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1 **Native paraneurial tissue and paraneurial adhesions alter nerve strain distribution**

2 **in rat sciatic nerves**

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19 *This study was approved by our institution's IACUC.*

1 **Abstract:**

2 Paraneurial adhesions have been implicated in the pathological progression of

3 entrapment neuropathies. Surgical decompression of adhesions is often performed, with

4 the intent of restoring nerve kinematics. The normal counterpart of adhesions, native

5 paraneurium, is also thought to influence nerve deformation and mobility. However,

6 influences of native or abnormal paraneurial structures on nerve kinematics have not

7 been investigated. We measured regional strains in rat sciatic nerves before and

8 immediately after decompression of native paraneurial tissue, and before and after

9 decompression of abnormal paraneurial adhesions, which formed within 6 weeks of the

10 initial decompression. Strain was significantly higher in the distal-femoral than the mid-

11 femoral region of the nerve before either decompression. Decompression of native and

12 abnormal paraneurial tissue removed this regional strain difference. Paraneurial tissues

13 appear to play a major role in distributing peripheral nerve strain. Normal nerve strain

14 distributions may be reconstituted following decompression, even in the presence of

15 paraneurial adhesions.

16

Introduction:

Peripheral nerves are dynamic structures that must withstand significant mechanical forces while reliably conducting electrical impulses. Because nerves traverse articulating joints and frequently follow paths outside of the normal plane of joint motion, they stretch and glide during movement, while maintaining structural and functional integrity.(Topp and Boyd, 2006) Animal models have demonstrated that when nerves are subjected to prolonged or excessive strain above physiological thresholds, their electrical function is compromised.(Clark et al., 1992; Tanoue et al., 1996; Wall et al., 1992) In humans, excessive traction related to mechanical impingement is believed to contribute to the progression of entrapment neuropathies, such as carpal and cubital tunnel syndromes.(Mackinnon, 2002; Mahan et al., 2015; Ogata and Naito, 1986; Rempel et al., 1999; Tanoue et al., 1996)

Peripheral nerves are believed to have several adaptations to protect against excessive strain. One such protective mechanism, observed in both animal and human cadaveric models, is the increased compliance of nerves at joint regions compared with non-joint regions (Mason and Phillips, 2011; Phillips et al., 2004), to accommodate increased strains in the vicinity of articulating joints.(Boyd et al., 2005; Topp and Boyd, 2006; Wright et al., 2001; Wright et al., 1996) This heterogeneity in nerve compliance is observed in nerves both *in vivo* and *ex vivo*, and allows them to accommodate the increased physiologic strain associated with synovial joint motion without resulting in mechanical failure. The structural basis for this heterogeneous strain profile has not been fully elucidated. Mason and Phillips revealed that rat median nerves possess subtle differences in collagen fibril geometry between joint and non-joint regions, possibly explaining these biomechanical differences.(Mason and Phillips, 2011) However, the same study also indicated that such structural variance is not conserved across all nerves,(Mason and Phillips, 2011) suggesting the possibility of additional adaptations that regulate local compliance.

46 The paraneurium, also referred to as mesoneurium, is another possible modulator of

47 local nerve compliance. It is composed of connective tissue that attaches the peripheral

48 nerve to its surrounding nerve bed, and also carries the nerve's extrinsic segmental

49 blood supply.(Lundborg, 1979) It has been hypothesized that that the paraneurium

50 serves a mechanical function, guiding the trajectory of peripheral nerves within their

51 beds and facilitating low-friction nerve "gliding".(Butler, 2000; Mazal and Millesi, 2005;

52 Millesi et al., 1995; Smith, 1966) Moreover, the paraneurium has a pathologic

53 counterpart frequently encountered in a surgical or other clinical setting:

54 adhesions,(Millesi et al., 1993) which often describe abnormal connections between two

55 tissue structures, such as two loops of bowel.(Smith, 1966) In peripheral entrapment

56 neuropathies such as cubital tunnel syndrome, paraneurial adhesions are often

57 implicated as causal agents of neural impingement and associated changes in traction,

58 abnormally tethering the nerve to its surrounding structures.(Abe et al., 2005; Foran et

59 al., 2016; Ochi et al., 2014; Topp and Boyd, 2006) Additionally, there is concern that

60 adhesions eventually contribute to intraneural fibrosis, resulting in intraneural structural

61 changes with deleterious mechanical and functional consequences(Ikeda et al., 2003;

62 Yamamoto et al., 2010), as well as increased risk for recurrent entrapment (Botte et al.,

63 1996; McCall et al., 2001; Steyers, 2002). The mainstay of surgical management of

64 these syndromes often involves some form of neurolysis or decompression,(Foran et

65 al., 2016; Millesi et al., 1993) with the intent of relieving mechanical effects of traction

66 and entrapment on the nerve.

67 Despite empirical evidence supporting a mechanical role for the paraneurium, as well as

68 a well-documented role for adhesions in the progression of entrapment neuropathy,

69 influences of native or abnormal paraneurial tissue on peripheral nerve strain have not

70 been examined. In this study we used a rat sciatic nerve model to investigate the effect

71 of both normal paraneurial tissue and paraneurial adhesions, and the effect of surgical

72 neurolysis of these tissues, on peripheral nerve strain.

Methods:

Rat surgeries and image analysis:

All procedures were completed under protocols approved by our institutional IACUC. Twelve 10-week-old male Lewis rats (Harlan (now Envigo), Placentia, CA) were studied in this experiment. Rats were kept in standard group housing, 3 rats to a cage, with a normal diet and water. The rats were induced and maintained under anaesthesia at 10 weeks of age with inhalational isoflurane delivered via nose cone. A 15 mm incision was made on the posterolateral thigh overlying the sciatic nerve with aseptic technique. The right or left sciatic nerve was chosen randomly for study by a random number generator (RANDOM.ORG). Using a gluteus splitting technique, the sciatic nerve was then carefully exposed using a combination of sharp and blunt dissection under a dissecting microscope from the level of the quadratus femoris muscle to the trifurcation of the tibial, common peroneal, and sural nerves. Care was taken not to disturb the paraneurial attachments of the nerve to the underlying nerve bed. Using a tissue marking pen, 5 epineurial markers were placed at about 4mm increments with the most proximal marker at the level of the quadratus femoris muscle and most distal marker at the level of the trifurcation (Figure 1). The region between the first and third marker was designated as "mid-femoral," while the region between the third and fifth marker was defined as "distal-femoral." A sterile ruler was placed into the wound to set scale, and pictures were then taken with the knee flexed to 90° and ankle maximally plantarflexed (relaxed configuration) and the knee extended to 0° and the ankle maximally dorsiflexed (stretched configuration). Next, the paraneurial connections from the level of the quadratus femoris to the trifurcation were sharply dissected with tenotomy scissors (decompression), with the knee in full flexion to minimize strain on the nerve during decompression. The knee and ankle were then placed into the two configurations above and pictures were again taken. The wound was then closed in layers with absorbable, braided 4-0 Vicryl suture (Ethicon, Somerville, NJ, USA).

Six weeks after the index surgery, rats were sacrificed with carbon dioxide and cervical dislocation, and the sciatic nerve was again exposed in the manner above under a

103 dissecting microscope. In all animals, adhesions were discovered connecting the sciatic

104 nerve to the surrounding muscle, muscle fascia, fat, and nerve bed (Figure 1) in a

105 distribution greater than the original native paraneurial tissues seen at week zero.

106 Epineurial markers were placed as above using a tissue marking pen, at 4mm

107 increments from the level of the quadratus femoris to the level of the trifurcation.

108 Adhesive tissue was perforated, but not released, to access the underlying nerve for

109 labeling. The knee and ankle were ranged through the same two joint configurations as

110 above and pictures were taken. Next, the adhesions from the level of the quadratus

111 femoris muscle to the trifurcation were sharply and fully dissected with scissors

112 (decompression), freeing the nerve from all soft tissue connections in this region. The

113 knee and ankle again were then placed into the two configurations above and pictures

114 were taken. With all images obtained, nerves were harvested from just above the level

115 of the quadratus femoris and just below the level of the trifurcation and placed into a

116 freezer at -80° future sectioning and analysis. Six sciatic nerves were also harvested

117 from 10-week old Lewis rats to serve as controls. Contralateral controls were not used,

118 due to the possibility of compensatory changes resulting from ipsilateral nerve damage.

119 Images were analyzed using ImageJ (NIH) and strain was calculated based on marker-

120 to-marker distances in the stretched position relative to the relaxed position.(Foran et

121 al., 2016; Mahan et al., 2015) Edges of each ink dot in each joint configuration were

122 used as fiducial markers, to improve accuracy of measurement. Average strain across

123 the entire exposed region, maximum strain between any two adjacent markers, and

124 regional nerve strain in the proximal and distal regions were calculated.

125

126 ***Nerve sectioning, trichrome, and immunohistochemistry***

127 All harvested nerves were thawed at -20°, embedded in Optimal Cutting Temperature

128 compound, and flash frozen in liquid nitrogen cooled isopentane. Several 10 µm cross-

129 sections were obtained using a cryostat (Leica CM 3050 S) proximally at the level of the

130 first tissue marker (at the level of the quadratus femoris) and distally at the level of the

131 fifth tissue marker (the level of the trifurcation). Sections were maintained in a freezer at

132 -20° between sectioning and staining.

133

A proximal and distal section of each nerve underwent trichrome straining to allow for analysis of gross structural morphology and connective tissue content. Slides were thawed at room temperature for 15 minutes and then placed into neutral buffered formalin. They were then stained with Harris Modified Hemotoxylin (HHS128, Sigma-Aldrich, St. Louis, MO) for 4 minutes, followed by Gömöri's Trichrome Stain (#24205, Polysciences, Inc., Warrington, PA) for 5 minutes. Slides were then successively washed in acetic acid, alcohol, and citrisolv, and finally mounted with Permount medium.

A proximal and distal section of each nerve also underwent immunohistochemical staining for phosphorylated neurofilaments (SMI31; axonal marker) and laminin (basal lamina marker). Sections were fixed with 10% formalin, permeated with 0.2% Triton X-100, and blocked using a solution of 10% normal goat serum and 3% BSA in PBS. For laminin and neurofilament staining, sections were incubated with primary antibodies against laminin (1:500, Sigma Aldrich) and phosphorylated neurofilament (1:1000, SMI31, Sigma-Aldrich) for 1 hour at room temperature. After rinsing with PBS, sections were then incubated with Alexa Fluor-594 goat anti-mouse and Alexa-Fluor-488 goat anti rabbit (1:200, Life Technologies) for 1 hour at room temperature. Samples were imaged using a widefield fluorescence microscope (Leica DM 6000 B) to analyze neurofilament localization and the internal structure of the nerve.

Axon counts were performed using a semi-automated approach, from 63X images of SMI-31 labeled sections. Images were binarized using Image J (NIH), and axon densities calculated by summing axon numbers from three randomly selected regions of interest (ROI) within each section, and dividing by the summed area of these ROIs. Axon counts were then estimated for the whole nerve by multiplying axon density by the intra-epineurial area of each section, measured from 20x images. Given the variability in attached extra-epineurial connective tissue following nerve excision, area-based measurements of connective tissue remodeling were unreliable. Therefore, trichrome images were scored semi-quantitively to determine the integrity of the epineurium and the infiltration of adhesion tissue into the nerve. Blinded images and scoring criteria

163   were presented to 5 individuals with knowledge of basic nerve architecture, and images

164   were scored based on a scale from 1 to 5. This scale reflects the circumferential extent

165   of fusion between extra-epineurial tissue and the interior of the nerve, indicated by the

166   loss of a clear external epineurial boundary (Table 1 and Figure 2). Sample images for

167   each score were presented to individuals as training, prior to blind testing of the full set

168   of images. Average scores from all 5 individuals for each image were used for statistical

169   analysis.

170

171   ### *Statistical analysis*

172   Average and maximum measured strains were compared using 2x2 two-way repeated

173   measures ANOVA with factors of time (0 or 6 weeks) and surgical intervention (pre- or

174   post-decompression). Proximal and distal strains were compared using 2x2 two-way

175   repeated measures ANOVA with factors of surgical intervention (pre- or post-

176   decompression) and region (proximal or distal), or factors of time point (0 or 6 weeks)

177   and region (proximal or distal). Student's t-test was used to test for significant

178   differences between epineurial integrity scores ($\alpha = 0.05$). Data are given as Mean

179   (SEM).

Results:

Nerve structure

At week 0, prior to decompression, nerves were slack with neutral knee and ankle, but incurred tension upon knee extension and ankle dorsiflexion (Figure 1A-B). At week 6 (42 days), the nerve trajectory in both neutral and stretched configurations was markedly altered, and overlying connective tissue was observed (Figure 1C-D). These changes did not appear to cause any apparent functional deficits, as assessed by toe-spreading. Gross vascular deficits were also not observed, based on minimal pooling of blood and a consistent coloration of nerves and visible regions of their targeted muscles. To investigate whether these extra-epineurial adaptations resulted in changes to the interior of the nerve, we performed immunohistochemistry. Blind scoring of tissue sections with trichrome labeling showed a significant decrease in epineurial integrity 6 weeks after decompression, compared to controls (Figure 2). However, immunofluorescence labeling of axons (phosphorylated neurofilaments, red) and basal lamina (laminin, green) revealed that connective tissue infiltration into the interior of the treatment group nerves was not observed (i.e., internal epineurial borders remained sharp), and nerve fiber architecture appeared normal in both mid-femoral and distal-femoral regions of the nerve (Figure 3). Axon counts (0 weeks: 5324 (434); 6 weeks: 5846 (312); p>0.37, n = 4) and axonal density (0 weeks: 9.9×10^{-3} (2.6×10^{-4}); 6 weeks: 9.8×10^{-3} (6.0×10^{-4}); p>0.92, n = 4) were not significantly different.

Nerve Strain

Average and maximum sciatic nerve strains were measured before and after decompression, at 0 and 6 weeks. Average strains (Mean±SEM) prior to decompression at week 0 and week 6 were 17.64% (4.5%) and 16.17% (2.14%), respectively. Average strains after decompression at week 0 and week 6 were 18.5% (3.35%) and 17.49% (4.5%), respectively. Maximum strains prior to decompression at week 0 and week 6 were 32.14% (6.35%) and 31.28% (3.9%), respectively. Maximum strains after decompression at week 0 and week 6 were 28.80% (4.15%) and 31.73% (5.02%), respectively. No significant differences were observed in either average or

210   maximum strains before and after decompression at either time point. We did, however,

211   observe significant regional differences in sciatic nerve strain (Figure 4). At 0 weeks,

212   strain in the distal-femoral region (i.e., just proximal to the trifurcation) was significantly

213   higher than that in the mid-femoral region (23.91% (5.4%) vs. 12.41% (4.07%),

214   $P<0.001$) prior to decompression, but this regional strain difference disappeared after

215   decompression (Figure 4A). Similarly, prior to decompression at 6 weeks, nerve strain

216   in the distal-femoral region was significantly higher than that in the mid-femoral region

217   (20.51% (3.86%) vs. 12.26% (2.23%), $P<0.05$); again, this regional strain difference was

218   not observed after decompression (Figure 4B).

Discussion:

Several studies have suggested a mechanical role for the paraneurium, in guiding nerve trajectory and in enabling controlled nerve gliding within its bed.(Butler, 2000; Mazal and Millesi, 2005; Millesi et al., 1995; Smith, 1966) Additionally, in an early investigation of peripheral nerve blood supply, Smith observed that nerves *in* situ displayed greater elasticity than those removed from their "mesoneuriual" attachments.(Smith, 1966) He noted, "The fact that an isolated nerve has far less capacity for stretching than one in situ suggests that it is mainly the mesoneurium which allows for adjustments in length of the nerve." Later, Millesi also described the apparent mechanical importance of the structure he termed the "paraneurium." He noted that this structure appeared to be designed to allow for nerve motion and caliber changes. He also recognized that the paraneurium could become fibrotic, forming adhesions: "shrinkage of the fibrotic paraneurium… may cause compression of the entire nerve trunk, like a stocking that is too tight."(Millesi et al., 1995) Although these investigators inductively reasoned that the paraneurium might modulate tensile nerve mechanics based upon gross observation, the nature of such a role remained to be tested.

In this study, we found that both normal paraneurium and its abnormal counterpart, adhesions, significantly impacted the distribution of strain in rat sciatic nerves. When normal paraneurial tissues were intact, regional differences in strain were observed, with increased strain in the distal-femoral region of the nerve compared to mid-femoral regions. Strikingly, an almost-identical strain gradient was recreated within 6 weeks after the initial decompression, in spite of differences in the trajectory of the nerve within its bed. This gradient was again eliminated the adhesion tissue was decompressed. The elimination of regional differences in strain by surgical decompression indicates that paraneurial attachments play an essential role in directing strain along the course of nerves. The underlying rationale for relative compliance of the more distal region of the nerve is not completely clear, but may result from proximity to the articulating knee joint (cf. (Boyd et al., 2005; Wright et al., 2001; Wright et al., 1996)), compliance required for trajectory changes of a trifurcation, or both.

249 Our data have several important clinical implications. The first is that surgeries that

250 divide paraneurium or adhesions may have unpredictable consequences. Peripheral

251 nerves are frequently dissected free of their paraneurial attachments during surgery,

252 either to mobilize them for purposes of exposure, or during decompression procedures.

253 However, releasing these nerves from their paraneurial tissues for purposes of

254 mobilization may redistribute strain along the nerve, as recently described for the ulnar

255 nerve, following its release during surgical treatment of cubital tunnel syndrome.(Foran

256 et al., 2016) More persistent redistribution of strain from nerve regions accustomed to

257 high strain to less compliant regions of the nerve may alter nerve susceptibility to

258 structural and functional injury. It is not unreasonable to posit that neuropraxia and

259 nerve injuries of greater severity, which are commonplace after these exposures, may

260 be a result of altered mechanical loading subsequent to neurolysis, rather than a direct

261 mechanical insult from retractors.(McConaghie et al., 2014)

262 Also compelling is our discovery that adhesion tissue appears to reconstitute strain

263 distributions seen in control nerves, in both orientation and magnitude. This finding

264 raises the possibility that the seemingly "random" generation of fibrous tissue exhibited

265 by adhesions may instead participate in or respond to an organized process that

266 attempts to recreate the normal neural biomechanical environment. This raises the

267 question of whether the formation of adhesion tissue is necessarily pathological.

268 Several strategies have been proposed to limit formation of adhesion tissue. However,

269 rarely is consideration given as to whether paraneurial structures – whether native

270 paraneurium or adhesions – may in some cases represent a positive biological

271 response, possibly in response to mechanical loads experienced, and perhaps even

272 favored, by peripheral nerves.(Anava et al., 2009; Pfister et al., 2004; Simpson et al.,

273 2013) It is worth noting that rats were not immobilized in this study. It may be that

274 immobilization, which is frequently recommended to patients following surgery or

275 traumatic injury, leads to an absence of mechanical stimuli, driving the formation of a

276 pathologic tether. Further research comparing nerve kinematics in the context of

277 adhesion formation with and without immobilization will be needed to investigate this

278 possibility.

279   An interesting methodological note is that paraneurial adhesion formation in our study

280   required little more than incision, dissection of the paraneurial structures about the

281   nerve, closure with absorbable braided sutures, and the passage of time. Gross

282   observations of fibrotic tissue, as well as histological findings indicating a loss of

283   epineurial integrity, confirm the formation of adhesions, despite use of reproducible and

284   conservative surgical technique. Similar phenotypes have also been previously reported

285   by Millesi, as indicators of adhesion formation.(Mazal and Millesi, 2005; Millesi et al.,

286   1995) The ease of forming adhesion tissue is of interest, as a prior study reported the

287   formation of adhesions in the sciatic nerves of rabbits by both cauterization of the nerve

288   bed as well as suturing of the nerve to the surrounding nerve bed, followed by 5-6

289   weeks passage of time.(Abe et al., 2005) This latter method of forming adhesions is

290   problematic for study purposes involving biomechanics, as suturing of the nerve to the

291   nerve bed in and of itself results in a mechanical tether that affects future strain

292   measurements.(Mahan et al., 2015) Our method of producing adhesions is highly

293   reproducible and does not require nerve suturing, which may be helpful to other

294   investigators in future studies.

295   Our study has several limitations. We measured nerve surface (epineurial) strain by

296   measuring displacement of epineurial markings, rather than directly measuring strain

297   with a microstrain gauge or other method. Despite the comparatively low resolution of

298   our approach, we avoided damage to the neural elements, and our nerve strain findings

299   were in keeping with prior studies.(Topp and Boyd, 2006) We also did not measure

300   nerve strain distal or proximal to the exposed region of the sciatic nerve. It is possible

301   that nerve strain following paraneurial decompression was transferred somewhere

302   along the course of an unexposed region of the nerve. Further investigations will be

303   needed to quantify this possibility, and would be aided by the development of

304   noninvasive approaches to imaging less accessible nerve regions. Additionally, while

305   we did not observe any pooling of blood or change in tissue coloration indicative of overt

306   ischemia, more investigation is needed to determine the functional effects of paraneurial

307   decompression on neural electrical conduction and blood flow. A final limitation is the

308   nature of adhesions that were created following surgical decompression of otherwise

309   healthy tissue. Such adhesions likely more closely resemble those formed following

310   surgical dissection than those formed following injury or during neuropathic progression.

311   Such conditions may result in damage to nerve fibers, an altered inflammatory

312   environment, and fibrosis, all of which could impact resultant mechanical sequelae.

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384

385 **Table Legend:**

Score	Criterion
1	Complete extracellular tissue infiltration through the epineurium and into the nerve.
2	An amount of extracellular tissue fusion with the epineurium greater than or equal to 50% of the circumference of the nerve.
3	An amount of extracellular tissue fusion with the epineurium greater than or equal to 20% but less than 50% of the circumference of the nerve.
4	An amount of extracellular tissue fusion with the epineurium less than 20% of the circumference of the nerve.
5	Complete absence of extracellular tissue fusion with the epineurium.

386

387 Table 1. Criteria for assigning Epineurial Integrity Scores, based on trichrome labeling.
388 Sample images for each score are shown in Figure 2. A score of 1 was not recorded for
389 any sections.

Figures

Figure 1. Macroscopic view of nerve trajectories just prior to the initial decompression (A-B, Day 0) and 6 weeks after the initial decompression, but before a second decompression (C-D, Day 42). (A) Nerve trajectory in a relaxed position at Day 0 (hip abducted, knee flexed 90°, ankle neutral). Also indicated on this image are sample epineurial markings used to make strain measurements. Exposed nerve was divided into two regions, mid-femoral and distal-femoral, for regional strain analysis. Ruler is demarcated in mm. (B) Nerve trajectory in a joint configuration inducing strain at Day 0 (hip abducted, knee fully extended (180°), ankle maximally dorsi-flexed). (C) Nerve trajectory in a relaxed position at Day 42 (hip abducted, knee flexed 90°, ankle neutral). (D) Nerve trajectory in a joint configuration inducing strain at Day 42 (hip abducted, knee fully extended (180°), ankle maximally dorsi-flexed).

Figure 2. Trichome labeling was used to assess epineurial integrity 42 days after decompression for sham operated and decompressed nerves. (A-D) Sample images of completely intact (Score 5, A) to largely disrupted (Score 2, D) epineurium, with images shown from each experimental group. Scale bars are 200µm. Epineurial integrity scores were assessed based on criteria provided in Table 1. No tissue sections were scored with "Score 1" in either experimental group. (E) Comparison of semi-quantitative scoring of epineurial integrity reveals decreased integrity after decompression.

Figure 3. Phospho-neurofilament/laminin staining of mid-femoral and distal-femoral regions of nerve cross-sections from sham operated (A-B) and decompressed (C-D) nerves show intact axonal and intra-epineurial structure in both experimental groups. Axons are indicated by phosphorylated neurofilaments (red), and basal lamina is indicated by laminin (green). Insets are shown below the full cross-sectional image. Scale bars are 100µm.

418 Figure 4. Regional rat sciatic nerve strains before and after the initial decompression (A,

419 Day 0) and before and after a second decompression 6 weeks after the initial

420 decompression (B, Day 42). Distal strain is significantly higher than proximal strain

421 (P<0.001) prior to decompression at both time points. 2-way repeated measures

422 ANOVA revealed an interaction effect of region and decompression on strain at Day 0

423 (p<0.027) and an effect of decompression on strain at Day 42 (p<0.05). Asterisks

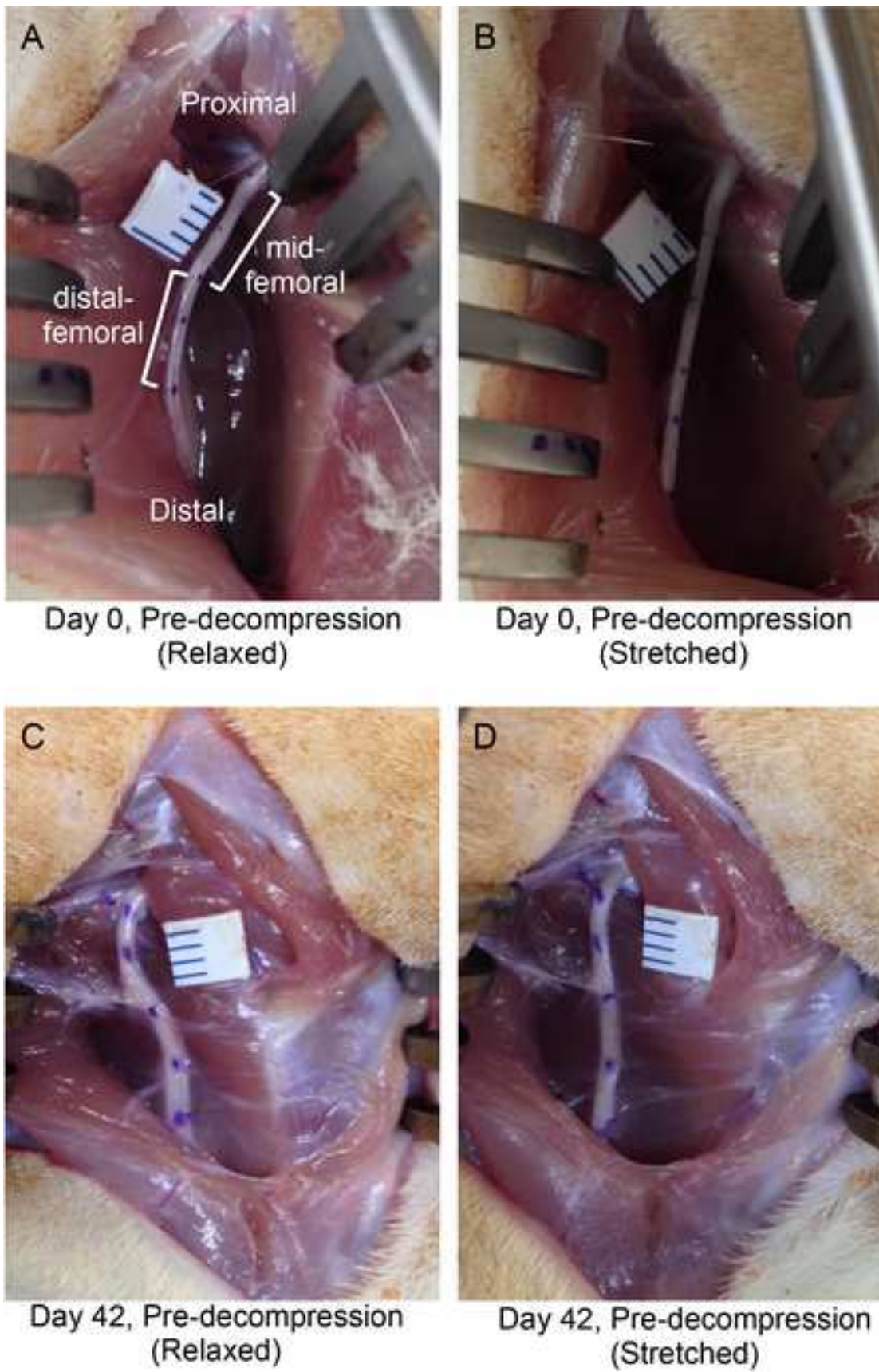424 indicate significant differences between individual groups based on post-hoc testing.
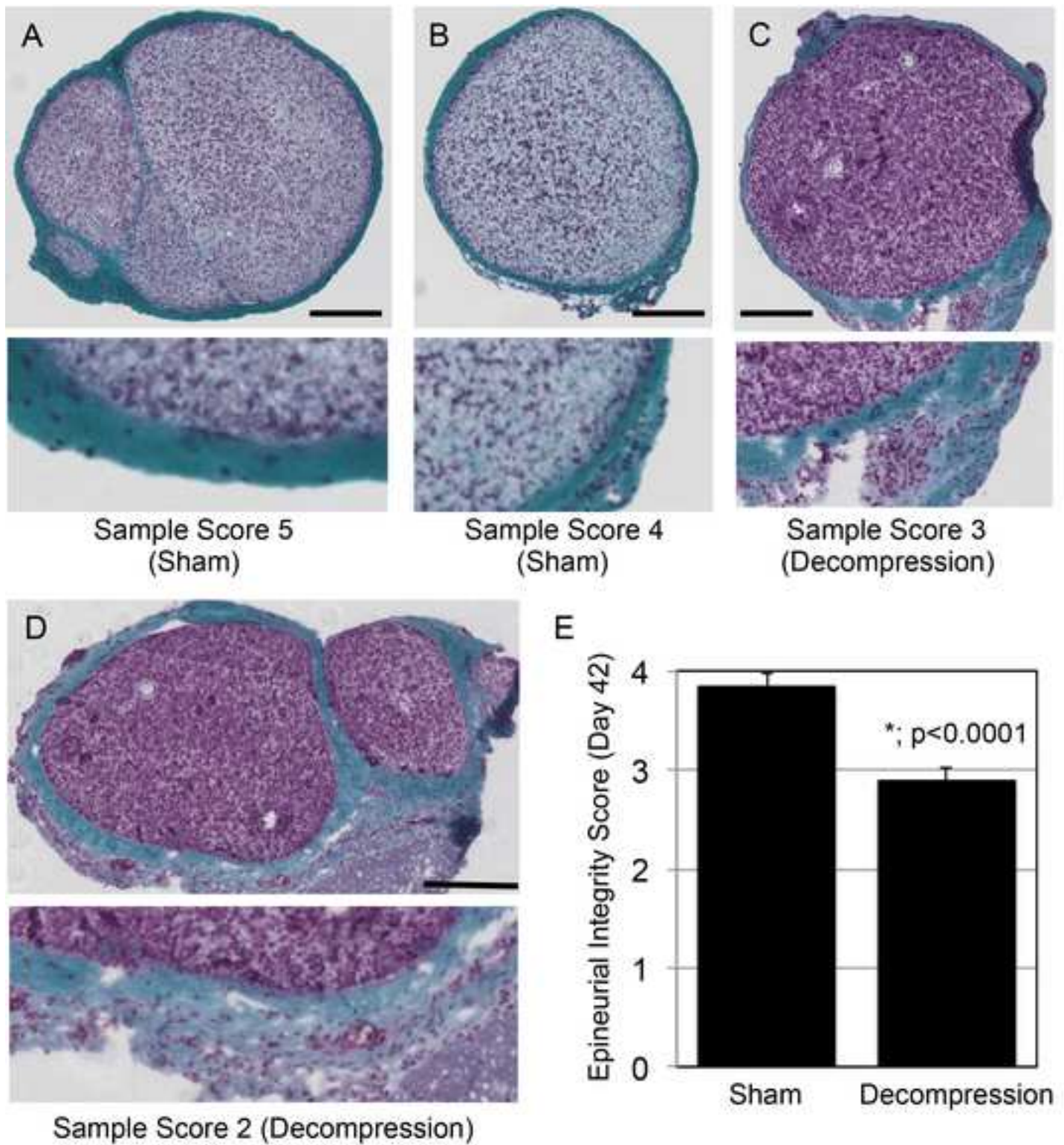
425

Figure 1

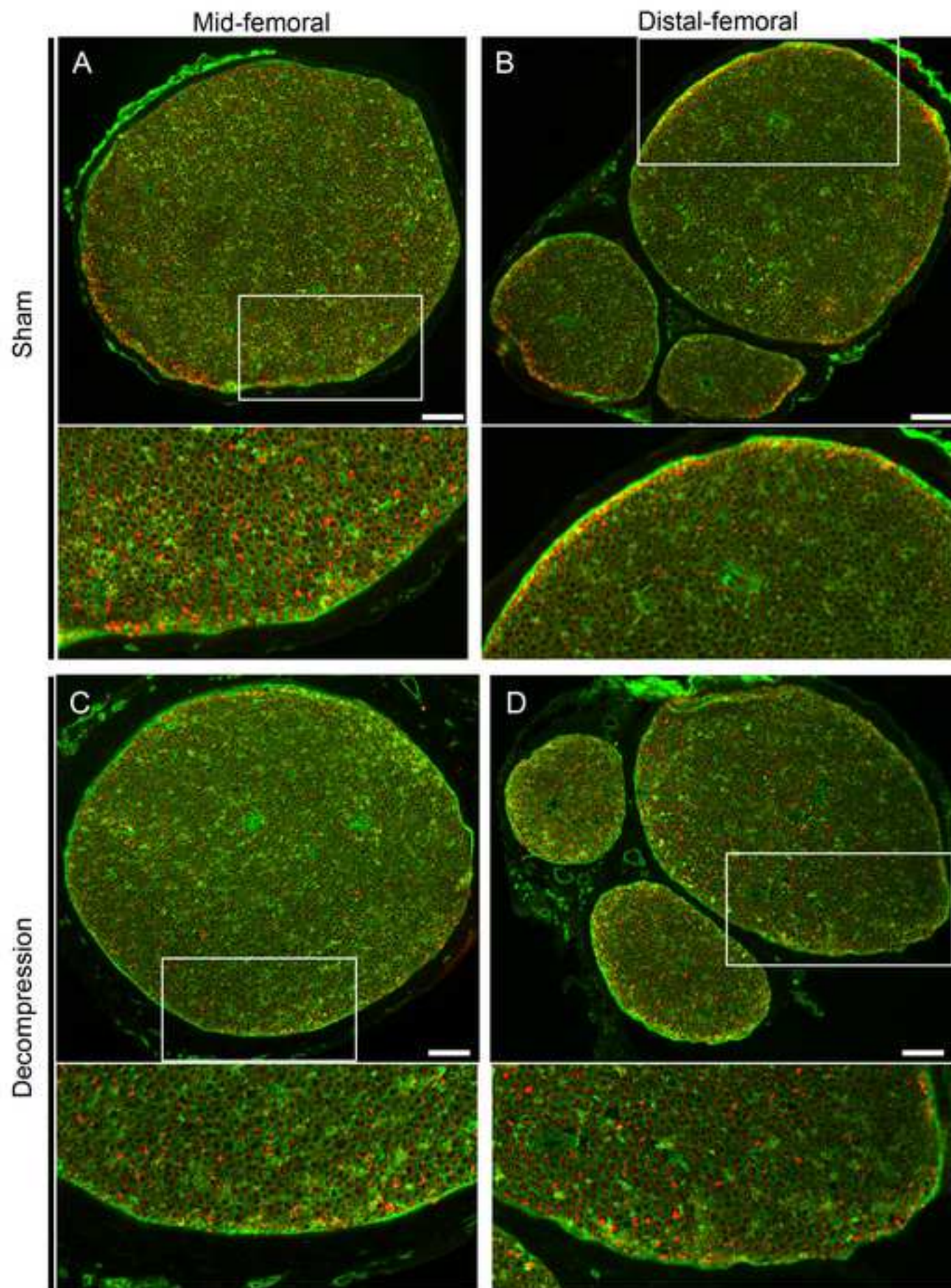Day 0, Pre-decompression (Relaxed)

Day 0, Pre-decompression (Stretched)

Day 42, Pre-decompression (Relaxed)

Day 42, Pre-decompression (Stretched)

Figure 1

Figure 2

A Sample Score 5
(Sham)

B Sample Score 4
(Sham)

C Sample Score 3
(Decompression)

D Sample Score 2 (Decompression)

E

Figure 2

Figure 3

Mid-femoral

Distal-femoral

Sham

Decompression

Figure 3

Figure 4

Figure 4