INTRODUCTION:
Although peripheral nerves are routinely subjected to mechanical forces, excessive or prolonged forces may induce neural injuries. Chronic nerve compression (CNC) injuries are the result of both ischemic and mechanical stimuli. Previous studies have shown that mechanical stimuli alone can have a direct effect on the nerve.1,2 During the early phases of CNC injury, Schwann cells undergo a dramatic process of demyelination, proliferation and apoptosis in the absence of Wallerian degeneration.1,3 It appears as if these alterations in Schwann cell behavior and ensuing changes in myelination are the direct result of mechanical stimulation on the nerve. Consistent with this hypothesis, Schwann cell proliferation, gene and protein expression are altered by applied mechanical stimuli.1 In this study we developed an in vitro system to isolate and define the role that mechanical stimuli play in the pathogenesis of CNC injuries.

METHODS:
All animal procedures are approved by the University’s IACUC. Dorsal root ganglion (DRGs) are harvested from E15 Sprague-Dawley rats and dissociated. After 4 days, purified P3 Schwann cells are added to the cultures. The media is supplemented with vitamin C to initiate myelination.

A custom-designed hydrostatic compression chamber was developed to apply a defined magnitude of stimulus to neural co-cultures. The system is pressurized with a diaphragm pump. Air flows out of the system to maintain constant gas exchange and a solid-state pressure sensor monitors the internal pressure. As pressurizing a liquid-gas interface increases the amount of gas dissolved in the media, a feedback control system is used to regulate dissolved O2 during pressurization. In addition, pH homeostasis is maintained by regulating CO2 flow into the incubator.

Lactate dehydrogenase (LDH) production is measured immediately following 1-7 days of static compression. The co-cultures are immunostained for S100, a pan-specific Schwann cell marker. Proliferating cells are labeled with bromodeoxyuridine (BrdU). The cultures were imaged using an Olympus IX71 inverted microscope and VisioPharm software.

RESULTS:
The cells maintained their morphological integrity after one week of being cultured in the compression chamber under normal culture conditions (37°C, pH= 7.1-7.3, dO2= 95-105%, no applied pressure). There is no morphological indication of axonal degeneration, as evidenced by detachment of axonal processes from the coverslips, or other alterations in cell structure/shape.

Cytotoxicity assay results showed that there was no significant production of LDH by the cultures with low levels of pressure (0.7221-0.886 PSI) for 24 hours. A pressure of 1.19 PSI was required to elicit a response from the cells. However, when a very low level of pressure (0.348 PSI) was applied for 7 consecutive days, the LDH levels increased. This data supports that chronic compression injury has a different effect on the nerve cells than acute compression injury.

DISCUSSION:
Several factors contribute to chronic nerve injuries, including mechanical stimulation, ischemia, and the immune response. Using an in vitro model, we are able to isolate the effects of mechanical stimuli on myelinated peripheral nerve cultures. Instead of inducing a cellular stress response, hydrostatic compression injury for 4 days in vitro stimulates Schwann cell proliferation. This data is consistent with previous studies which indicate that Schwann cells are mechanosensitive cells.1 Future studies will focus on further defining the role that mechanical stimulation plays in the demyelination associated with CNC injury.

REFERENCES: