INTRODUCTION
This project serves a great purpose to the military community, since approximately 5-6% of military injuries involve some form of major peripheral nerve injury (PNI) [1]. Once these nerve injuries occur, voluntary muscle function is impacted in the limbs. Novel treatments to restore and initiate nerve regeneration and expedite recovery would help greatly with curing peripheral nerve injuries [1].

PNI leads to Wallerian degeneration, in which nerve tissue distal to the injury site degrades. This causes deterioration of the neuromuscular junction (NMJ), the lab’s approach is to target the NMJ while developing therapeutic treatments. The NMJs are specialized chemical synapses formed at the sites where the end branches of the axon of a motor neuron communicate with a target muscle cell [2]. Since the NMJ is being affected in PNIs, past research has involved the controlled release of anti-inflammatory drugs or neurotropic factors at the NMJ. Intramuscular electrodes have been widely studied to modify, restore, or bypass a damaged or diseased portion of the nervous system [3]. This electrically controlled drug release system would depend on a conducting polymer and carbon nanotube (CNT) framework to load drug molecules on the CNTs, which would then be released at the NMJ [3].

Literature research has shown effective use of dexamethasone (DEX), a corticosteroid that suppresses inflammation, in the electrically controlled drug release system mentioned above [3]. However, the one problem is that this system is dependent upon the use of water during the loading of the drug into the CNTs. Since the future direction of the research involves the potential use of anabolic steroids, the project’s unique approach is to capture and release weakly-water soluble drugs.

OBJECTIVE
The feasibility of the poly(3,4-ethylenedioxythiophene) (PEDOT), DEX, and CNT coating and the efficacy of the DEX release in in-vitro studies. PEDOT serves as the conductive polymer in the study. The parameters that will be measured include the charge loading capacity of the electrode, the impedance of the electrode, verification of DEX release, and presence of the nitrite ion in solution post-application of lipopolysaccharide (LPS) in cell culture with the use of HAPI cells.

SUCCESS CRITERIA
The in-vitro testing with dexamethasone is successful provided the following criteria are met: (1) an increase in charge storage capacity of the electrode due to the increased surface area from the rough conductive polymer morphology, (2) a decrease in impedance in the electrode from the increase in surface area of the available material (3) absorption only at the DEX wavelength (240nm) in the drug-release solution, and (4) a decrease in the LPS stimulated nitrite ion (NO) production in the cells.

METHODS
The electrically controlled drug release paradigm involves the use of glassy carbon electrodes in order to characterize the release of the drug. Before proceeding with the polymerization process, the electrodes have to go through an extensive washing procedure in order to remove any past residue. In addition, the CNTs must be treated with acid in order to functionalize them. Post-functionalization, the CNTs will be loaded with the drug through sonication.

Post-cleaning of electrodes and loading of drug into CNTs, the next steps involve the polymerization of the electrodes. During the polymerization process, the drug-loaded CNTs are coated onto the electrode with the conductive polymer. The instrument required for this is the Gamry, which comprises of a three-electrode setup. The chronocoulometry experiment of the Gamry allows for the PEDOT-DEX-CNT coating on the electrode. Two of the metrics required for the success criteria of this project demand data to be collected before and after the polymerization process. Cyclic voltammetry and electrochemical impedance spectroscopy scans must be run before and after electrode polymerization in order to measure changes in charge storage capacity and impedance, respectively.

Following electrode polymerization, the drug will be released from the electrode and submitted to absorbance tests in order to verify that the compound released is dexamethasone. Repeated chronoamperometry is performed on the Gamry to release the drug. The release solution is then analyzed with a spectrophotometer to analyze at which wavelength light is absorbed. The typical wavelength at which dexamethasone is absorbed is 240nm.

In order to determine the bioactivity of released dexamethasone, a Griess assay is performed on LPS-stimulated HAPI cells to detect the presence of NO in solution. A combination of HAPI cell media, interferon gamma, and LPS is created and applied to pre-passaged HAPI cells. Two groups will be used for this experiment: one group will be the LPS-stimulated HAPI cells and the second group will be the LPS-stimulated HAPI cells combined with the dexamethasone release solution. After incubating both groups of cells for 24
hours, they solutions are subject to the spectrophotometer to analyze the NO content.

RESULTS
As shown in Figure 1, there is an increase in the charge storage capacity of the electrode. The area under the two curves represents the charge storage capacity, and it is evident that the area under the curve for the electrode post-polymerization is greater compared to pre-polymerization.

![CV Voltammogram, Electrode 3 and Impedance, Electrode 3](image)

Figure 1. Cyclic Voltammetry scan of electrode 3 (left). Figure 2. Electrochemical Impedance Spectroscopy scan of electrode 3 (right). In both curves, the blue curve signifies post-polymerization and the red curve signifies pre-polymerization.

As shown in Figure 2, there is a decrease in the impedance of the electrode based on the starting point of the impedance curve. Following the dexamethasone release, the absorbance study showed positive results as the curve for absorbance showed a component of the solution being released at around 240 nm, which indicates the release of dexamethasone. Lastly, as seen in Figure 3, there was a significant decrease in the LPS-stimulated NO production in HAPI cells post-application of dexamethasone.

![Released Dex attenuates LPS stimulated NO production in HAPI cells](image)

Figure 3. NO production comparison between dexamethasone and non-dexamethasone treatment on LPS-stimulated HAPI cells.

DISCUSSION
As shown in the results section above, the in-vitro tests with dexamethasone were successful. The success criteria of all the studies were mostly met, with the exception of the absorption of the dexamethasone.

As shown with the increase in charge storage capacity, it was indicative that the PEDOT-DEX-CNT coating was properly deposited onto the electrode. Initially, when performing the cyclic voltammetry on a bare electrode, there should be no charge since nothing is on the electrode. If there is a reading, that comes from trace amounts of material from past studies. However, after the CV scan is performed post-polymerization, the increase in charge storage capacity comes from increased surface area from the PEDOT. The decrease in impedance also serves as a viable factor in analyzing whether or not the coating has been placed on the electrode. Impedance is defined as the measure of opposition that a circuit presents to a current when a voltage is applied. Because there is more material and increased surface area presented from the conductive polymer, it is easier for current to flow and therefore decreased impedance. The absorbance results did not show definitive release of dexamethasone due to the lack of a peak. Based on an absorbance spectra measured from a standard DEX solution, there is a peak which indicates the wavelength at which dexamethasone is most absorbed. A similar peak is required in experimental results to show what component of the solution was released and at what wavelength it was most released. Because there was no peak shown in the absorbance results, there is no way to know definitely whether dexamethasone was properly released. Lastly, since the released dexamethasone was able to reduce the NO production by nearly half, it is clear that the drug is bioactive.

There were a couple of limitations to this study. First, the loading of drug into the CNTs was trivial based on the properties of the CNTs themselves. Being very small and highly dense structures, there is no definitive way to know whether the dexamethasone is housed within the CNT framework. Second, performing a drug release in a weakly-water soluble environment poses issues.

Future steps include in-vitro experimentation for drug release with lower concentrations of dexamethasone. Doing so will allow the possibility of generating a peak during absorbance data. If the concentration of dexamethasone is too high, then there will just be a maximum amount of drug absorbed at the starting wavelength rather than varying amounts of drug release across a range of wavelengths. Second, provided dexamethasone proves to be a viable drug in in-vitro and in-vivo studies, then electrode polymerization and drug release will be carried out with a different anabolic steroid, such as testosterone or nandrolone.

ACKNOWLEDGMENTS
Thanks to the principal investigator Dr. Tracy Cui, graduate mentor James Eles, and other scientists at the NTE Lab.

REFERENCES