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Analysis of porcine nerves as *ex vivo* model for upper limb neuro-electrode implantation studies

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Abstract: In upper limb amputees implantation of prostheses equipped with electrodes to innervate respective musculature might improve sensitivity and life quality. Besides different types of prostheses being available, there is still a need for devices that provide more comfort and functionality to patients. Prior to prosthesis implantation magnetic resonance imaging (MRI) or ultrasound are suitable methods for imaging peripheral nerves but are limited in description of nerve dimension. Using X-ray micro-computed tomography (micro-CT) exact dimensions of nerves can be obtained. However, since *ex vivo* micro-CT analysis on human nerves is rarely possible, the underlying study analyzed whether porcine nerve tissue is comparable to human nerve tissue and thus suitable for nerve electrode implantation studies. Therefore, micro-CT analysis was performed at 10 different proximal to distal sections of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* to determine the diameters of nerves and fascicles (n = 1). The diameters of the porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were 2.99 ± 0.28 mm, 4.40 ± 0.38 mm and 7.89 ± 0.98 mm, respectively. The diameters of the human *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were measured at 10 different regions using a caliper and were 2.93 ± 0.71 mm, 3.65 ± 1.06 mm and 2.94 ± 1.11 mm, respectively (n = 2). Fascicular diameters of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were 0.22 ± 0.09 mm, 0.26 ± 0.06 mm and 0.29 ± 0.07 mm, respectively. Our preliminary results showed that nerve diameters were similar between pigs and humans. Espcially, further analyses of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* are necessary to conclude whether porcine nerve tissue is suitable for electrode implantation studies.

Keywords: nerve tissue, fascicles, micro-computed tomography, porcine, human, *ex vivo*

1 Introduction

Loss of the upper extremity due to amputation has a huge impact on lives of affected people [1]. Especially lack of innervation of arms impedes daily activities. Improvement in sensitivity and life quality of amputees might be achieved by implantation of prosthesis equipped with electrodes to innervate muscles of the upper limbs [1].

Different types of upper limb prostheses are available. Passive prostheses do not allow movement of the affected limb but have a cosmetic purpose. In body-powered prostheses movement can be achieved via muscle strength that instigates flexion or extension in manufactured joints. Moreover, there are electric prostheses composed of two electrodes that can be activated via muscle tension. Nowadays, multi-electrodes in prostheses are implanted that can address multiple regions of the upper limbs [2].

However, there is still a need for prostheses that are better adapted to the human body since patients often discard them due to less comfort and improvable functionality. One reason for this is that currently available prostheses do not fully transfer signals to axons [3]. Therefore, further investigation is necessary regarding the upper limb implantation of neural electrodes.

For the surgical approach of nerve electrode implantation, knowledge about nerve dimension is necessary, such as diameter of nerves as well as number and area of fascicles [4].

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Since *ex vivo* analysis on human nerves is rarely feasible, examination of nerve tissue comparable to human nerves obtained from animals might be applicable.

Thus, the aim of the underlying study was to analyze whether porcine nerves are comparable to human nerves and subsequently suitable for upper limb neural electrode implantation studies.

2 Materials and methods

2.1 Animals

Experiments were performed *ex vivo* on porcine nerve tissue. Therefore, female German Landrace pigs (*Sus scrofa domesticus*) were provided by the Research Institute for Farm Animal Biology in Dummerstorf, Germany. Pigs were killed and dismembered by a butcher prior to obtaining the brachial plexus with *Nervus ulnaris, Nervus medianus* and *Nervus radialis* from the right upper forelimb (Fig. 1). Nerves of one pig (weight: 92 kg, age 5 months) were analyzed.

Since nerve tissue was collected as a by-product from slaughtering, ethics committee approval was not required.

2.2 Body donors

Body donors were provided by the Institute of Anatomy of the Rostock University Medical Center to obtain N*ervus ulnaris, Nervus medianus* and *Nervus radialis.* The nerves of two upper arms were dissected.

The Ethics Committee of the University of Rostock approved the use of the bodies for research purposes under number A 2016 0083.

2.3 X-ray micro-computed tomography

Nervus ulnaris, Nervus medianus and *Nervus radialis* of one pig *(n* = 1*)* were analyzed using X-ray micro-computed tomography (micro-CT). Prior to micro-CT analysis samples were fixed in Bouin's solution (No. HT10132, Sigma-Aldrich Chemie GmbH, Germany) for 24 h. To increase X-ray contrast, samples were incubated in Lugol´s solution (No. 1.09261, Sigma-Aldrich Chemie GmbH) for 72 h at room temperature under constant rotation.

Subsequently, micro-CT analysis was conducted using a SKYSCAN 1273 (Bruker Corp., USA) at a spatial resolution of 7 µm voxel^{-1}. Nerves were scanned with a source voltage of 55 kV and a current of 140 µA.

2.4 Geometric measurement of nerves and fascicles

Measurement of porcine nerve diameters was conducted after micro-CT analysis using the software ImageJ (Rasband W., National Institutes of Health, USA). Diameters were measured in the upper right *Nervus ulnaris, Nervus medianus* and *Nervus radialis* at 10 different proximal and distal sections of each nerve. Subsequently, diameter means were calculated.

Porcine fascicles of the upper right *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were scanned using micro-CT (Fig. 2), subsequently counted and diameters determined after measuring the fascicular area.

Analysis was performed on one slice of each nerve analyzed that contained the highest number of fascicles. Prior to determining fascicular diameters, the area of each fascicle was measured by outlining the perimeter manually using the software ImageJ. Finally, diameters of nerves or fascicles were calculated using Excel 2016 (Microsoft Corporation, USA).

Human diameters of *Nervus ulnaris, Nervus medianus* and *Nervus radialis* of the upper arm were determined using a caliper. Diameters were measured at 10 different proximal to distal sections of each nerve and mean diameters were calculated.

3 Results

3.1 Dissection of porcine nerve tissue

To expose porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* of the right upper forelimb skin at the positions of cervical vertebra 8 (C8) and thoracic vertebra 1 (Th1) was incised using a scalpel. Subsequently, musculature of the neck and the upper right forelimb was carefully thrust aside to expose the *Plexus brachialis* and eventually *Nervus ulnaris, Nervus medianus* and *Nervus radialis* that originate from the *Plexus brachialis* (Fig. 1).

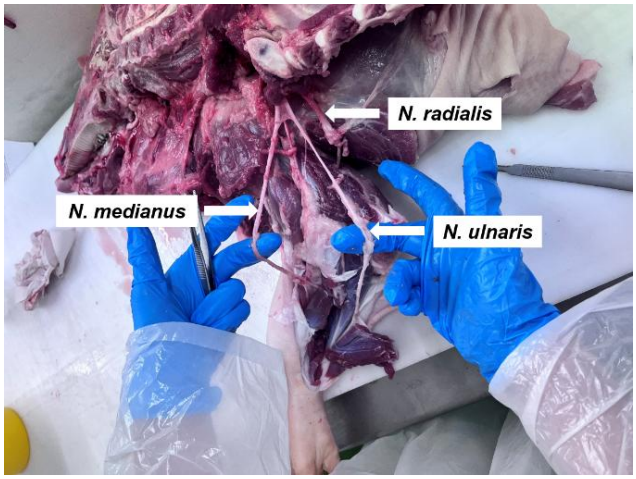

Figure 1: Dissection of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis.*

3.2 Dissection of human nerve tissue

Human *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were obtained from body donors and provided by the Institute of Anatomy of the University Medical Center of Rostock, Germany. Formalin-fixed specimens were dissected for subsequent measurement of diameters using a caliper (Fig. 2).

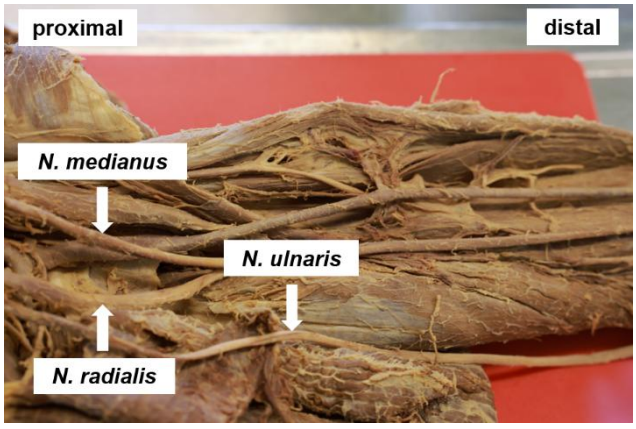

Figure 2: Dissection of human *Nervus ulnaris, Nervus medianus* and *Nervus radialis.*

3.3 Measurement of porcine and human nerve diameters

Diameters of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* as well as the fascicles were measured using micro-CT. Analysis of fascicles was performed by measuring the fascicular area and counting the number of fascicles. An exemplary micro-CT image of the porcine *Nervus medianus* including fascicles of the upper right forelimb is shown in Figure 3.

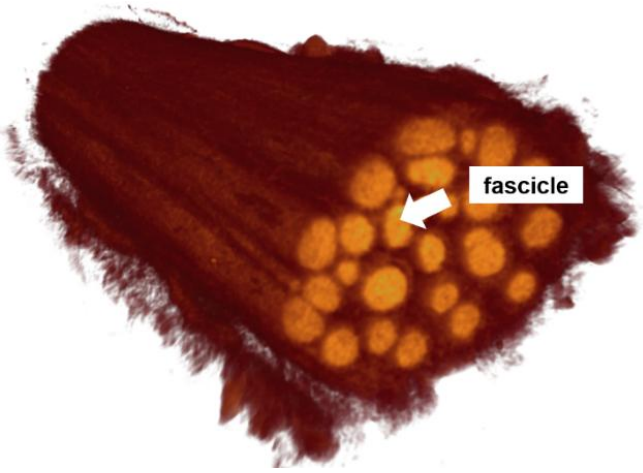

Figure 3: Micro-CT image of fascicles of the porcine upper right *Nervus medianus.*

Micro-CT analysis revealed that diameters of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were 2.99 ± 0.28 mm, 4.40 ± 0.38 mm and 7.89 ± 0.98 mm, respectively.

Diameters of human *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were determined using a caliper. Caliper measurement of diameters of human *Nervus ulnaris, Nervus medianus* and *Nervus radialis* showed values of 2.93 ± 0.71 mm, 3.65 ± 1.06 mm and 2.94 ± 1.11 mm, respectively (Table 1).

Table 1: Mean diameters and standard deviations [mm] of the upper *Nervus ulnaris, Nervus medianus* and *Nervus radialis* (10 segments each) of pigs and humans.

Diameter [mm]	*N. ulnaris*	*N. medianus*	*N. radialis*
Porcine (*n = 1*)	2.99 ± 0.28	4.40 ± 0.38	7.89 ± 0.98
Human (*n = 2*)	2.93 ± 0.71	3.65 ± 1.06	2.94 ± 1.11

Fascicular diameters of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* were 0.22 ± 0.09 mm, 0.26 ± 0.06 mm and 0.29 ± 0.07 mm, respectively.

The number of fascicles differed in *Nervus ulnaris, Nervus medianus* and *Nervus radialis.* The lowest number of fascicles was counted in *Nervus ulnaris* (17), followed by *Nervus medianus* (25). *Nervus radialis* showed the largest number of fascicles (37). Respective values are depicted in Table 2.

Table 2: Mean porcine fascicular diameters and standard deviations [mm] as well as number of fascicles of the right *Nervus ulnaris, Nervus medianus* and *Nervus radialis (n* = 1*)*.

Porcine	*N. ulnaris*	*N. medianus*	*N. radialis*
Diameter [mm]	0.22 ± 0.09	0.26 ± 0.06	0.29 ± 0.07
Fascicle number	17	25	37

4 Discussion

In the underlying study porcine *Nervus ulnaris, Nervus medianus* as well as *Nervus radialis* were analyzed by micro-CT. Diameters of the respective human nerves were measured using a caliper. Our preliminary data revealed similar diameters of these nerves in pigs and humans.

Determining nerve and fascicular dimensions is important for upper limb prosthesis implantation, especially for placing electrodes. Prior to prosthesis implantation magnetic resonance imaging (MRI) or ultrasound are common methods for imaging nerve tissue [5], but are limited in description of nerve dimension. Whereas determination of number and diameter of nerves and fascicles using MRI or ultrasound is rather challenging [4], micro-CT represents a useful tool to perform this analysis [6]. We consider micro-CT assessment as an excellent method to investigate nerve tissue since it allows 3D measurements of specimens, in contrast to histological analyses that offer 2D results.

Brill *et al.* (2017) conducted an extensive analysis in histological cross sections of the human ulnar, median and radial nerve that depicted fascicular diameters of 0.57 ± 0.39 mm, 0.6 ± 0.3 mm, 0.5 ± 0.26 mm, respectively [4]. In the underlying study values for the same nerves in pigs were 0.22 ± 0.09 mm, 0.26 ± 0.06 mm and 0.29 ± 0.07 mm, respectively. This shows that porcine fascicular diameters analyzed in the underlying study by micro-CT were about half the size compared to histologically obtained results for humans by Brill *et al.* (2017). However, it should be considered that different methods were used for nerve tissue fixation in the histological analysis of human tissue and in the micro-CT analysis of porcine nerve tissue, which might have influenced the results obtained. Fixation of specimen usually causes shrinkage of tissue. Still, the possible differences in shrinkage by the here used fixation techniques actually cannot be estimated.

It has been shown that the number of fascicles in human nerves is greater than in animal nerve tissue [7], which might result in overall larger diameters of nerves. However, our results showed that overall diameters of porcine and human *Nervus ulnaris, Nervus medianus* and *Nervus radialis* values were similar, except for *Nervus radialis*, which was larger in pigs. This might be explained by the large number of fascicles in the porcine *Nervus radialis.* Moreover, nerve diameters might be correlated to the size of the animal.

Still, further analyses are needed to verify the promising results obtained in the underlying study, to make a final assessment whether porcine peripheral nerves are suitable for nerve electrode implantation studies.

5 Conclusion

Our preliminary results of the micro-CT analysis showed that overall diameters of porcine *Nervus ulnaris, Nervus medianus* and *Nervus radialis* of the upper right extremity were comparable to human *Nervus ulnaris, Nervus medianus* and *Nervus radialis*.

However, the number of experiments needs to be increased to conclude whether *N*ervus *ulnaris, Nervus medianus and Nervus radialis* are suitable for neural electrode implantation studies in pigs as a model for human applications.

Author Statement

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Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration and has been approved by the authors’ institutional review board or equivalent committee.

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