

## Collagen fiber angles as a function of compression and depth within the nerve

Michael B Christensen<sup>1,2\*</sup>, Reza Behkam<sup>3</sup>, Melissa R Chao<sup>4</sup>, Amanda C Stark<sup>2</sup>, Jonathan P Vande Geest<sup>3</sup>, Julie M Barkmeier-Kraemer<sup>1</sup>

<sup>1</sup>Department of Surgery, University of Utah, Salt Lake City, UT

<sup>2</sup>National Center for Voice and Speech, University of Utah, Salt Lake City, UT

<sup>3</sup>Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

<sup>4</sup>Department of Bioengineering, University of Utah, Salt Lake City, UT

**Keywords:** collagen; second harmonic imaging; compression

### Introduction

The epineurium of peripheral nerves, of which collagen is a main constituent, serves as a protective layer for underlying nerve structures. It has previously been shown that collagen fibers within the epineurium align in the presence of uniaxial tension [1]. However, changes in collagen fiber angles as a function of compression have not previously been reported. Further, changes in collagen fiber angles as a function of depth, both in naïve and compressed nerves, has not been reported. Therefore, we investigated collagen fiber angles within the epineurial space both as a function of depth and compression in adolescent and juvenile pigs.

### Methods

Recurrent laryngeal nerves (left and right) were harvested from both adolescent (3-4 months) and juvenile (1-3 weeks) pigs. Harvested nerves were cut into three adjacent sections, with two being compressed and the other serving as a non-tested control. Sections were compressed using a hydrostatic column in a custom built chamber. Following compression, nerves were fixed at pressure via fluid exchange using 10% formalin. Nerve diameters were measured using digital calipers. 100µm thick sections were collected using a cryostat and imaged using second harmonic generation (SHG) imaging with a Bruker Ultima multi-photon confocal microscope. Collagen fiber angles were evaluated using a custom Matlab code. Fiber angles were normalized so that the mean fiber angle for each section was equal to zero (zero being the direction along the length of the nerve).

### Results

Non-tested sections from adolescent pigs had a mean diameter of 1.04mm, while compressed nerves had a mean diameter of 0.78mm ( $p=0.03$ ). Non-tested sections from juvenile pigs had a mean diameter of 0.31mm, while compressed nerves had a mean diameter of 0.27mm ( $p=0.35$ ).

Fiber angle measurements as a function of compression are shown in Figure 1A. Compression to the nerves from the adolescent pigs appeared to significantly alter the distribution of fiber angles, while compression to the nerves from juvenile pigs did not.

Examining collagen fiber angles as a function of depth, collagen fibers tend to be more aligned deeper in the nerve in adolescent pigs, both naïvely and after compression (Figure 1B), while depth did not appear to change fiber angle distributions in the juvenile pigs.

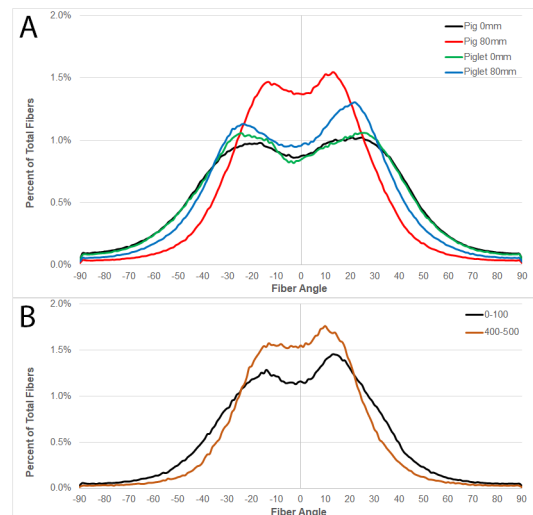


Figure 1: A) Compression seemed to significantly alter fiber angle distributions after compression in adolescent, but not juvenile, pigs. B) Deeper sections demonstrated fiber angle profiles that were more aligned than superficial sections both naïvely (not shown) and after compression (shown) in adolescent, but not juvenile, pigs.

### Discussion

Compression significantly altered nerve diameter and collagen fiber angle distributions in adolescent, but not juvenile, pigs. This may be due to a more mature development of collagen in the older pigs, which allows the collagen to absorb compressive forces by aligning and becoming denser. The inability of collagen fibers to make similar changes in the young pigs suggests that these nerve may be more susceptible to compressive damage.

### Funding

This work was funded by the NIH R01-DA942367

### References

[1] Williams *et al*, J Biomech Eng, 136(8), 2014