6.5 ± 1.5 µm, respectively ($p < 0.01$).

The fitted data from all 129 fascicles (M1) resulted in a coefficient of determination R^2 of 0.53, while the R^2 of the model accounting for location (proximal vs distal, M2) as an explanatory variable was 0.69. In addition, the Chi-square test between the two models was significantly different ($F = 34.4$, $p < 0.01$), signifying that the location of the nerve influences the relationship between fascicular diameter and perineurium thickness.

Therefore, two distinct first-order linear regression models were fitted to the data to estimate perineurium thickness based on fascicular diameter (in microns); the model coefficients are presented in Table I.

TABLE I. MODEL COEFFICIENTS TO PREDICT PERINEURIUM THICKNESS BASED ON THE FASCICULAR DIAMETER

Model	**Coefficients**			
	Intercept	***Slope [µm]***	***p-value***	***R^2***
Distal UN	2.257170	0.020925	<0.001	0.6005
Proximal UN	2.193727	0.015799	<0.001	0.7099

The observed data and the fitted models are shown in Fig. 3.

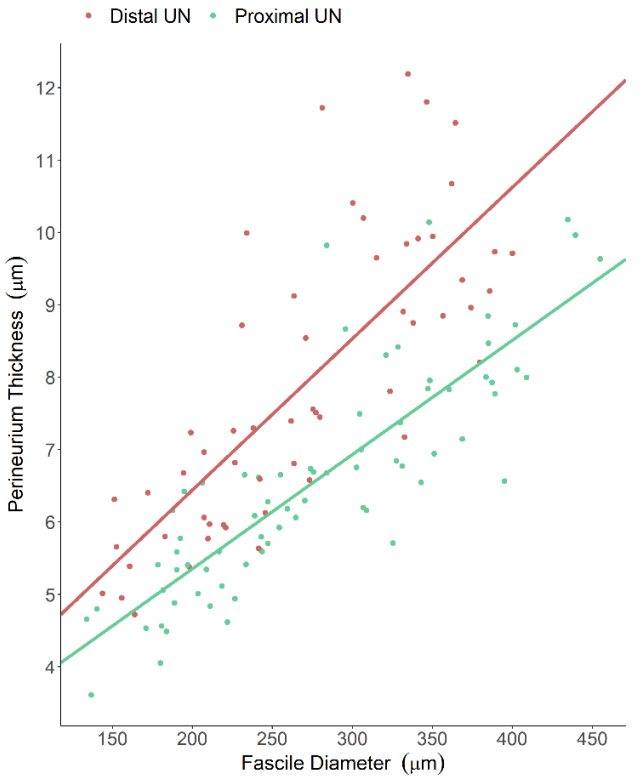

Fig. 3. Relationship between fascicular diameter and perineurium thickness for distal and proximal segments of the ulnar nerve.

A clear linear relationship between fascicular diameter and perineurium thickness can be observed in Fig. 3, with a higher intercept and slope for the distal UN. Residual analysis, through Normal Q-Q plots and scale-location plots, showed that the data followed a normal distribution with homogeneous variances.

IV. DISCUSSION

Several studies have demonstrated the impact of different neural structures on the activation patterns of peripheral nerve fibres [7], [20], resulting in modelling platforms that allow the control of several nerve parameters, with the main goal of improving the predictive values of computational models. Perineurium resistivity has received increasing attention among these parameters because of its substantial effect on activation or block thresholds. As the perineurium resistance is dependent on its thickness, this study aimed to investigate the relationship between fascicular diameter and perineurium thickness across 129 fascicles on distal and proximal sections of the UN in the forearm of pigs.

The results corroborate the literature [10], [11] showing that a linear relationship exists between the fascicle diameter and perineurium thickness, which is important to maintain the same circumferential tension to resist a given internal pressure [21]. Furthermore, the results showed that this relationship is dependent on the location of the nerve section, with distal sections of the nerve exhibiting a greater relative thickness of the perineurium. It is not yet fully understood why the relative thickness of the perineurium increases distally within a somatic nerve, and possible explanations include changes in the intrafascicular pressure [10] or, because fascicles are more numerous in distal segments of a nerve, the nerve requires more resistance to pressure and stretching.

Many studies have reported the advantages of using pigs over rodents for developing peripheral nerve interfaces and stimulation parameters [11], [16]. However, the majority of these studies were conducted assessing the vagus nerve. In one case, the authors reported an intercept of 3.440 and a slope of 0.02547 [11], both parameters smaller than the ones reported in this manuscript.

The information on the somatic nerve of pigs is still sparse, and the pig model has gained popularity in peripheral nerve research because of its close resemblance to humans. In this study, equations were supplied to estimate perineurium thickness based on fascicular diameter, so the results can be used to design nerve models. Furthermore, the data shows that the location at which the nerve is assessed must also be considered in the model.

A limitation of the current study is the investigation of only two sections of the ulnar nerve, with approximately 7 cm distance from the two segments. It would be interesting to assess the perineurium thickness at multiple locations of the same nerve, from the origin to the extremity, to understand if the increase in relative thickness follows a linear function with distance. If true, this data could be used to generate a single predictive model of perineurium thickness for the whole nerve length.

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