INTRODUCTION: Peripheral nerves typically exhibit viscoelastic behavior and exhibit a load-elongation curve similar to other soft tissues such as tendon. The collagenous architecture of the epineurium, perineurium, and endoneurium allow the nerve to regularly withstand tensile forces in the elastic region of their stress-strain curve without suffering permanent functional deficit. However, nerves will incur structural damage that may preclude functional recovery when strained beyond their yield point. Because the endoneurium contains a much lower ratio of collagen to myelin, it has been shown that endoneurial collagen will experience permanent damage before the external epineurium. Nerves strained to their yield point will experience a loss of endoneurial elasticity, which is indicated by wavy, undulating collagen fibers. This behavior can be defined as fiber tortuosity, and is represented as a ratio of fiber path length to fiber end-to-end length. Additionally, nerves strained above their yield point may lose endoneurial continuity, also indicating permanent damage. The fact that internal damage precedes external signs of damage poses a significant clinical dilemma since injured nerves may not show macroscopic signs of damage when neural injury is first presented. Therefore, it is often difficult to prospectively predict which nerve injuries will require surgical reconstruction and which will heal spontaneously.

We have previously used Second Harmonic Generation (SHG) imaging in a preliminary study to assess the time-related changes to the internal architecture of the nerve after inducing a general stretch injury in vivo. However we have not used SHG microscopy to assess the relationship between strain and internal structural damage in the nerve. The relationship between applied strain and internal structural damage is essential for the purpose of utilizing SHG microscopy as a tool for predicting when severe damage has occurred. Therefore, the objective of this study was to relate the amount of internal damage with the applied strain to better understand when and how severe nerve damage is presented. We hypothesize that SHG images will reveal internal damage at applied strains near 14% applied strain, and that all nerves will fail when strained to 20%. We hypothesize that damage will be indicated by loss of endoneurial or epineurial continuity, the onset of tortuous behavior, and internal disorganization.

METHODS: Median nerves (n=27) from female Sprague-Dawley rats were used in this study. Six nerves were monotonically loaded to failure using an Instron machine to determine the elastic properties of the rat median nerve, the point of failure, and the appropriate strain levels to be used later in the study. Median nerves were strained to 4% (n=6), 10% (n=5), 14% (n=7) and 20% (n=6). A nominal load of 0.5N was applied to each nerve using a device that has been previously developed and validated in our lab. Nerves were then fixed in the applied nominal load with a consistent starting length of 23mm and a first set of images was taken using the appropriate parameters for SHG microscopy. Each nerve was then loaded to 4%, 10%, 14%, or 20% of the 23mm starting length and the applied strain was held for 5 minutes and released. After 5 minutes at rest, the nominal load of 0.5N was reapplied for an additional 5 minutes, and a second set of images were obtained. Both sets of images were taken at three distinct regions within the 23mm segment of the nerve. Sets of images were compared to one another and were analyzed for co-linearity and parallel fiber arrangement, loss of epineurial or endoneurial continuity, and indications of tortuous behavior. Fiber tortuosity is found by calculating the ratio of fiber path length to end-to-end fiber length, however specific tortuosity values are not included due to raw data sample number.

RESULTS: Monotonic loading tests showed that the median nerves failed, on average, at 1.99N or 19.4% strain. The elastic region for all nerves began at approximately 0.25N, thus we chose 0.5N as an appropriate nominal load, for it fell within the low elastic region of all nerves that were mechanically tested. Nerves imaged after application of the nominal load revealed a distinct linear fiber appearance, indicating the removal of any slack from endoneurial collagen (Fig 1A). Nerves that were subsequently strained to 4% (Fig 1B) and 10% (Fig 1C) showed no signs of damage or structural alteration after the nominal load was reapplied, and the response was homogenous throughout length of the nerve. Nerves strained to 14% behaved variably, showing complete failure (n=4), no damage (n=2), tortuous behavior (n=1), or loss of epineurial continuity (n=1) after the nominal load was re-applied. The tortuous behavior (Fig 1D) indicated that the nerves had been strained above their plastic limit, thus losing their original elasticity. The loss of endoneurial continuity demonstrated how internal damage can precede external, epineurial damage (Fig 1E). Nerves loaded to 20% also behaved variably, with exactly one half (n=3) showing complete failure and the other half (n=3) showing noticeable fiber tortuosity after the nominal load was re-applied (Fig 1F).

DISCUSSION: We have previously used SHG microscopy to non-destructively generate images that reveal changes in the internal collagen microstructure of nerves subjected to in vivo stretch injury and how the structure changes over time post injury. This study now relates the degree of internal, microstructural damage with the amount of applied strain using images generated through SHG microscopy. SHG images have allowed us to now identify a critical strain value, in the region to and above 14% strain, where significant internal collagen damage is likely to occur. SHG images have indicated that nerves will behave consistently along the linear region of their stress strain curve up to this critical strain region, after which they may behave variably. Nerves will either experience no significant structural damage, or they will accrue permanent damage as indicated by tortuous behavior or loss of endoneurial continuity. Furthermore, we found that nerves also behave variably when subjected to 20% strain, a value that had previously led to complete failure. This finding is also consistent with the idea of a critical strain region for peripheral nerves, where it is possible that the collagen architecture and organization of individual nerves primarily determines whether or not they will experience permanent damage. Our study shows that SHG microscopy is capable of revealing significant internal damage after applying various levels of strain. This study provides insight into how SHG microscopy can be used to analyze the severity of neural damage that accrues over time when various strains are applied to the median nerve in vivo, and how neural structure changes during regeneration and healing.
