COLLAGEN FIBER ANGLES AS A FUNCTION OF COMPRESSION AND DEPTH WITHIN THE NERVE

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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, have not been reported. Therefore, we investigated collagen fiber angles within the epineurial space both as a function of depth and compression in both adolescent and juvenile pig recurrent laryngeal nerves to determine if compression affects fiber alignment similar to nerves in tension, and also determine if any such alignment is uniformly distributed through the depth of the nerve.

MATERIALS AND 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.

For analysis of collagen fiber angles, All tested and control samples were equilibrated in a 30% sucrose solution before sectioning using a Leica CM1950 cryostat (Leica, Bannockburn, IL). Two pigs and two piglets were used for the analysis of fiber angles. 100 µm thick longitudinal sections were serially collected and placed in wells. The depth of the section within the nerve was determined based on the distance from the exterior surface, regardless of nerve orientation (top vs. bottom). Type I The ccollagen fibers were visualized via second harmonic generation (SHG) imaging using a Bruker Ultima Multi-photon confocal microscope with a Nikon LWD 16x water immersion objective (numerical aperture of 0.8) and Prairie View Software. A Coherent Cameleon Vision IR laser was tuned to a wavelength of 800 nm, and detection was performed using non-descanned PMT detectors. SHG signals were discriminated from background autofluorescence using a 460LP dichroic and 377/50 nm bandpass filters, limiting detected signal to a range of 377-402 nm. XY pixel size was 0.81x0.81 µm, with a 7 µm z-step size. Collagen fiber angles were evaluated using a custom MATLAB code [2, 3]. Fiber angles were normalized so that the mean fiber angle for each section was equal to zero (with the assumption that a zero angle is the direction along the length of the nerve). A total of 13,129,369 fiber angles were used for analysis.

RESULTS

Collagen fiber angles in compressed pig nerves demonstrated a more narrow distribution of fibers than the non-compressed nerves (Figure 1A). Results from piglet nerves demonstrated a more minimal narrowing of the distribution (Figure 1B). Evaluation of fiber angles as a function of depth reveals that fibers deeper within the pig nerves tend to have more narrow distributions in both non-compressed and compressed nerves, while angles of fibers within the piglet nerves tend to have similar distributions regardless of depth (Figure 1C-F).

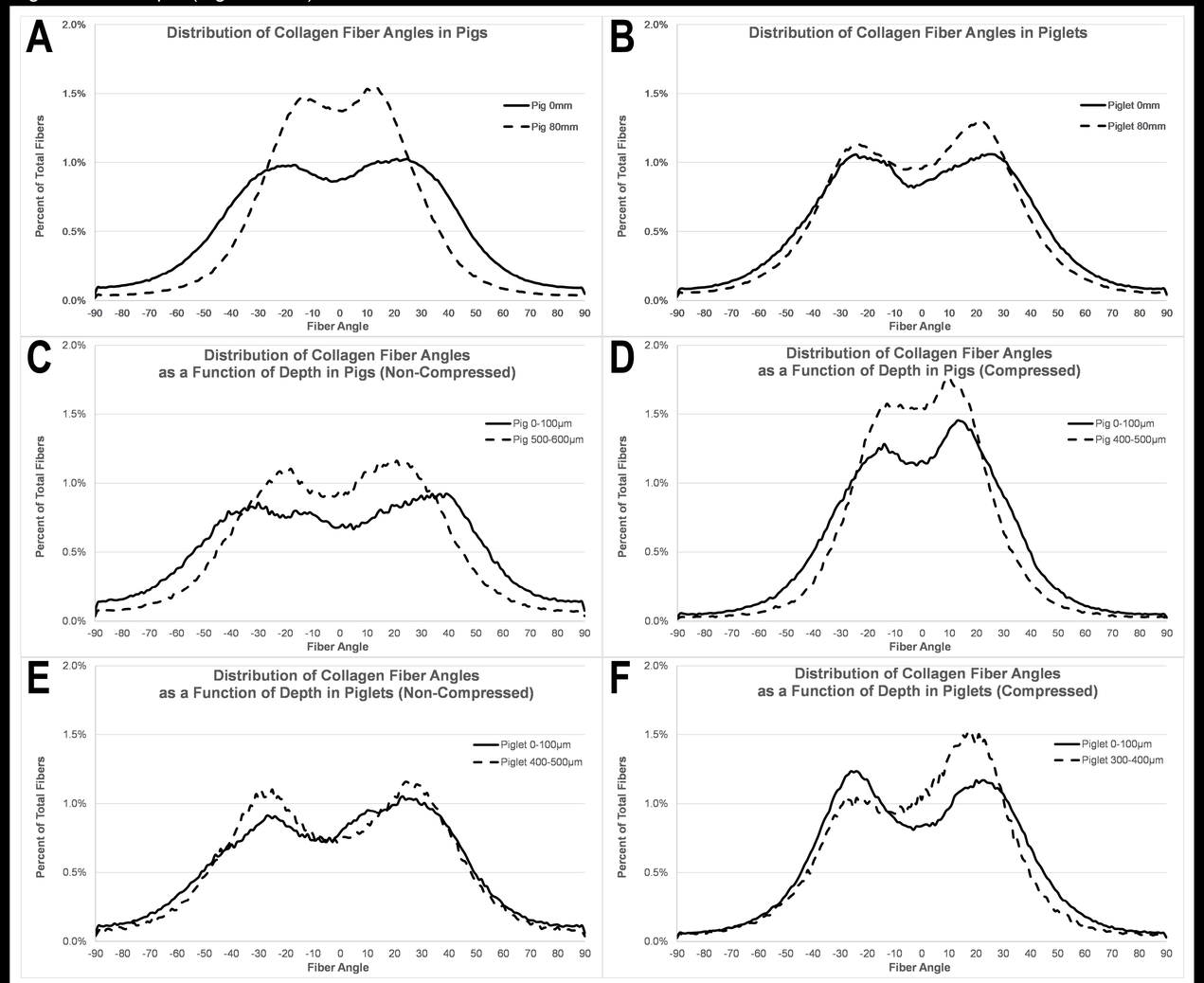

Figure 1: Distribution of the collagen fiber orientation angles. A) Distribution on fibers in pigs at 0 and 80 mmHg. B) Distribution of fibers in piglets at 0 and 80 mmHg. C) Distribution of fibers as a function of depth in non-compressed pig RLN. D) Distribution of fibers as a function of depth in compressed pig RLN. E) Distribution of fibers as a function of depth in non-compressed piglet RLN. F) Distribution of fibers as a function of depth in compressed piglet RLN.

	Control	Compressed
Pig	1.036 ±0.407	0.782 ±0.291*
Piglet	0.314 ±0.191	0.270 ±0.103

Table 1: Diameter (mm) of RLNs from pig and piglet groups in both control and compressed nerve samples. Data are represented as mean ±stdev, *p<0.05 compared to control samples. Nerves from adolescent pigs showed a significant decrease in nerve fiber diameters following compression at 80 mmHg, while no significant reduction in nerve diameters from piglets was observed.

CONCLUSIONS

This study demonstrated a trend towards increasing orientation under compression in the pig RLN. While this increased organization of the fibers may be necessitated by the decreased cross-sectional area experienced in these nerves as a result of compression, it is unclear if this increased organization plays a functional role in nerve protection. Notably, this trend towards increased orientation was not noted in the piglet RLN. This may be due to the more homogenous nature and lack of development of the epineurial space in piglets, as has been previously studied [4].

Our results also suggest that collagen fibers deeper within the nerve in pigs may be more aligned with the nerve than more superficial fibers, under both non-compressed and compressed states (Figure 1C-D). This may be due to the more limited relative epineurial space deeper within the nerve, but it is again unclear if these apparent differences in fiber alignment translate to any functional differences. It also appears that the degree of alignment with depth relative to more superficial fibers did not drastically change following compression, suggestion that alignment due to compression is uniformly distributed across the nerve. Depth did not appear to alter fiber angles within the piglet RLN, presumably because of the homogenous nature of the nerve in these immature animals [4].

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